TREATMENT OF ORGANIC EFFLUENT AND THE PRODUCTION OF SINGLE - CELL PROTEIN

Thesis submitted to the University of Kerala
in partial fulfilment of the requirements
for the degree of
Doctor of Philosophy in Microbiology

By

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February 1987

To

my father

and

late mother

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February 4, 1987

This is to certify that the thesis entitled "TREATMENT OF ORGANIC EFFLUENT AND THE PRODUCTION OF SINGLE-CELL PROTEIN" herewith submitted by Shri V.B.Manilal, in partial fulfilment of the requirements for the Ph.D. Degree in Microbiology of the University of Kerala, is an authentic record of the research carried out by him under our guidance and supervision and that no part thereof has been presented for any other degree.

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ACKNOWLEDGEMENTS

I express my sincere gratitude to Dr. C. Balagopalan, Scientist and Head, Division of Post-Harvest Technology, Central Tuber Crops Research Institute, Trivandrum, for his excellent guidance throughout the period of the work. Grateful thanks are expressed to Dr. C. S. Narayanan, Head, Division of Food, Regional Research Laboratory, Trivandrum, for his guidance during the study.

I wish to place on record my profound sense of gratitude and indebtedness to Dr. A. D. Damodaran, Director, RRL, for his continuous encouragement during the course of this work and for providing necessary facilities. I must thank Dr. A. G. Mathew, Deputy Director, RRL, Trivandrum, for the encouragement and personal guidance.

My thanks are due to Mr. C. Chandrasekhara Bhat and Mr. Ajith Haridas, Scientists, RRL, Trivandrum, for the help in the computer study. I am extremely thankful to Mrs. K. P. Padmakumari, Mrs. Beena Symon and Mr. S. G. K. Pillai for their assistance in the analyses.

The financial support rendered by the Council of Scientific and Industrial Research is gratefully acknowledged.

I wish to thank all my colleagues for their cooperation.

V. B. MANILAL

PREFACE

Cassava (Manihot esculenta, Crantz) is a major source of starch in many parts of the world. The processing industry is facing serious and constant pollution problems, so the treatment of effluents from the cassava starch industry is important. Conventional biological methods are found to be efficient in the treatment of organic effluents in which different microorganisms are involved in the oxidisation process of the organic matter. Introduction of specific microorganisms in the treatment is economically viable to reduce the pollution hazard with the concomitant production of valuable products like single-cell proteins. Anaerobic digestion is proved to be effective in the treatment of many other effluents containing heavy load of organic matter.

The thesis deals with the studies carried out for the development of microbiological methods to treat cassava starch factory effluents, in six chapters.

The origin of cassava starch factory effluents during the extraction of starch from the tubers, and the review on the aerobic as well as anaerobic treatment of similar effluents are presented in the Chapters 1 and 2. Characteristics of the effluents collected from different starch factories were analysed and the rate of degradation of organic matter by natural contamination under ambient conditions were assessed. The effluents

were treated with yeasts and yeast-like organisms and the solid wastes with Aspergillus niger. Anaerobic treatment of cassava starch factory effluent for the generation of methane gas was studied, subsequently the reduction of pollution load was assessed. The methods employed and the results obtained are presented in Chapters 3 and 4. The discussions of the results are incorporated in Chapter 5 of the thesis. In the 6th Chapter the significant observations are summarised.

Two publications entitled "Physico-chemical and microbiological characteristics of cassava starch factory effluents" (J. Root Crops, 9 (1 & 2): 27-31, (1983)) and "Amyloglucosidase and cellulase activity of Aspergillus niger in cassava starch factory wastes", (Proceedings of the National Seminar on "Utilization of tropical tuber crops" held in Central Tuber Crops Research Institute (ICAR) Trivandrum, 1985) were also made from the study.

CONTENTS

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Cassava (Manihot esculenta, Crantz), popularly known in India as Tapioca is one of the major tuber crops, grown in more than 80 countries of the humid tropics. It is one of the most efficient photosynthetic plants known which accumulates starch in adventitious roots. The cassava tubers have an average composition of 60-65% moisture, 30-35% carbohydrates (predominantly starch) 0.2-0.6% ether extractives, 1-2% crude protein and comparatively low content of vitamins and minerals (Omole, 1977).

The cassava starch industry is one of the major agro-based industries usually located in the cassava growing tracts of the tropical countries. Starch is mainly used in the food, textile, paper and pharmaceutical industries. The process of starch extraction requires large quantities of water in the beginning and the water is separated and discharged during the final stages. These discharges (effluents) contain high amount of carbonaceous matter in soluble and insoluble forms. Most of the starch factories are usually situated on the banks of rivers or lakes and it has become customary to discharge the effluents from the factory to rivers or lakes. These effluents pose a serious threat to the environment and quality of life in the rural areas where the factories are located. Effective treatment of effluents from the industry will result in better environmental conditions. In order to develop efficient and

effective low-cost technology, the physico-chemical and microbiological characteristics of the effluents which are subjected to varying environmental influences have to be studied in detail.

The ubiquity of microorganisms and their exploitation by man is well established in the biological process of waste water treatments. Based upon the conditions required for the growth and thereby degradation of waste materials, the microbiological treatments are classified as aerobic and anaerobic.

Various types of microorganisms proliferate in the organic effluents depending upon the available substrates and conditions. Cassava starch factory effluents being rich in starch can promote the growth of microorganisms which liberate amylolytic enzymes for the hydrolysis of starch to glucose. The sugars thus formed in the starch factory effluents could be utilized for the production of microbial biomass rich in protein. From time immemorial, the ability of yeasts to utilize sugars for the production of protein rich biomass has been exploited in food and feed industry.

Co-culturing of yeasts for effluent treatment and single-cell protein (SCP) production has been attempted elsewhere (Lemmel et al., 1979). Solid state fermentation has been reported to be a relatively low-cost appropriate technology for the upgradation of substrates rich in starch to protein rich animal feed (Senez et al.,1983).

The co-ordinated metabolisms of different bacterial groups efficiently decompose the hydrolysable carbohydrates into methane and carbondioxide under anaerobic conditions (Bryant, 1967). The anaerobic fermentation of carbohydrates is of particular importance in the effluent treatment system because of its potentiality in the waste stabilization.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Organic Effluents from Cassava Starch Factory

Starch production from cassava tubers is an increasingly important agro-industry in tropical countries. Cassava starch is one of the major raw materials in food and pharmaceutical industries.

The cassava tubers contain about 20-30% starch which is distributed in the cellulosic matrix (Mangalakumari, 1983). Extraction of starch from tubers is generally followed by a wet process and it requires voluminous amount of water. Basically the extraction of starch consists of washing of tubers, mechanical peeling, rasping, grinding, sieving, regrinding, sieving and dewatering (Fig.1). During off-seasons dried chips of cassava tubers are also processed for starch extraction. All these procedures aid the separation of starch granules which come out in the aqueous medium from the natural loci. This is further separated and collected for processing. The recovery of starch even in the modern starch factories is not complete and some amount of it along with fibrous wastes accumulate on the sieves. This is washed out as primary effluent.

The starch present in the water (starch milk) is separated by settling and centrifugation. Waste water coming out of the settling tank and centrifuge is called as secondary effluent which mainly contains soluble organics and unseparated

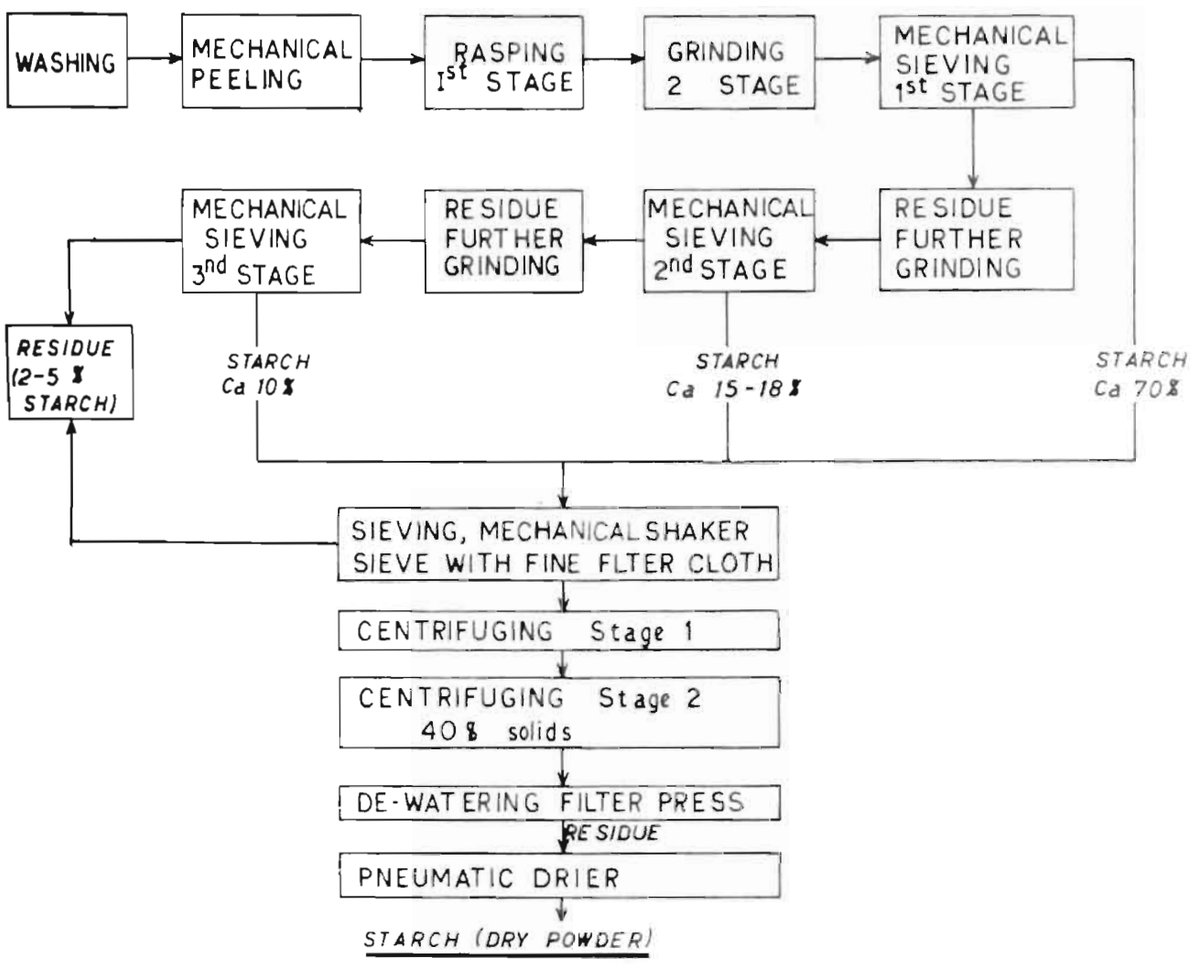


FIG. 1. FLOW DIAGRAM FOR THE EXTRACTION OF STARCH FROM CASSAVA TUBER

starch granules. The extracted starch is further dried and bagged for various end uses.

Essentially the extraction methods are the same in large-scale and small-scale starch factories. In the former, extraction of starch is done efficiently with modern equipments. Waste water produced at the time of washing the tubers contain negligible amount of organics. Effluents produced from the remaining steps altogether comprise, unrecovered starch, cellulose fibres, soluble carbohydrates, nitrogenous compounds, etc. and these are discharged into the nearby lakes or streams or fields, thus causing deleterious effects on the environment.

2.ii. Microbial Degradation of Organic Matter

The biological material on earth is continuously subjected to decomposition by microorganisms. King (1981) has defined biodegradation as the ability of compounds to undergo change, usually simplification of structure by the action of microorganisms.

Biodegradation will depend upon the presence of microorganisms possessing genetically determined biochemical ability to initiate and to proceed attack on the molecular structure of the material undergoing decay (Pike and Ridgway, 1985). The rate of biodegradation will be determined by the number of active microorganisms, the dilution rate and other conditions in the system and concentration of the substrates which may be

toxic to microorganisms at higher concentrations.

Biodegradation is measured either by estimating the primary compounds or by the ultimate dissolved organic compounds. Uptake of oxygen, production of carbon dioxide and rate of growth of microorganisms are also taken into account in certain cases (Anon, 1981).

Normally the pollution load is assessed by estimating the biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

Organic matter decomposition serves two functions for the microflora, providing energy for growth and supplying carbon for the formation of new cell material. Carbon dioxide, methane, organic acids and alcohols produced are merely waste products as far as microbial development is concerned.

Under aerobic conditions, usually 40% of the substrate carbon is assimilated and the remainder is released as carbon dioxide or accumulated as waste products.

During the decomposition process by fungi, 30-40% of the substrate carbon metabolized is used to form new mycelium. Population of aerobic bacteria, assimilate 5 to 10% of the substrate carbon while anaerobic bacteria incorporate only about 2 to 5% of the substrate carbon into new cells (Waksman, 1929).

The rapidity with which a given substrate is oxidized

will depend upon its chemical composition and the physical and chemical conditions in the surrounding environment. Temperature, oxygen supply, moisture, pH, available minerals and C:N ratio of the substance are the chief environmental influences (Anon, 1967). The maximum rate of decomposition of carbonaceous materials takes place at 30-40°C. Above 40°C, the rate of decomposition declines except in those special circumstances where thermophilic decomposition is initiated (Anon, 1967).

Acharya (1935) demonstrated that the decomposition of rice straw is most rapid aerobically, slower in water logged conditions and least pronounced under complete anaerobiosis. The decay of the plant constituent is affected in the same way i.e. the process is depressed as the supply of oxygen diminishes. Microorganisms grow readily in liquid culture media provided oxygen supply is ample; on the other hand, high moisture levels reduce microbial activities not as a result of the water itself but rather indirectly, by hindering the movement of air and thus reducing the oxygen supply. The pH not only influences growth rates and enzymatic potentialities of microorganisms but also governs the type of microorganisms associated with decomposition. Decomposition typically proceeds more readily in neutral than in acidic range.

A number of investigators have reported that the rate of decomposition of plant materials depends upon the nitrogen content of the tissue. Protein-rich substrates are metabolized

most readily (Miller et al., 1936). Cellulose and hemicellulose decompose slowly when compared to water soluble substances. Lignins are highly resistant to microbial decay and hence relatively slow in decomposition.

The main products of aerobic carbon mineralization are carbon dioxide,water and cells. In the absence of oxygen, organic carbon is incompletely metabolized to intermediary substances. Accumulation of abundant quantities of methane, carbon dioxide and smaller amounts of hydrogen is also reported. At the same time, the energy obtained during anaerobic fermentation is low,resulting in the formation of fewer microbial cells per unit of organic carbon degraded. In cases where sufficient carbohydrates are available most of the oxygen is utilized on the surface layers before it could penetrate deeply into the liquid layer, and thus the transformation of the lower depth is almost entirely anaerobic.

The establishment of microbial growth in the cassava starch factory effluents is quite possible since the effluents contain large amount of carbohydrates (Manilal et al., 1983).

2.iii. Single-cell Proteins from Organic Materials and Wastes

A new generic term "single-cell protein" (SCP) was coined during the First International Conference on Microbial Proteins convened in 1967 at the Massachusett Institute of Technology (MIT), Cambridge, Massachusetts, USA to denote crude or refined

sources of protein whose origin is unicellular or multicellular organisms, i.e. bacteria, yeasts, molds and algae" (Mateles and Tannenbaum, 1968).

The use of microorganisms for the conversion of carbohydrates, lignocellulose and other industrial wastes into food and feed, rich in protein is advantageous due to the following characteristics of microorganisms.

(a) Microorganisms have a very fast growth rate (Thaysen, 1956).

(b) They can be easily modified genetically for growth on a particular substrate and under particular cultural conditions.

(c) Their protein content is quite high, varying from 35 to 60%.

(d) They can be grown in slurry or on solids.

(e) Their nutritional values are as good as those of other conventional foods rich in protein (Rox, 1982).

Several types of microorganisms such as algae, bacteria, yeasts and filamentous fungi were studied as potential sources of SCP on various substrates like hydrocarbons (n-alkanes, gas oil, crude oil having high wax content, natural gases, petrochemicals, etc.) and agro-industrial wastes (molasses, bagasse,

cereal straw, dairy wastes, sulfite waste liquor, fruit processing wastes, cereal grain wastes, potato processing wastes, wood hydrolysates, feed lot run-off, etc.).

Algae

Micro-algae were first suggested by Spoehr and Milner (1949) as a protein source and immediately after that Chlorella species was introduced into pilot plant scale production (Little, 1953). Tannenbaum and Steven (1971) suggested that SCP derived from algae and other microorganisms would fulfil a major food need for mankind. Mutants of Chlorella vulgaris were studied for its higher growth rate, quality proteins and biomass production (Passera et al., 1973; Garrett et al., 1976; Allen et al., 1977). It consisted of about 42% true protein, 23% carbohydrates, 16% lipids and 9% nucleic acids. Dor and Inka (1975) have suggested the production of Scenedesmus obliquus in dialysis tubes suspended in raw sewage. The culturing of Scenedesmus acutus and Scenedesmus quadricaula was proved useful for SCP production (Venkataraman et al., 1977, 1979). The growth of Spirulina maxima in effluents from secondary waste water treatment plants was studied by Ayala et al. (1984).

Bacteria

The potentiality of heterotrophic bacteria have been studied as SCP sources (Hamer, 1973). Bacterial utilization of methane for SCP production was proposed by Wolnak et al.

(1967) and Hardwood (1972). Methane was used as a carbon source for the growth of Methylomonas flagellata and Methylococcus capsulatus (Wilkinson et al., 1974; Morinaga et al., 1976). Bacterial cells were also grown on sugars and many fractions of plant and animal biomass. Continuous cultivation of Bacillus megaterium on collagenous by-product was reported by Bough et al. (1972). The growth of Pseudomonas fluorescens and Cellulomonas species on different substrates were studied by many workers for single-cell protein production (Dunlap, 1969; Callihan and Dunlap, 1971; Abbott et al., 1973; Daly et al., 1974; Irwin, 1974; Callihan and Irwin, 1974; Iglesias et al., 1979; Lee et al., 1979; Shuler et al., 1979).

Mixed culturing of Azotobacter chroococcum and Azotobacter agile with Candida and Torula yeasts could achieve efficient utilization of sucrose and better yields of SCP (Panosyan and Avakyan, 1974).

Filamentous fungi

Certain higher fungi have been consumed by humans for centuries. Commercial development of fungal SCP other than mushrooms has not been very common. The cellulolytic and lignolytic activity make some fungi desirable for cultivation on lignocellulosic wastes for economical production of SCP (Tusé, 1984).

With the exception of Cladosporium resinae, filamentous

fungi have not been seriously cultured as potential source of SCP on hydrocarbon substrates because of their slow growth rates and morphology (Walker and Cooney, 1973). Species of Penicillium, Scopulariopsis, Aspergillus, Trichoderma, Cladosporium, and Fusarium were grown on the ground pine (Pinus radiata) bark/ extract of the pine bark and the necessity of addition of nitrogen for economic production of SCP was demonstrated by Updegraff et al. (1975). Han et al. (1976) grew Aureobasidium pullulans on acid hydrolysate of rye grass (Lolium multiforum) straw and claimed the achievement of fungal protein, superior to Candida utilis. Compared to Aspergillus niger, Pestalotia species and Penicillium chrysogenum were found effective in converting alkali-treated rice straw to fungal protein (Sitaram et al., 1978). The work of Daugulis and Bone (1978) has supported the production of Phanaerochaete chrysosporium protein from inexpensive substrates like pine, maple, cedar tree barks, etc. Fermentation of rye grass hydrolysate with Aureobasidium pullulans, waste paper hydrolysate with Scytalidium acidophilum was motivated to produce fungal protein (Beily et al., 1979; Ivarson and Morita, 1982). Chaetomium cellulolyticum was tried on carbohydrates and found effective in the production of SCP (Pamment et al., 1978; Moo-Young and Chahal, 1979; Hecht et al., 1985). Meiering et al. (1979) have reported that Aspergillus fumigatus could be utilized to form fungal protein. Single-cell proteins from rotten vegetables and fruits with Aspergillus flavipus, from brewery spent liquor with Aspergillus niger, from orange waste liquor

with Sporotrichum pulverulentum, etc. were also reported (Hang et al., 1975; Karapinar and Melahat, 1982; Ali et al., 1984).

Yeasts

Probably the most experience in microbial food manufacture has been with yeasts (Reed and Peppler, 1973). Rapid growth rates, ability to assimilate a variety of substrates, high protein content, and with the exception of the sulfur containing aminoacids, good aminoacid profiles have made certain yeasts prime candidates for SCP production. Saccharomyces cerevisiae has been studied extensively for SCP production. Recently Candida utilis has been also studied widely as a source of SCP, because of its ability to grow on pentoses as well as hexoses which enabled the production of SCP from waste lignocellulosic hydrolysate (Rose, 1979). Baker's yeast can in fact be regarded as a specialized form of single-cell protein. The commercial production of Baker's yeast has been extensively reviewed (Hoogerheide, 1969; Burrows, 1970; Harrison, 1971; Reed and Peppler, 1973; Rosen, 1977; Burrows, 1979).

Utilization of gas oil for the production of SCP with an yeast isolate was studied by Vadalkar et al. (1969 and 1971) and with Trichosporon species was studied by Singh et al. (1970) and Barua et al. (1970). The fermentation efficiency of Saccharomycopsis lipolytica, Candida lipolytica, Cunninghamella elegans, Candida guilliermondii, etc. was determined in n-alkane

media for the production and evaluation of SCP (Bassel et al., 1973; Gerasimova et al., 1975; Shishkanova and Finogenova, 1979). Many workers have investigated the growth of Candida lipolytica and Candida guilliermondii on different hydrocarbons (Goma et al., 1974; Kazantsev et al., 1975; Gradova and Koval'skii, 1978). The yeast biomass grown on hydrocarbon was further evaluated for animal and human consumption.

Many workers have used organic substrates like ethanol, by-product of fermentation industry (slop), peat hydrolysate, enzymatic hydrolysate of agricultural waste, molasses with nitrogenous waste, chemical hydrolysate of pith, cattle manure liquor with straw hydrolysate, enzymatic hydrolysate of cassava, vinasse, malt extract, date palm juice, molasses, corn stover hydrolysate, rape seed oil meal, lemonade processing waste, potato processing waste and pineapple cannery waste for the cultivation of Candida utilis (Oleinikova, et al., 1975; Kvasnykov et al., 1976; Foda et al., 1976; Shishkova et al., 1976; Ramos et al., 1979; Kamel and Basil, 1979; Lemmel et al., 1979; Hang, 1980; Moo-Young et al., 1980; Konstantinova et al., 1980; Gonzalez and Moo-Young, 1981; Garg et al., 1983; Rale and Vinay, 1984). Dairy wastes were found as a good substrate for the production of SCP with various organisms like Saccharomyces fragilis, Saccharomycopsis and Rhodotorula, Kluyveromyces fragilis, Candida curvata, Trichosporon cutaneum Candida pseudotropicalis (Barnes, 1976; Giec et al., 1978; Moon et al., 1978; Desanches et al., 1980; Barraquio et al., 1980). Green et al. (1976) have successfully grown Geotrichum

candidum on stick water which is a by-product of fish processing and oil industry. Optimization of the conditions for the growth of Trichosporon cutaneum on xylose, xylan and hemicelluloses, Candida tropicalis, Rhodotorula marina and Saccharomyces cerevisiae on enzymatic hydrolysate of agricultural waste, Candida rugosa on maize gur, Candida tropicalis on hydrolysate of blue green algae, Candida ingenes on supernatant of anaerobic fermentation waste, Pichia kudriauzevii on hydrolysate of shell fish chitin, Hansenula sydoviorum on hydrolysate of Parthenium, Torulopsis candida on crude palm oil were carried out and recommended as potential source of SCP (Sheholokova et al., 1975; Henry et al., 1976; Beily et al., 1978; Chaudry et al., 1978; Volfova and Kyslikova, 1979; Henry and Thomson, 1979; Araujo et al., 1980; Jeong-Sankoh et al., 1983; Rale and Vakil, 1984).

Nutritional and safety evaluation of SCP was the subject of detailed study for many years and numerous publications and review articles are available (Mateles and Tannenbaum, 1968; Yudkin, 1973; Calloway, 1974; Tannenbaum and Wang 1975; Garattini et al., 1977; Rose, 1979; Rehm and Reed, 1983). Several processes have been described for treatment of effluents with concomitant production of saleable product. Development of new processes of that type may be generated to alleviate the present constraints of SCP production.

Treatment of organic effluents and production of single-cell proteins

The use of submerged fermentation technology for utilization of simple sugars and polysaccharides has been well documented. As a result of the treatment of effluents with microorganisms, the product is clean water and SCP is the by-product. The efficiency of the fermentation is thus proportional to the reduction of BOD/ and COD of the waste stream.

Single-cell protein from molasses

Molasses is the by-product of cane and beet-sugar production. It is the traditional carbon source for the production of the yeast Candida utilis and Saccharomyces cerevisiae. Molasses requires little or no pretreatment before it is used for SCP production. Fermentation is controlled between a range of pH 3.5 and 4.5 and temperature 25-35°C depending on the strain of yeasts.

Single-cell protein from whey

Whey is the residual liquid after fat and casein have been removed from milk. Many microorganisms were used for SCP from whey experimentally and a list of names was already presented by Meyrath and Bayer (1979). Yeast biomass is the major commercial product obtained from whey. In the Vienna process the sweet whey as such is fermented

with Candida intermedia, a fast growing acid tolerant yeast for SCP production. Bel Fromageries process and Kiel process are the other two processes commonly applied for the production of yeasts from whey (Meyrath and Bayer, 1979).

Single-cell protein from spent sulfite liquor

The spent sulfite liquor is the waste produced from wood pulp mills. This serious pollutant has a BOD of 25,000-50,000 mg/l. It has been reported that about 90% of BOD could be removed by fermentation (Forage and Righelato, 1979).

The fermentation of the sulfite waste liquor was started in Sweden with S. cerevisiae. Two types of processes are common in commercial operation. Majority of the processes for example, in North America, Europe and Russia make use of Candida utilis for SCP production (Kretzschmar, 1962; Peppler, 1970). Robinson (1952) reported that these processes were established in Germany during World War II. The salts of nitrogen, potassium and phosphorus must be added into the culture to compensate the deficiency in sulfite waste liquor. An ammonia based pulp mill sulfite liquor is used to produce about 5000 tons of dried C. utilis annually to be used as food (Peppler, 1978). In the Pekilo process, Paceilomyces varioti has been successfully used to reduce the BOD of paper factory effluents and also for the production of SCP (Forss and Passinen, 1976). The spent liquor contained 32 g/l reducing substances, and the yield of biomass was 55% based on the reducing substances consumed. The mycelial

concentration in the fermentors reached 17 g/l. This process could remove 83% of its BOD load. Cell mass obtained in practice had a crude protein content of 52-57% and its composition was comparable to the feed yeast produced from spent sulfite liquor.

Single-cell protein from potato processing waste

The basic Symba process was developed by Jarl (1969) to cultivate yeasts on starch. Mixed culturing of Candida utilis and Endomycopsis fibuliger on starch could result in a product rich in Candida utilis. The growth of E. fibuliger enhanced the production of glucoamylases and converted the starch to monomers which was consumed by the fast growing Candida utilis.

Application of Symba process for the treatment of effluents from the processing factories of potato, corn and rice was suggested to reduce pollution load (Jarl, 1969). The Symba process was in operation for some years after 1973 to produce SCP from potato processing wastes. The BOD value of the waste water was lowered from 10,000-20,000 mg/l to 1,000-2,000 mg/l by fermentation. It was reported that the process yielded 250 kg dry yeast h^{-1} by treating 20 m^3 effluents h^{-1} (Skogman, 1976).

Single-cell protein from food processing effluents

A process for growing C. utilis in confectionary effluent

was commercialised. The effluent consisted of 3-4% solids mainly glucose and sucrose, and had a COD of 30,000-40,000 mg/l. The pilot plant studies had demonstrated that the process could remove 75% of the COD and produce 1.5 tons of dry yeast product daily (Forage, 1978).

Submerged fermentation of cassava starch factory effluents

Cassava starch extraction indiscriminately discharges large amounts of effluents consisting of starch, cellulose, hemicellulose and reducing sugars. The conversion of carbohydrates into microbial biomass will result in the reduction of pollution hazard and accumulation of valuable SCP. Multiplication of torula yeast in the tapioca starch waste water was studied in Thailand (Thanh and Wu, 1975). About 99% of the volatile acids were removed after 24 hrs of aeration. The total COD removed was about 66% for nitrogen deficient waste and 75% in the nitrogen added waste. Microbial treatment of starch factory effluents was reported by Balagopal et al.(1977). A strain of Candida yeast was cultured in the supernatant effluent after separation of solids and the reduction of BOD and COD was assessed.

As discussed elsewhere, starch constituted more than half of the waste materials. The above two studies neither described the utilization nor removal of the easily degradable starch from the effluent.

2.iv. Role of Solid State Fermentation in the Production of Single-Cell Protein

Solid state fermentation (SSF) is a process whereby an insoluble substrate is fermented with sufficient moisture but without free water. In liquid state fermentation (LSF), the substrate is solubilized or suspended as fine particles in a large volume of water. In most liquid state fermentation systems, the substrate concentration is adjusted in the range of 0.5 to 6.0% depending upon the density of the substrate (Chahal, 1985). Although SSF has its own inherent problems it requires no complex controls and has many advantages over LSF which requires rigorous control of pH, temperature, aeration, foaming and agitation (Moo-Young et al., 1978).

The moulds are preferred for solid state fermentation because of their invasive growth pattern. The filamentous branches grow in the interparticle spaces and it can produce a wide range of enzymes to degrade carbohydrates. A number of filamentous fungi have been grown on solid substrates for the extraction of enzymes or for single-cell protein production.

Chaetomium cellulolyticum has been successfully grown on various substrates like pulp and paper mill solid wastes, pretreated straw, raw and acid- and alkali- treated hardwood saw dust (Moo-Young et al., 1977, 1978; Chahal et al., 1981 ; Hecht et al., 1985). Hamment et al. (1978) developed a modified

process to eliminate the problems of large quantities of post fermentation solid residues. Rogers et al. (1972) have attempted to produce SCP from ground refuse and wood pulp using Penicillium. Penicillium species have been successfully grown on corn stem, corn cobs, wheat bran, and cotton linters (Korculanin et al., 1976). Production of SCP from pine bark using various species of the filamentous fungi like Penicillium, Aspergillus, Trichoderma, Cladosporium and Fusarium has been done, but biomass yield on this heavily lignified substrate was too low for economic production (Updegraff et al., 1975). Penicillium notatum and Penicillium digitatum were grown on wastes from starch producing and potato processing plants (Stakheev and Babitskaya, 1978). Fungal protein production from the waste products after olive oil extraction has been attempted using the organisms, Aspergillus oryzae, Aspergillus niger, Sporotrichum pulverulentum and Trichoderma viride (Karapinar and Worgan, 1983). Citrus peel, coffee waste, barley husk, maple wood, etc. have also been used as substrates for SCP production using the organisms Aspergillus niger, Trichoderma koningii and Polyporus ancepus. In cassava fermentations with Aspergillus niger Raimbault (1977) reported a dried product containing 25-30% true protein.

Amylases (α-amylase and amyloglucosidase)

Amylases, the starch degrading enzymes, have numerous biotechnological applications, e.g. in the production of syrups containing oligosaccharides, maltose and glucose.

Enzymatic degradation of starch on an industrial scale has been practised for many years and this has replaced to a considerable extent the traditional acid-catalysed process.

α-amylase (α-1,4-D-glucan glucanohydrolase, EC 3.2.1.1., endoamylase) is distributed widely in microorganisms (Fogarty, 1983). It hydrolyses α-1,4-glucosidic linkage in amylose, amylopectin and glycogen in an endofashion, but α-1,6-glucosidic linkages in branched polymers are not affected. The properties and mode of action of α-amylases depend upon the sources of the enzyme.

Amyloglucosidase (EC 3.2.1.3., glucoamylase, α-1,4-D-glucan glucohydrolase, γ-amylase) is an exoacting enzyme that yields β-D-glucose (Ono et al., 1965) from non reducing chain of amylose, amylopectin and glycogen by hydrolysing α-1,4-linkages in a consecutive manner. It also hydrolyses α-1,6 and α-1,3 linkages although at a much slower rate than α-1,4-linkages. For example, maltose is hydrolysed at about 40 times the rate for isomaltose, and the linear polysaccharide pullulose is apparently quite resistant to hydrolysis, the rate being about 2% of that for starch. Amyloglucosidases occur almost exclusively in fungi (Fogarty, 1983), and the enzymes used commercially originate from strains of either Aspergillus niger or Rhizopus species where they are used for the conversion of malto-oligosaccharides into D-glucose. Fleming (1968) classified amyloglucosidases into two major groups, one yielding total hydrolysis of starch and dextrins and the second yielding 80% hydrolysis

of starch and 40% conversion of β-limit dextrins to glucose. Fogarty and Benson (1982 b) reported that the enzyme from A. niger has relatively high affinities for starch, maltotriose, and maltose.

Cellulase (Endoglucanase)

The ability to produce extracellular enzymes is widespread amongst fungi. In the search for good producers of cellulolytic enzymes, however, only relatively few fungal species have been selected by various research workers; such fungi are Trichoderma reesi, Chaetomium cellulolyticum, Aspergillus terreus, etc. (Mandels and Reese, 1957; Chahal et al., 1977; Miller and Srinivasan, 1979).

Aspergillus species are preferred for the production of β-glucosidase, and also for the total hydrolysis of cellulose. Lots of β-glucosidase production have been reported with Aspergillus awami, A. phoenicus and A. niger. Endo-β-(1,4)-glucanases (1,4-β-D-glucan 4-glucanhydrolase, EC 3.2.1.4.) are one group of several components of cellulase complex (Bisaria and Ghosh, 1981) which hydrolyses β-1, 4-glucosidic linkages randomly in native cellulose.

2. v. Biogas from Organic Matter

Biogas production by anaerobic digestion of non-fossil biomass has attracted considerable interest in recent years in its application to rural situation since this is one of

the methods of supplementing the fuel supply (Joshi, 1945; Sun, 1959; Williams, 1977; Shelat and Karia, 1977; Martinez and Muldas, 1981). Methane is the major constituent of natural gas obtained from the anaerobic digestion of biomass (Klass, 1976).

The anaerobic digestion process offers a number of advantages over other forms of energy conversion: (1) It produces a relatively clean gaseous product which can be readily upgraded (Ghosh and Klass, 1977). (2) It efficiently converts the carbon of organic matter to a usable fuel. (3) Many types of organic matter including organic substances with high moisture content are potential feed stocks (Ghosh and Klass, 1976). (4) It is safe, reliable and interconvertible (Ghosh and Klass, 1976; Picken and Fox, 1981; Picken and Hassan, 1982; Held and Konig, 1982; Ahlers, 1982). Anaerobic digestion of agricultural, industrial and municipal waste is very important for environmental protection since it combines waste stabilization with net energy supply and also permits the use of ungassified solid or liquid residues as fertilizer or animal feed or fish feed.

Since Volta identified methane in marsh gas in the 18th century, the fact that organic matter decomposition under anaerobic conditions will produce methane has been known (Meynell, 1976). In 1895 the gas collected from a specially designed septic tank was used for street lighting in the city of Exeter in England (Bell et al., 1975).

Anaerobic digestion has been used as a method of treatment of municipal sewage and a number of anaerobic sludge treatment plants are in operation in several parts of the world (Fowler, 1907; Acharya, 1954; McCabe and Eckenfelder, 1955; Goold, 1959). Mosey (1980) reported that in Wales (England) half of the sludge produced has been treated in this way. Considerable interest has been shown in this area during the last few decades, including methane production from manures, food and industrial wastes, crop residues, garbage, etc., and recently it has been applied to waste treatment processes (Fullen, 1953; Schroepfer, 1955; Loehr and Agnew, 1967; Ricci, 1975; Stafford et al., 1980; Edeline et al., 1986).

The studies conducted on the anaerobic digestion of cattle manure have contributed much to the development of advanced processes in biomethanation (Desai, 1951; Hart, 1963; Anon, 1974; Varel et al., 1977; Ghose and Amit, 1981; Newell, 1981; Rodriguez and Werner, 1982; Wellinger, 1985).

The potentiality of the substrates like piggery waste and poultry manure for biomethanation has been reported by many workers (Fry 1961; Jewell et al., 1976; Callander, 1979; Summers and Bonsfield, 1980; Hobson, 1981; Fisher, 1981; Van Velsen, 1981; Hill, 1983; Hashimoto and Andrew, 1983; Aubart and Fanchille, 1983).

The studies began with batch digestion of sewage sludge

(Fowler, 1907; Acharya, 1954) and these have further led to continuous and advanced digestion methods capable of attaining greater volatile solids (VS) reduction, and methane yield (Ghosh et al., 1975; Bryant, 1979; Temper et al., 1982; Rebillat, 1982).

The application of anaerobic digestion in industrial waste treatment has progressed due to the intensive investigations of many workers. Van den Berg and Lentz (1972) have reported the anaerobic digestion of pear waste. The same workers applied the technique of anaerobic digestion to treat the effluent from food processing plant waste (1977 and 1979). Cillie et al. (1969) demonstrated the application of anaerobic digestion in the treatment of waste water. Distillery waste and sugar industry waste were also anaerobically treated to produce methane (Barry et al., 1982). Anaerobic digestion of waste waters and pulp from coffee processing industry was studied by Calzada and Rolz (1984) and they reported a higher productivity in the two-phase digestion (up to 6-4 volumes of gas per volume of reactor per day).

Biochemistry of the process

During the process of anaerobic digestion a large amount of organic matter is destroyed., but 90% of the energy available in the substrate is retained in the easily purified gaseous product, methane (Barnard and Hall, 1983). Different types of

microorganisms are involved in the process, the main biological agent being bacteria. Fermentative ciliates, flagellates, protozoa and anaerobic fungi may also contribute in some ecosystems (McInerney and Bryant, 1981).

In anaerobic digestion the complex molecules such as polysaccharides, proteins, and lipids are hydrolysed to fatty acids and other organic acids, alcohol, ammonia, sulfide, carbon dioxide and hydrogen (McInerney and Bryant, 1981). Propionates and longer chain fatty acids, some organic acids, and alcohols are probably decomposed by a second intermediate group of bacteria called the obligate H_2-producing (i.e. proton reducing) acetogenic bacteria (Bryant, 1979), but only a few species have been reported. Acetate and some times other acids are produced from H_2 and CO_2 by some other bacteria (Balch et al., 1977; Ohwaki and Hungate, 1977). These two steps are carried out by a hardly separable group of fastly growing obligately anaerobic bacteria which rapidly utilize the metabolites of fermentative bacteria.

However, there are two types of methanogenic bacteria which are able to use these materials. One type utilizes H_2 and CO_2. The second type converts acetic acid (or its salt acetate) to CO_2 and CH_4 (Mobson, 1978), which is a quantitatively important reaction since about 70% of methane produced is derived from the methyl group of acetate (Scammell, 1975). The successful operation of a digester relies on the

correct balance between these two groups of bacteria and this occurs within the pH range 6.6 - 7.6 (Chittenden et al., 1980). The methanogenic bacteria multiply at a slower rate than the acid formers, and they are more sensitive to environmental changes (McInerney and Bryant, 1981).

Temperature, nitrogen content and retention time

Methane is formed over a wide temperature range from 0°C to 97°C. The optimum operating temperature range for mesophilic methane fermentation is 30-40°C (Wellinger and Kaufmann, 1982). It is well known that within certain limits an increase in the temperature may result in an increase in gas yield (Malina, 1964). Below this, digestion proceeds slowly (Temper et al., 1982); however, temperature tends to favour the acid forming bacteria and the system becomes more suceptible to a reduction in pH. The accumulation of volatile acids inhibits the methane-forming bacteria, and eventually stops fermentation (Ghose and Amit, 1981). The fermentation rate is considerably faster at the thermophilic range (Cowley and Wase, 1981). However, the increased heat requirement, and higher levels of CO_2 in the biogas, are the major disadvantages.

The presence of nitrogen is an important factor and a C:N ratio close to 30:1 is generally considered to be desirable (Bell et al., 1975; Cowley and Wase, 1981).

Efficient fermentation requires a minimum retention time below which it ceases due to the insufficiency of microbial population (Lawrence, 1971). The loading rate is influenced by the dry matter content of the feed stocks, and dilution and retention times, are interdependent (Kirimhan, 1983).

Reactor designs and methodology

The most common technology of biomethanation was based on the process of one-step completely mixed methane digestion. Full scale digesters of this kind have been referred to as "first generation digesters". These digesters can be operated on semi-continuous way under the following conditions with the volumetric loads up to 4 kg dry matter, day^{-1} m^{-3} of digester and mean residence time as low as 10 days (Melchior et al., 1982). In completely mixed one-step semi-continuous methane digesters without active biomass recycle, as is found in the case with most 'first generation' methane digesters, a constraint arises from the low specific growth rate of the methanogenic bacteria. A world-wide survey confirmed that the efficiencies of 'first generation' digesters must be limited to $3m^3$ of gas ($2\ m^3$ of methane) day^{-1} m^{-3} of digester (Nyns et al., 1980).

The two-step process of methane digestion often appears more reliable than the one-step process for a wider variety of substrates. This could be mainly due to the accumulation

of a large variety of intermediary metabolites during fermentation other than acetate and dihydrogen in the separate steps. The second stage of fermentation always maintains a high level of proton reducing bacterial population which in turn prevents the accumulation of the deleterious volatile fatty acids (Asinari, et al., 1980).

Though the early emphasis on waste treatment has led to the development of reactor designs operated with a low or negative energy balance, they were unsuitable for other agricultural and industrial wastes. The continued search for alternative energy production routes has resulted in recent years in the development of 'second generation' reactor designs which are capable of dealing high strength liquid wastes with favourable net energy yield (Newell, 1981). When attempts were made to operate conventional digesters at high loadings and short hydraulic retention times unbalanced digestion and ultimately process failure were encountered (Ghosh and Henry, 1981). However, the development of two-phase anaerobic digesters solved this problem (Pohland and Ghosh, 1971; Ghosh et al., 1975).

Several types of anaerobic treatment systems are available at present on a commercial scale, such as contact reactor (Meynell, 1976; Bell et al., 1975), the upflow anaerobic sludge blanket (UASB) process (Mosey, 1980; Stafford et al., 1980), the anaerobic filter (Hughes et al., 1952), fluidized

bed (Jeel, 1980; Switzenbaum, 1981) etc.

The anaerobic treatment of stillage from alcohol industry was examined with a modified upflow anaerobic sludge blanket called the upflow-flock reactor, and achieved 95% COD reduction. A preliminary study of the digestibility of silage effluent in an upflow anaerobic filter (UAF) was carried out by Barry et al. (1982). The downflow stationary fixed film (DSFF) reactor is one of several types of retained-biomass reactors developed to achieve high rates of methane production and waste treatment (Kennedy et al., 1981; Vanden Berg and Kennedy, 1981 b). The active biomass growing on stationary supports could not readily be washed out and reactors could therefore withstand high hydraulic as well as organic loads. The reactors were found capable of handling dilute and concentrate substrates (4 to 130 g COD/l) and of changing readily from one waste to another at high rates of methane production. Efficient digestion with high methane yield was demonstrated by the anaerobic filter for the digestion of high strength liquid waste and solid biomass residues (Colleran, et al., 1982).

Chen et al. (1985) evaluated the performance of anaerobic fluidized bed system designed for the recovery of methane from liquid waste, with an emphasis on the substrate utilization and gas production rates under different operating conditions. In that system the COD removal was in the range of 75 to 98% when evaluated at COD loadings between 5.8 to 108 m^{-3} day^{-1},

hydraulic retention times of between 4.45 to 8.1, and feed COD concentration between 480 to 9,000 mg dm^{-3}.

Messing (1982) operated an anaerobic two-stage immobilized microbe processor with a sewage feed. It was designed for the efficient conversion of the carbon in biodegradable molecules to methane and for the effective transfer of that gas.

Anaerobic digestion of starch factory waste

Frostell and Staffanstrop (1983) have evaluated the modified anaerobic-aerobic biological treatment of starch industry waste waters. More than 90% COD removal and 97-99% BOD removal was achieved by this treatment. The attempt made in the treatment of waste from a Dutch maize starch factory (Zetmeelbedrijven de Bijenkorf) employing UASB anaerobic process was effective to reduce the COD load to 400 - 800 mg/l (Original 1,500-11,000 mg/l) at a short retention time of 15-21 hours (Zeevalkink and Maaskant, 1984). Trevalyan (1975) suggested that most of the organic compounds can be digested by anaerobic means relatively easy with the exception of fibrous materials.

Present Work

The problems associated with the discharge of cassava starch factory effluents into rivers and lakes have been discussed by many workers (Thanh and Wu, 1975; Balagopal et al., 1977; Suseela et al., 1980; Kunhi et al., 1982). Though the effluent discharge is a serious threat, very little attempt has been made to reduce the pollution load generated by the cassava starch factories. An attempt was therefore made for the microbiological treatment of effluents from a cassava starch factory located on the banks of Ashtamudi lake, Kerala, India, which has been polluted with the discharge from the factory.

Experiments were laid out for the characterization of the physico-chemical nature of the effluents, and enumeration of various microorganisms of the discharged effluents from a large-scale and a small-scale starch factory. The potential microorganisms especially yeasts were studied with a view to treat the effluents.

Investigations were made to assess the rate of degradation of cassava starch factory effluents by the proliferation of natural contaminants under ambient conditions.

Utilization of cassava starch factory effluents as a substrate for SCP production employing various yeasts and

yeast-like organisms was explored by submerged fermentation technique. Attempts were also made to convert the cassava starch factory wastes into fungal biomass by solid state fermentation.

In the cassava starch factory an obvious source of organic material for the process of anaerobic digestion is available as effluents discharged during the extraction of starch from the cassava tubers, but the work on the anaerobic digestion of cassava starch factory effluent is scanty. The possibility of utilizing cassava starch factory effluents for biomethanation was tried by anaerobic digestion at different conditions. The extent of reduction of pollution load was assessed during fermentation and biogassification.

Materials and methods, results and discussions are presented in the succeeding chapters.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.i. Collection of Samples

Effluent samples were collected from small-scale as well as large-scale cassava starch factories in the Kerala State of India. The primary and secondary effluent samples were separately taken from the phase of discharge. They were brought to the laboratory in black polythene jerry cans, and kept at 4-7°C for short time and at -7°C for long time storage. Generally the analyses were completed within 5 days of collection. Necessary precautions were taken for the collection of effluent samples as described in AOAC (Horwitz, 1975).

3.ii. Physical, Chemical and Biochemical Analyses

3.ii.1. Ash

Known volume of thoroughly mixed sample was taken in a silica crucible and evaporated to dryness. The crucible was then transferred to an air-oven adjusted to a temperature of 105°C and dried the residue to constant weight. Ash was determined by ignition of the dried residue in a muffle furnace at 550°C as explained in AOAC (Horwitz, 1975).

3.ii.2. Settling property of solids in the effluents

The settling property of solids in the effluents was determined by keeping the samples in measuring cylinders for

15 min. Settled solids were separated by decanting the supernatant, and total solids in the supernatant solutions were estimated.

3.ii.3. Estimation of total solids (TS) and suspended solids (SS)

Total solids and suspended solids were determined according to the ASTM Standards (1979). Total solids were estimated by evaporation in a water bath at 100°C and by drying the residue to constant weight at 103°C in an air-oven. Measured volume of well mixed sample was filtered through a filter crucible (Gooch crucible with an evenly distributed asbestos fiber) that previously has been prepared and dried for 1 hr at 103°C, cooled in a dessicator and weighed. After filtration, the content was washed with water and air dried for several minutes and oven dried at 103°C for 1 hr. Cooled in a dessicator and the weight of the solids was accounted as suspended solids.

3.ii.4. Biochemical oxygen demand and chemical oxygen demand

BOD estimation was done by incubating the samples for 5 days at 20°C in the presence of acclimated biological system. The difference in the oxygen content of the sample at the beginning and at the end of incubation was taken as the measure of BOD. Oxygen was determined by employing the method of Winkler and COD determinations were carried out using the

dichromate method described in AOAC (Horwitz, 1975).

3.ii.5. Biogas analysis

The biogas was analysed by gas chromatography using a Hewlett Packard Model 5840 A gas chromatograph with a built-in electronic integrator.

Column 6' 1/8" dia carbosieve	
Column temperature	- 60°C for 5 min. and then temperatur grammed to 180° per min. and held at final temperature for 10 min.
Injection temperature	- 100°C
T.C.D. temperature	- 300°C
Carrier gas	- Hydrogen at a flow rate of 20 ml per min.

The gaseous compounds were identified using authentic samples.

GC-MS analysis of the biogas was carried out in a Hewlett Packard Model 5995 GC-MS system using 15 meter cross-linked methyl silicone flexible silica column of i.d. 0.02 mm.

3.ii.6. Carbon estimation

The organic carbon in the sample was estimated by oxi-

dising it to CO_2 in the presence of oxygen in a catalytic combustion tube at 900°C (Robinson, 1939). The carbon dioxide generated during the combustion was estimated by absorbing it in potassium hydroxide.

In a porcelain combustion boat, 0.5 g material was taken. The boat was then placed inside the heating zone of combustion tube furnace, keeping temperature at 900°C. A stream of oxygen was passed into the combustion tube and close circuit connection was established with carbon burette of the carbon and sulfur determination apparatus (Cat. No.GL 37.01 Toshniwal Brothers Pvt. Ltd.) filled with acidulated water to zero level. After equalizing the level of the burette and levelling bottle, the gas was twice or thrice passed into absorption vessel, till the absorption of CO_2 in KOH solution was completed. The gas was then passed back to burette from absorption vessel and difference in the level was noted on an adjacent scale. The reading arrived on the scale of carbon burette was multiplied by the correction factor from the pressure temperature chart. The result so obtained was the carbon percent in the sample.

3.ii.7. Hydrocyanic acid

The presence of hydrocyanic acid (HCN) was examined as per the method given in AOAC (Horwitz, 1975). The colour of the sodium picrate paper (filter paper dipped in 1% picric acid and 10% sodium carbonate solution) was turned to orange

and then to brick-red in the presence of HCN.

3.ii.8. Nitrogen and biomass protein

Nitrogen was estimated by the Kjeldahl method as described in AOAC (Horwitz, 1975). From the nitrogen value the biomass protein was calculated (Mckenzie and Wallace, 1954). Nitrogen-enriched materials were thoroughly washed with water till the supernatant wash water was free of nitrogen. The residue was then analysed for nitrogen content.

3.ii.9. Determination of effluent pH

The pH of the thoroughly mixed sample was determined using a digital pH meter, Elico Model - L 1-120.

3.ii.10. Extracellular protein

The extracellular protein was determined by the method of Lowry et al. (1951).

3.ii.11. Reducing sugar

Samples were centrifuged at 1200 g for 15 min. to separate the solids. The reducing sugar was determined in the supernatant using dinitrosalicylic acid reagent (Miller, 1959).

3.ii.12. Starch

Mixed 200 ml of sample with 20 ml of concentrated HCl (sp. 1.125) and refluxed for 2½ hrs. Collected the filtrate

and estimated for reducing sugar following the method of Miller (1959) using glucose as the standard.

3.ii.13. α-Amylase

α-Amylase was estimated by the procedure of Manning and Campbell (1961) at 60°C and pH 6.0. (One unit of α-amylase activity is that quantity of enzyme which hydrolyses 1 mg of starch per minute under specified conditions of assay).

3.ii.14. Amyloglucosidase

The amyloglucosidase activity in the cassava starch factory waste during the growth of Aspergillus niger was estimated as per the method of Ramasesh et al. (1982), a modified procedure of Miles Chemical Laboratory (1963). The activity was expressed in International Units (I.U.). (An I.U. is equal to the μM of glucose released per minute per gram under the defined conditions i.e. temperature 60°C, pH 4.2 and 4% starch solution).

3.ii.15. Cellulase

The cellulase assay was based upon the increase in reducing groups following incubation of carboxy-methyl-cellulose with enzyme solution prepared from the effluents. Assay was carried out in acetate buffer (0.1 M, pH 5.0) at 40°C for 24 hrs. Bacterial activity was prevented by the addition of antibiotic solution. Reducing sugar formed by the enzyme action was

estimated using dinitrosalicylic acid reagent (Miller, 1959). (The specific activity was expressed as the mg of glucose liberated/24 hr/100 mg protein). The protein content of enzyme extract was determined by the method of Lowry et al. (1951).

3.ii.16. Endoglucanase

During the growth of A. niger in primary starch factory waste the endoglucanase activity was estimated in the substrates. Subsamples of the same enzyme extract (3.ix.1) , which was prepared for the estimation of α-amylase and amyloglucosidase (3.ii.13 and 3.ii.14), were used for the endoglucanase assay. The assay was carried out following the method of Petterson and Porath (1966). (The specific activity was expressed as the mg of glucose liberated/24 hr/100 mg protein). The protein in the extract was estimated as per the method of Lowry et al. (1951).

3.ii.17. Amylase

Subsamples drawn from the effluent samples (primary and secondary) kept in measuring cylinders were centrifuged at 1200 g for 15 min. to remove the solids. The supernatant solutions were used as the enzyme source for assaying amylase and cellulase activity.

Potato starch solution (1%) was used as the substrate in acetate buffer (0.01 M, pH 4.8). Enzyme source was added and incubated at 30°C for 24 hrs. Bacterial growth in the assay system was arrested by adding antibiotic solution. Aliquots

were analysed for reducing sugar using dinitrosalicylic acid reagent (Miller, 1959).

The amylase activity was expressed as mg of glucose liberated per hour/100 mg protein. The method of Lowry et al. (1951) was followed for estimation of the protein content of the enzyme extract.

3.iii. Microbiological Methods

3.iii.1. Tests for coliforms

The coliform bacterial counts were made following the most probable number (MPN) method (Collins, 1976).

3.iii.2. Identification of mould isolates

Mould identification was carried out as per the descriptions given by Subramanian (1971) and Barnett and Hunter (1972).

3.iii.3. Identification of yeasts

The yeasts were identified as per the methods given by Barnett et al. (1979)

3.iii.4. Microbial enumeration

Samples collected in sterile conical flasks were transferred quantitatively to measured volume of sterile water for the preparation of suitable dilution. One millilitre of aliquot was transferred to sterile petri-dishes and 15-20 ml of appropriate

media . poured in the conventional manner. Martin's rose bengal agar (Martin, 1950) with streptomycin, malt-yeast-glucose-peptone (M-Y-G-P) agar with streptomycin, Kuster's agar, and nutrient agar (Difco) were used for the enumeration of fungi, yeasts, actinomycetes and bacteria respectively. Petri-dishes for the enumeration of bacteria and yeasts were incubated at 37°C and 28 ± 2°C respectively for 48 hrs. In the case of actinomycetes and fungi the plates were incubated at 28 ± 2°C for 144 and 72 hrs respectively. Bacterial isolates were maintained on nutrient agar slants and yeasts on M-Y-G-P agar slants. Potato dextrose agar slants were used for the maintenance of fungi and actinomycetes.

3.iii.5. Microbial proliferation in starch factory effluents at ambient conditions

Primary and secondary effluents collected from the cassava starch factory were kept separat ly in 2 glass containers (measuring cylinders). Excessive evaporation was prevented by keeping at room temperature. Subsamples were drawn for examination of bacteria, yeasts and fungi. Serial dilution technique was followed for the enumeration of microorganisms using nutrient agar, M-Y-G-P agar and rose bengal agar for bacteria, yeasts and fungi, respectively. The nutrient agar plates were incubated at 37°C. Bacterial growth in M-Y-G-P agar plates and rose bengal agar plates were suppressed by adding streptomycin.

The plates for yeasts and fungi were further incubated at 28°C. The colonies developed after appropriate incubation were counted and the population was expressed as number of colonies x 10^X, where X denotes the dilution rate. These experiments were continued from the first day of collection of samples to the tenth day, both for primary and secondary effluents.

3.iii.6. Morphological grouping of bacteria

Morphological characters were determined as described by Harrigan and McCance (1966). Bacteria were grouped on the basis of their gram reaction, shape, size and sporulation.

3.iv. Fermentation of Cassava Starch Factory Effluents with Yeasts and Yeast-like Organisms

Many cellulosic and starchy materials can be fermented with microorganisms (Litchfield, 1983). In the present study cassava starch factory effluents were used.

3.iv.1. Substrate preparation

Fresh primary effluent samples were collected and enriched with modified basal media (Paca, 1982). Basal media composition was:

$(NH_4)_2SO_4$	0.28%
KH_2PO_4	0.07%
$CaCl_2\ 2H_2O$	0.01%
$MgSO_4\ 7H_2O$	0.02%
NaCl	0.01%
Yeast extract	0.01%

The samples were mixed thoroughly and distributed at the rate of 100 ml in 500 ml Erlenmeyer flasks and sterilized at 15 lbs pressure for 20 min.

3.iv.2. Culture

Two isolates; Candida valida and Geotrichum candidum obtained from cassava starch factory effluents and three cultures received from National Collection of Industrial Microorganisms (NCIM), Pune, India; Candida utilis-3400, Endomycopsis fibuliger and Endomycopsis magnusi were maintained on M-Y-G-P agar slants.

3.iv.3. Inoculum preparation

All the cultures except E. fibuliger were transferred aseptically to M-Y-G-P broth, distributed at a level of 100 ml in 500 ml Erlenmeyer flasks. E. fibuliger was grown in potato-dextrose-peptone (P-D-P) broths under aseptic conditions. The inoculated flasks were incubated on a gyrorotary shaker kept at room temperature (30 $\pm$ 2^{o}C). After 24 hrs of incubation the flasks were removed from the shaker and the cells were separated from the broth by centrifuging at 1200 g for 15 min. The cells were further washed and suspended in sterile normal saline. The sterile media was inoculated with the organisms at the rate of 5% (v/v) containing the following cell counts.

C. valida	- cell count	70 x 10^6/ ml
G. candidum	"	120 x 10^6/ml
C. utilis	"	300 x 10^6/ml
E. fibuliger	"	11.6 x 10^6/ml
E. magnusi	"	8.4 x 10^6/ml

3.iv.4. Analysis

Samples were drawn at intervals of 0, 24, 48, 72, 96 and 120 hrs for the following analyses.

a. Cell count

The number of cells in the culture media was counted under microscope, PZO Warzawa Biological MB.10 by means of a Neubauer (improved) chamber (0.100 mm, 1/400 qmm).

b. Reducing sugar

Samples were centrifuged at 1200 g for 15 min. to separate yeast cells and solids. The supernatant was analysed for free reducing sugar as explained by Miller (1959).

c. Starch

Known volume of culture broth was centrifuged at 1200 g for 15 min. and sedimented solids were analysed for starch as described previously in 3.ii.12.

d. Biomass protein

Protein content of the biomass was estimated using the

method described in 3.ii.8.

3.v. Fermentation of Cassava Starch Factory Secondary Effluent with C. utilis and E. fibuliger

Instead of the primary effluent, secondary effluent was fermented with C. utilis and E. fibuliger separately. The effect of enrichment was also studied by fermenting non-enriched secondary effluent.

Cell density of the suspension of C. utilis and E. fibuliger was 715 x 10^6/ml and 10.5 x 10^6/ml respectively in all the experiments and both were inoculated at the rate of 4.0%. Though the experiments were conducted in the same manner described in 3.iv. it was terminated at 72 hrs.

3.vi. Co-culturing of C. utilis with E. fibuliger and C. utilis with E. magnusi in the Primary Effluent of Cassava Starch Factory

Unlike the methods followed earlier, here two cultures were simultaneously added in the sterile primary effluent and the experiments were repeated in the same manner described in 3.iv. Cell density of the suspension of E. fibuliger, E. magnusi and C. utilis were 1060 x 10^6/ml, 15.5 x 10^6/ml and 2180 x 10^6/ml respectively. The E. fibuliger and E. magnusi were inoculated at a rate of 4.0% and C. utilis at a rate of 2.0%. The cell

numbers were determined by serial dilution technique using M-Y-G-P agar for C. utilis and P-D-P agar for E. fibuliger and E. magnusi. The analyses of the samples included COD, BOD and pH in addition to the parameters described in 3.iv.4.

3.vii. Co-culturing of C. utilis and E. fibuliger in the Secondary Effluent of Cassava Starch Factory

The secondary effluent was fermented by co-culturing of C. utilis and E. fibuliger. Non-enriched secondary effluent sample was also fermented in the same manner. Procedure followed here was the same as described in 3.iv. Chemical oxygen demand of the effluents of fermented sample was also estimated using the same technique described earlier (3.ii.4). Serial dilution technique was followed for the estimation of C. utilis and E. fibuliger using M-Y-G-P agar and P-D-P agar respectively.

3.viii. Co-culturing of C. utilis and E. fibuliger in the Primary Effluent of Cassava Starch Factory under Monitored Conditions

Co-culturing of C. utilis and E. fibuliger was carried out in a 7 l. Chemap Laboratory Fermentor. The conditions monitored in the fermentor were:

Temperature	-	30°C
pH	-	5.0
Stirring	-	250 rpm
Aeration	-	0.5 vvm

The population of C. utilis and E. fibuliger was maintained at 700 x 10^6/ml and 680 x 10^6/ml respectively in the inoculum samples. E. fibuliger was added at 4.8% level and after 4 hrs C. utilis was added at the rate of 2.4%. Samples were drawn at 0 hr to 60 hr at 4 hr intervals and analysed for protein, reducing sugar, starch, COD and BOD. Total cell counts were also simultaneously made as described in 3.vii.

3. ix. Fermentation of Cassava Starch Factory Wastes with Aspergillus niger

Primary effluent collected from starch factory was concentrated and dried. Two kinds of samples were prepared: the first sample with a nutrient solution containing the following ingredients (Hecht et al., 1985)

KH_2PO_4	20.0 g/l	$FeSO_4 7H_2O$	50.0 mg/l
$(NH_4)_2SO_4$	30.0 g/l	$MnSO_4 H_2O$	15.6 mg/l
$MgSO_4 7H_2O$	3.0 g/l	$ZnSO_4 7H_2O$	14.0 mg/l
$CaCl_2 2H_2O$	4.0 g/l	$CoCl_2 6H_2O$	3.7 mg/l

and the second sample without the addition of nutrients. In both cases the moisture level was adjusted to 60%.

A strain of Aspergillus niger (RRL-T_2) was used for the study. The fungal culture was maintained on potato dextrose agar slants. One week old culture maintained at 30°C was used for the preparation of fungal spores.

The samples weighed exactly 50 g were distributed in 500 ml Erlenmeyer flasks and autoclaved at 121°C for 20 min. Fungal spore suspensions were prepared in sterile water containing 1 percent tween 80. Two millilitres of spore suspension (11 x 10^6/ml) was inoculated to each flask. Samples of both kinds were incubated at 30°C for 120 hrs.

The samples were drawn at different intervals for total protein analysis after fermentation with A. niger. Analysis of protein was carried out by the same method followed in 3.ii.8.

3.ix.1. Extraction and analysis of enzymes

Samples drawn at different intervals (0, 24, 48, 72, 96 and 120 hr) were homogenised with distilled water by stirring for 15 min. The extract was analysed for α-amylase, amyloglucosidase and endoglucanase.

3.x. Anaerobic Fermentation of Cassava Starch Factory Effluents for Methanogenesis in Batch Digesters

3.x.1. Digestion apparatus

Single stage batch digesters were fabricated in the laboratory.

Erlenmeyer flasks of one litre capacity which served as digesters were stoppered with bend tube inserted rubber corks (Fig. 5A). Similarly seven litre glass bottles were also used

as batch digesters and each one was stoppered with a bend tube inserted rubber cork (Fig. 5B).

Two hundred litre metallic (tin) barrel was cut open on the upper side and a metallic rod was fixed vertically in the centre of the lower side. Another metallic barrel half inch less than the diameter of the main (first) barrel, and opened on the lower side was inserted in. One metallic pipe with slightly higher inner diameter than the metallic rod fixed in the main drum was inserted through the middle of the upper side of the smaller barrel for easy movement. Hence the smaller barrel could easily slide over the central rod of the main barrel according to the pressure of the biogas produced from the main barrel. Provisions were also made for the collection of gas released by providing two valves (Fig. 5C).

3.x.2. Feed stock preparation

Primary effluent collected from the cassava starch factory was used in this study. The effluent was enriched with nitrogen (urea) to give a C:N ratio around 30:1. Solid content of the sampleswas adjusted to 8.2% and the pH was then raised to 7.0 with sodium hydroxide.

Feeding material

Total solids	-	8.2%
Carbon	-	18.84%
Enrichment (urea)	-	0.5 g/l.
pH	-	7.0

The feed stocks prepared were fed at the rate of 400 g in 1 l., 5 kg in 7 l. and 150 kg in 200 l. digesters respectively. Digesters were then incubated at ambient temperature (30 ± 3°C) and biogas generated from the digesters were collected by the downward displacement of acidified water in the case of 1 l. and 7 l. digesters, but in the case of the 200 l. digesters, the gas production was calculated by measuring the increase in height of the smaller barrel from the level of solution in the digester. The effect of nitrogen enrichment was also studied by adding urea at the rate of 0.5 g/l of effluent.

3.xi. Anaerobic Fermentation of Cassava Starch Factory Effluents for Methanogenesis in Semi-Continuous Digesters

3.xi.1. Digestion apparatus

Single stage digesters were designed with 2 l. Erlenmeyer flasks (Fig. 5D). There were three openings in the digester, one inlet to feed the digester with fresh effluents, one upper outlet for gas collection and the second lower outlet for effluent (digested) removal. The digester was kept on a magnetic stirrer and the gas outlet was connected with a rubber tube to a measuring cylinder, filled with acidfied water, which was clamped upside down. The other two openings were closed temporarily.

A portion of 1 l. digested material from the batch digester was transferred to the semi-continuous digester. The

cessation of gas production from this digested sludge was tested for 30 days.

3.xi.2. Feeding material

Total solids	-	8.03%
Carbon	-	18.84%
Enrichment (urea)	-	0.5 g/l
pH	-	7.0
COD	-	65,000 mg/l

The above material was kept at 5°C and brought to room temperature before feeding. Fresh samples were added to the digester containing 1 l. digested material through the inlet of the digester. Two experiments were conducted with 20 ml (1,300 mg COD) and 100 ml (6,500 mg COD) of feeding material till the gas production was reduced almost to negligible amount. Since it was found that after 4 days of digestion the rate of gas production was negligibly low, subsequent experiments were continued only for 4 days. The other experiments were conducted by adding 40 ml (2,600 mg COD), 120 ml (7,800 mg COD) and 140 ml of the effluents (9,100 mg COD) to the digester. The experiments were repeated 5 to 6 times. Care was taken to maintain the total volume of effluents constant whenever fresh samples were added.

The solution in the digester was mixed thoroughly thrice a day for 2 min. each time with a magnetic stirrer. All the

experiments were carried out at room temperature (30 ± 3°C).

Biogas produced from the digester was collected quantitatively by the downward displacement of acidified water kept in measuring cylinders. The pH profile and total solids of the effluent samples from the digester were determined periodically as per the methods described earlier.

Enumeration, screening and determination of the physiological traits of different organisms were carried out in triplicate unless otherwise stated.

3.xii. Statistical analysis

Statistical analysis was carried out to understand the significant differences between the control and treated samples. The method described by Chatfield (1978) was followed for the purpose.

Significance tests

Significance test is a numerical method for testing a hypothesis. On the basis of the data obtained during the study it is of great use to know the acceptability of the observations by this method also. The following formula was used for the calculation of the t-values

$$t_{cal} = \frac{(\bar{X}_1 - \bar{X}_2)\sqrt{n}}{\sqrt{\frac{n\,(S_1^2 + S_2^2)}{2\,(n-1)}}}$$

where

t_{cal} - calculated t-value

$\bar{X}_1$ - mean value of the treated sample

$\bar{X}_2$ - mean value of the control sample

S_1^2 - sample variance of the treated sample

S_2^2 - sample variance of the control sample

n - number of observations in each sample (sample size)

CHAPTER 4

RESULTS

4. RESULTS

4.i. Physico-chemical and Microbiological Characteristics of Cassava Starch Factory (C.S.F.) Effluents

The easily degradable carbohydrates present in the cassava starch factory effluents support the growth of microorganisms and subsequently cause imbalance of natural conditions in and around the place of discharge (Figs. 2A,2B,2C & 2D). In order to study the extent of pollution load, samples of effluents collected from various starch factories in the State of Kerala, India, were analysed. Attempts were also made to understand various microorganisms present in the effluents. Results of the systematic analytical study of the effluents are consolidated here. Samples of effluents discharged at different phases of the starch extraction in small-scale and large-scale starch factories were separately analysed to understand the various physico-chemical and microbiological characteristics of the effluents.

4.i.1. Physico-chemical characteristics of primary effluent from large-scale starch factory

The thick creamy-white acidic slurry obtained from large-scale starch factories during the primary stage of starch extraction was apparently different from other effluents primarily due to the presence of higher quantity of organic matter (Table 1 and Fig. 2.1A). The BOD of the primary

effluent was in the range of 13.20 - 14.30 g/l which reached about one third of COD (38.22 - 48.82 g/l). Significant amount of the solids were present in the suspended form. By acid hydrolysis, more than 50% of the solids were converted as reducing sugar which was in the range of 22.61 - 29.28 g/l. Small amount of reducing sugar in the free form was also observed (0.425 - 1.85 g/l). Exceptionally low concentration of nitrogen (0.097 - 0.182 g/l) was one of the unique characteristics of the effluent.

The primary waste material produced from the small-scale cassava starch industry was semi-solid in consistency with a moisture content of 70-80%.

4.i.2. Physico-chemical characteristics of secondary effluent from large-scale starch factory

The secondary effluent of the large-scale cassava starch factory was a light creamy-white coloured acidic water which was separated at the final stages of starch extraction (Fig. 2.1B). The BOD and COD of the effluent samples were in the range of 3.60 - 7.05 g/l and 3.80 - 12.05 g/l respectively. This effluent also contained some starch, free sugars, and fibrous matter which contributed to the total solids of 3.20 - 9.60 g/l (Table 1). Nitrogen content of the secondary effluent was also negligible as in the case of primary effluent (0.065 - 0.086 g/l).

4.i.3. Physico-chemical characteristics of the secondary effluent from small-scale starch factory

There was little difference between the secondary effluent of small-scale and large-scale starch factories (Table 2). The BOD was in the range of 3.87 - 6.02 g/l and COD was in the range of 4.87 - 9.60 g/l. Total solids and suspended solids were 4.0 - 6.60 g/l and 1.87 - 2.96 g/l respectively. The presence of starch and free reducing sugar was also observed in the secondary effluent (1.59 - 3.02 g/l and 0.64 - 2.075 g/l respectively).

4.i.4. Microbial load of primary effluent from large-scale starch factory

Various kinds of microorganisms were observed in the effluent which varied from sample to sample (Table 3). Compared to all other microorganisms, bacterial number was the highest ($0.70 \times 10^5 - 10.0 \times 10^5$/ml). The yeast and mould counts were in the range of $1.20 \times 10^3 - 14.75 \times 10^3$/ml and $3.60 \times 10^3 - 20.0 \times 10^3$/ml respectively. Population of actinomycetes was found minimum ($0.90 \times 10^3 - 10.0 \times 10^3$/ml).

4.i.5. Microbial load of secondary effluent from large-scale starch factory

Among the different microorganisms observed in the secondary effluent, the bacterial population was the highest

and it ranged from 199.5 x 10^5/ml - 400.0 x 10^5/ml (Table 4). Yeast count was also high (13.13 x 10^3 - 30.0 x 10^3/ml) when compared to moulds and actinomycetes (1.60 x 10^3 - 2.70 x 10^3/ml and 1.58 x 10^3 - 3.0 x 10^3/ml respectively).

4.i.6. Microbial load of secondary effluent from small-scale starch factory

Substantial proportion of the microbial load of the effluent was due to bacteria and it was in the range of 5.50 x10^5-15.45 x 10^5/ml. The yeast count was also high but it was lower than bacteria (12.0 x 10^3 - 35.0 x 10^3/ml). The population of mould was almost equal to yeast (Table 5). Number of actinomycetes present in the samples was in the range of 1.5 x 10^3 - 2.0 x10^3/ml.

Wide spectrum of microorganisms were identified in the fresh effluent samples (Table 6). Bacterial population, consisted of gram- negative coccobacilli, gram-positive cocci, gram-positive rods, spore formers and non-spore formers.

The filamentous fungi such as Aspergillus, Penicillium, Fusarium, Rhizopus, Monocillium and Humicola were found in the effluents. The yeasts harboured in the effluent samples were Candida valida (Fig. 3A), Geotrichum candidum (Fig. 3B) and Saccharomyces species. The presence of coliform bacteria was also observed in the effluent samples ($\geq$ 1600/100 ml).

In addition to the above, the presence of hydrocyanic

acid in the fresh effluent sample was detected. Hydrocyanic acid could not be detected in samples kept beyond 3 days.

4.ii. Biochemical Changes and Microbial Proliferation in the Cassava Starch Factory Effluents Under Ambient Conditions

4.ii.1. Biochemical changes of cassava starch factory effluents at ambient conditions

a. Amylase activity

Determination of amylase activity in the primary effluent kept at ambient conditions was continued for 10 days and the results are given in Table 7 and Fig. 2.5. There was no amylase activity at the beginning of incubation. On the second day the specific activity of amylase was 0.46 and it was almost doubled on the next day (0.86). Maximum activity was obtained on the fourth day (0.96) and there was a gradual decrease in the activity on the fifth, sixth and seventh days. The corresponding values were 0.76, 0.42 and 0.33 respectively on the fifth, sixth and seventh days. However on the eighth day the specific activity was slightly higher than the previous day. There was a reduction in the activity of amylase on the tenth day.

From the negligible level (marked as 0 activity in Table 8) of amylase activity in the secondary effluent at the beginning of incubation, it was raised to 0.42 on the second day by incubation at ambient conditions. It further increased to 0.73 on the third day. On the fourth day the activity was

diminished to 0.45 before getting the maximum activity on the fifth day (0.95). Some reduction in the amylase activity was observed on the sixth day (0.35). Then it gradually increased to 0.52 on the seventh day and to 0.62 on the eighth day. A decreasing trend was observed thereafter.

b. Reducing sugar content

The results obtained for the reducing sugar content of the primary effluent are given in Table 7. Initial reducing sugar content of the primary effluent was 0.036% (w/v) which was reduced to 0.015% on the next day. The reducing sugar values for the successive 8 days were 0.013%, 0.011%, 0.01% 0.009%, 0.01%, 0.009%, 0.007% and 0.008% respectively (Fig. 2.6).

In the secondary effluent the initial reducing sugar content was 0.031% (w/v). It was diminished to 0.016% on the second day. The sugar content further decreased to 0.012% on the third day and 0.01% on the fourth day. There was slight increase in the sugar content on the fifth day i.e. 0.017%. Thereafter a gradual reduction in the reducing sugar levels was observed (Fig. 2.6). The reducing sugar levels were 0.011%, 0.005%, 0.005%, 0.004% and 0.004% on the sixth, seventh, eighth, ninth and tenth days respectively (Table 8).

There was no cellulase activity in the primary as well as secondary effluents through out the 10 days of incubation.

c. Biochemical oxygen demand, chemical oxygen demand and pH changes in cassava starch factory effluents at ambient conditions

When the primary effluent was kept at ambient conditions for 10 days the BOD of the primary effluent was increased from 14.71 g/l to 15.36 g/l (Table 7). It was observed that during the 10 days of incubation, COD was decreased from 37.20 g/l to 35.30 g/l. The effluent became more acidic as indicated by the pH decrease from 4.4 to 3.0 (Fig. 2.7).

In the case of secondary effluent the initial BOD of 3.57 g/l was reduced to 1.94 g/l and COD of 3.95 g/l to 2.58 g/l at the end of 10 days storage at ambient conditions (Fig. 2.8). Corresponding variation in pH of the secondary effluent was from 4.5 to 3.7 (Table 8).

d. Effect of gravity settling

The result of gravity settling experiments indicated the separation of 41.30% of total solids and thereby a reduction of 48.42% BOD and 49.53% COD of the secondary effluent during 15 min. (Table 9 & Fig. 2.3). But the gravity settling did not help the separation of solids in the primary effluent (Fig.2.2)

4.ii.2. Microbial proliferation in cassava starch factory effluents at ambient conditions

The growth of microflora in the effluents discharged from starch factory after different incubation periods was

studied in detail. The main focus was on bacteria, yeasts and fungi (Table 10 and 11).

Rapid multiplication of the bacterial cells was noticed in the primary effluent through out the period of observation up to ninth day of incubation and the bacterial population was 0.09 x 10^6/ml cells on the ninth day of incubation. Afterwards there was no increase in the bacterial population (Fig. 2.4).

The yeast count of the primary effluent was 0.6 x 10^6/ml on the first day. The number of cells suddenly increased to 4.0 x 10^6/ml on the second day and again increased to 30 x 10^6/ml on the third day. Further multiplication of yeasts in the primary effluent was not as rapid as earlier (39.4 x 10^6/ml, 54.4 x 10^6/ml, 82 x 10^6/ml and 100 x 10^6/ml on the fourth, fifth, sixth, and seventh days respectively. A stationary phase in the growth was observed from the seventh day except small variations in the yeast cell counts on the eighth (93.64 x 10^6/ml)ninth (97.0 x 10^6/ml) and tenth days (99.80 x 10^6/ml).

Maximum growth of mould was noticed on 6th day (18.59 x 10^6/ml) during incubation at ambient conditions. After a lag in the growth on seventh, eighth and ninth days the mould number again increased to 18.0 x 10^6/ml on tenth day.

Bacterial population in the secondary effluent at the beginning was 30.08 x 10^6/ml. There was a gradual increase

in the population from the second day onwards and maximum load was observed on the tenth day (500 x 10^6/ml). In the case of yeasts there was a gradual increase in the population right from the beginning and the rate of multiplication of yeast cells was comparatively faster than bacteria and fungi. On the first day of observation the yeast population was 2.4 x 10^6/ml and on the eighth day, maximum population was found (491.79 x 10^6/ml).

The mould count was comparatively lower in the secondary effluent. The total mould count in the initial stages of observation was 0.04 x 10^6/ml and the maximum count of 4 x 10^6/ml was observed on the fifth day. A decline phase of fungal growth in the secondary effluent at ambient conditions began on the sixth day with a corresponding population of 3.96 x 10^6/ml.

4.iii. Submerged Fermentation of Cassava Starch Factory Effluents with Yeasts and Yeast-like Organisms for the Production of Single-Cell Protein

The primary and secondary effluents of cassava starch factory were fermented aerobically for the conversion of the carbohydrates into SCP. Five cultures including yeasts and yeast-like organisms were used in the present studies (Fig. 3A, 3B, 3C, 3D & 3E).

4.iii.1. Performance of C. valida, C. candidum, C. utilis, E. fibuliger and E. magnusi in the primary effluent of cassava starch factory

a. Candida valida

Significant reduction in the starch content was not observed during 5 days growth of C. valida (Table 12). The initial starch content was 2.23%(w/v) and the final starch content after 5 days of incubation was 2.04%. There was a gradual increase in the total cell count of yeasts in the media which multiplied initially using the free sugar available and as a result the initial reducing sugar concentration of 0.016% was decreased to 0.006% by 24 hr incubation. The initial cell count in the medium was 21.2 x 10^6/ml and the cell numbers increased to 30.0 x 10^6/ml and 50.7 x 10^6/ml at 24 and 48 hrs respectively. The corresponding protein content was also maximum at 48 hr (0.105% w/v) as against the initial protein content of 0.065%. There was a gradual reduction in the biomass protein content supporting the values of cell numbers after 48 hrs of incubation. The cell count at 72 hr of incubation was 42.3 x 10^6/ml and the corresponding percentage of protein was 0.102 . A reduction in the cell count and protein content was observed during further incubation (Fig. 3.3 and 3.4).

b. Geotrichum candidum

The results on the performance of G. candidum in the primary effluent are given in the Table 13. Though the initial starch content was 2.34% after 4 days of incubation

there was not much reduction in the total starch content (1.95%). The initial reducing sugar was 0.025% and there was significant reduction in the reducing sugar during 24 hrs of incubation (0.009%). At the end of observation (120 hr) the total reducing sugar content was only 0.007%. The cell count increased gradually during the initial 3 days and thereafter the cell count was maintained uniformly throughout the period of observation. The initial cell count was 54.35 x 10^6/ml and on the third day it was increased to 101.0 x 10^6/ml and thereafter there was no increase in the cell count. Similarly the protein content also increased from 0.125% to the highest concentration of 0.179% during 72 hrs of incubation. The protein content decreased thereafter (Fig. 3.4).

c. Candida utilis

The utilization of starch during 5 days of incubation of C. utilis in the effluent was only 0.08% (Table 14). But the content of free reducing sugar of the effluent was reduced from 0.032% to 0.009% during 24 hrs of incubation. Further assimilation of sugar was very slow as it had reached a level of 0.007% by 120 hr of incubation. Though the initial cell count was only 15.96 x 10^6/ml it reached 64.58 x 10^6/ml after 72 hrs of incubation. After 72 hrs there was a reduction in total cell count and protein content (Figs. 3.3 and 3.4).

d. Endomycopsis fibuliger

The conversion of starch into reducing sugar was

obviously appreciable during the growth of E. fibuliger (Table 15). The initial starch of 2.38% was reduced to 0.45% by 120 hr of incubation (Fig. 3.1). Incessant growth of the organism led to the increase of cells from 0.55 x 10^6/ml to 105.62 x 10^6/ml and a corresponding increase of protein from 0.029% to 0.182%. During the growth phase, the hydrolysed sugar was assimilated as a reduction in reducing sugar was observed after 48 hrs at which the sugar content was 0.075%. On the fifth day the sugar content was slowly reduced to 0.032% (Fig. 3.2).

e. Endomycopsis magnusi

About 1.82% starch out of 2.74% was degraded by E. magnusi during 120 hrs of fermentation. Maximum reducing sugar content was observed at 48 hr (0.071%), unlike the cell number and protein content which was found maximum at 120 hr (89.94 x 10^6/ml and 100.159% respectively). But significant protein increase was observed after 48 hrs (Table 16).

4.iii.2. Performance of C. utilis and E. fibuliger in the enriched and non-enriched secondary effluents of cassava starch factory

a. Candida utilis

In both enriched and non-enriched secondary effluents the starch was not utilized by C. utilis (Tables 17 and 18)

During 72 hrs of incubation in the enriched and non-enriched secondary effluent samples only 0.01% of starch was utilized by C. utilis. Free sugar was readily utilized in the enriched effluent during different days of incubation (Fig. 3.6). The initial sugar content of 0.025% was reduced to 0.008% by 72 hr of incubation. In the non-enriched samples the initial free sugar was 0.030% and it was reduced only to 0.012% at 72 hr of incubation. The cell density after 72 hrs of incubation was 375 x 10^6/ml and 255 x 10^6/ml in the enriched and non-enriched effluent samples respectively. The corresponding protein content was 0.213% and 0.191% respectively for enriched and non-enriched effluents.

b. Endomycopsis fibuliger

Nearly 50% of the starch present in the enriched secondary effluent was converted to reducing sugar by the hydrolytic activity of E. fibuliger during 72 hrs of culturing (Fig. 3.5). But degradation of starch was slightly lesser in the non-enriched secondary effluent (44.0%). Initially in the enriched samples utilization of reducing sugar was faster than the non-enriched samples (Table 19 and 20). By 72 hr, the reducing sugar level was 0.008% but the corresponding figures in the non-enriched sample was 0.010%.

Maximum cell counts were found in the 48 hrs old samples (375.72 x 10^6/ml). But in the non-enriched effluent maximum

cell count was recorded as 335 x 10^6/ml at 48 hr of incubation (Fig. 3.7). The protein content of the enriched samples increased from 0.096 to 0.193% during 72 hrs of incubation. At the same time in the non-enriched samples the protein content reached only to 0.175% (Fig. 3.8).

4.iii.3. Co-culturing of C. utilis with E. fibuliger and C. utilis with E. magnusi in the primary effluent of cassava starch factory

a. Candida utilis and Endomycopsis fibuliger

The results given in the Table 21 show that there was degradation of starch at a significant rate during the growth of E. fibuliger and C. utilis (3.23% to 1.53%). Reducing sugar content increased from 0.05 % to 0.58% at 48 hr and this again reduced to 0.168% at 120 hr (Fig. 3.10). There was profound growth and multiplication of C. utilis and E. fibuliger in the effluent and the C. utilis count was 281.08 x 10^6/ml and that of E. fibuliger was 118.75 x 10^6/ml at 120 hr of incubation (Fig. 3.11). Corresponding to the increase in the cell count there was increase in the protein content (protein content increased from 0.13% to 0.295%). During 120 hrs of growth a significant and gradual reduction of COD from 68.29 g/l to 6.71 g/l and BOD from 20.82 g/l to 5.12 g/l were observed (Fig. 3.14). At the end of observation the pH of the effluent was found to be lowered from 4.80 to 3.20.

b. Candida utilis and Endomycopsis magnusi

There was a reduction in the starch content from 3.25% to 1.66% during 120 hrs growth of the organisms (Fig. 3.9). There was also a reduction in the reducing sugar content from 0.051% to 0.008% during 48 hrs of incubation (Table 22). The reducing sugar content suddenly increased and reached 0.811% at 120 hr of incubation. The growth of E. magnusi was found to be quite slow (0.59 x 10^6/ml to 8.67 x 10^6/ml with 120 hr). In the case of C. utilis the cell count increased to 133.74 x 10^6/ml by 96 hr from the initial count of 39.95 x 10^6/ml. At 120 hr of incubation the cell count was again reduced to 85.4 x 10^6/ml (Fig. 3.12). The biomass protein was gradully increased from the initial level of 0.061% to the maximum of 0.134% at 96 hr of incubation and it was declined thereafter (Fig. 3.13). The BOD and COD content was minimum at 24 hr of incubation (3.64 g/l and 3.86 g/l respectively). A gradual increase was observed in the BOD and COD content during successive hrs of incubation. During the study, there was a sharp reduction in the pH from 6.00 to 3.35.

4.iii.4. Co-culturing of C. utilis and E. fibuliger in the enriched and non-enriched secondary effluent of cassava starch factory

In the enriched secondary effluent there was 50% reduction of starch by 72 hr when C. utilis was co-cultured with E. fibuliger (Table 23 and Fig. 3.15). The reducing sugar

content gradully reduced from 0.047% to 0.008% during incubation (Fig. 3.16). Maximum cell count was observed at 24 hr (365 x 10^6/ml) and slow reduction with time was noticed (Fig. 3.17). Initial protein content of 0.154% was enhanced to 0.27% during growth of the organisms (Fig. 3.19). Considerable reduction of COD was achieved by the growth of the above organisms (65%).

In the non-enriched samples the starch reduction during culturing of C. utilis with E. fibuliger was about 30% by 72 hr of growth (Table 24). The reducing sugar content increased slightly (from 0.023% to 0.028%) and again reduced at the end of observation (0.015%). The cell count also increased from the initial 70.68 x 10^6/ml to 375 x 10^6/ml by 48 hr. Correspondingly an increase in the protein level was also observed (0.148% to 0.218%) at 48 hr of incubation. The COD removal was about 58% by 72 hr (Fig. 3.20).

4.iii.5. Co-culturing of C. utilis and E. fibuliger in the primary effluent of cassava starch factory under controlled conditions

In the laboratory fermentor co-culturing of C. utilis and E. fibuliger in cassava starch factory primary effluent resulted in faster utilization of starch and sugar by the organisms (Table 25). By 28 hr of incubation, the starch content was reduced from 1.92% to 0.40%. But thereafter the

degradation of starch was slow. Similarly the sugar utilization was at a rapid rate in the beginning (Fig. 3.22) and resulted in 0.01% at 28 hr, later much reduction was not observed (.004% at 48 hr). E. fibuliger multiplied at a moderate rate and reached a maximum cell density of 288.0 x 10^6/ml from the initial 28.25 x 10^6/ml at 28 hr. Thereafter a reduction in the cell count was observed (Fig. 3.23). The cell division was comparatively rapid in the case of C. utilis and was maximum at 24 hr (1107.5 x 10^6/ml). When the incubation was continued further there was a reduction in the cell number rather than increase. The protein content also increased from 0.0876% (w/v) to 0.5407% (w/v) by 28 hr (Fig. 3.21). After 28 hrs of incubation, there was slight reduction in the protein content and reached 0.515% w/v at 60th hr of incubation. A maximum biomass protein content of 22.07% (w/v) was obtained at 28 hr of fermentation (calculation was made on the basis of raw primary effluent with a protein content of 0.51% w/w). The initial BOD value of 11.25 g/l has come down to 1.06 g/l during 28 hrs of incubation and initial COD value of 28.25 g/l has diminished to the minimum of 1.95 g/l during 32 hrs of incubation. Further incubation slightly increased the BOD and COD values to 1.18 g/l and 2.32 g/l respectively at 60th hr (Fig. 3.24).

4.iv. Solid State Fermentation of Cassava Starch Factory Waste with Aspergillus niger

4.iv.1. Accumulation of fungal protein during the growth of Aspergillus niger on cassava starch factory waste

The solid state fermentation of cassava starch factory waste with A. niger has enhanced the protein content as shown in the Tables 26 & 27 and Fig. 4.7.

Initial biomass protein present in the enriched material was 1.60% (w/w). A steep increase in the protein content was observed during the first three days (7.0% on the third day). Only 0.7% additional increase in the protein content was achieved during the next two days of incubation.

In the case of non-enriched samples the initial protein content was 1.10% and this was increased to the maximum level of 3.70% on the third day of incubation. There was no further increase in the protein content during the fourth and fifth day of incubation.

4.iv.2. Extracellular protein

The protein present in the water extract of the fermented material was estimated and is presented in Tables 26 and 27 and Fig. 4.6 as extracellular protein.

The extracellular protein present initially in the enriched material was 0.742% (w/w). It gradually increased

during incubation and the maximum value was obtained on the third day (2.505%). A reduction in the amount was noticed subsequently on fourth (2.202%) and fifth (1.844%) days of incubation.

Initial extracellular protein content of the non-enriched sample was 0.395% and it had slowly reached a level of 1.627% on the third day. During the fourth and fifth days the extracellular protein contents were 1.426% and 1.468% respectively.

4.iv.3. α-Amylase activity

Higher activity of α-amylase was observed in the enriched cassava starch factory waste when compared with the non-enriched waste during the growth of A. niger (Tables 26 & 27 and Fig. 4.3). Maximum activity was found at 48 hr of incubation (0.018 units) and later the activity retarded. In the non-enriched waste the α-amylase activity was constant (0.003 units) from 48 hr to 96 hr of incubation.

4.iv.4. Amyloglucosidase activity

The amyloglucosidase activity of Aspergillus niger in cassava starch factory waste was assayed and results are given in Tables 26 & 27 and Fig. 4.4. The activity was expressed in International Units (I.U.).

Higher activity of amyloglucosidase was found in the enriched sample than the non-enriched sample. At the end of 24 hr incubation, there was an activity of 5.90 I.U. in the enriched sample. The maximum activity of 24.62 I.U. was found on the second day of incubation. There was a drastic decrease in the enzymatic activity on the third (8.77 I.U.), fourth (1.88 I.U.), and fifth day (1.30 I.U.).

The amyloglucosidase activity of A. niger in the non-enriched cassava starch factory effluent was 6.51 I.U. on the first day. The maximum enzymatic activity of 16.45 I.U. was observed on the second day of incubation. Thereafter a gradual reduction in the activity was recorded (9.40 I.U., 2.05 I.U., and 8.19 I.U. on the third, fourth and fifth days of incubation respectively).

4.iv.5. Endoglucanase activity

The estimation of endoglucanase activity of A. niger in the cassava starch factory effluent was carried out at different intervals and results were expressed as specific activity of the enzyme (Tables 26 & 27 and Fig. 4.5).

The maximum enzyme activity of A. niger in the cassava starch factory waste during solid state fermentation was

observed on the first day (8.35). On the second day the specific activity was only 2.8 and on the third, fourth, and fifth days the specific activities of endoglucanase were 4.53, 6.04 and 6.0 respectively.

In the case of non-enriched samples, maximum specific activity was observed on the fifth day of incubation (17.0) eventhough an increased specific activity of endoglucanase was observed on the second day (16.67).

4.iv.6. Starch, reducing sugar and pH in the cassava starch factory waste during the growth of A. niger.

a. Starch

The starch content of the samples was estimated at different intervals of fermentation and the results obtained are presented in Tables 26 & 27 and Fig.4.1. The starch content of the enriched waste was 56.85% at the beginning of fermentation There was a slight reduction in the total starch during 24 hrs of incubation. A drastic reduction in the starch content was observed after 24 hrs (41.85%, 33.15%, 14.55% and 13.05% respectively on 48, 72, 96 and 120 hrs) of fermentation of the material with A. niger.

In the non-enriched sample the reduction of starch content was conspicuous after 48 hrs of incubation. During five days of culturing, the initial starch content of 58.40% was reduced to 25.0%.

b. Reducing sugar

The reducing sugar content in the enriched substrate used for fermentation was negligible in the beginning (0.006%). This has gradually increased to 0.130% in 24 hrs and maximum concentration of 0.252% was obtained after 48 hrs of incubation (Table 26 and Fig. 4.2). But there was a gradual decrease in the concentration of reducing sugar during the subsequent three days ranging from 0.104% to 0.039%.

In the non-enriched samples there was a gradual increase in the reducing sugar. Though the sugar concentration was 0.006% at 0 hr it has increased to 0.393% after 120 hrs of incubation (Table 27 and Fig. 4.2).

c. pH

The pH was measured at different intervals of fermentation and the values are presented in Tables 26 & 27. The pH decreased to 3.0 and 2.85 respectively in the case of enriched and non-enriched samples from an initial pH of 5.4.

4.v. Biomethanation of Cassava Starch Factory Effluents

4.v.1. Biomethanation of primary effluent of cassava starch factory in batch digesters

a. Nature and composition of gas samples collected from anaerobic digesters

The qualitative analysis of biogas obtained from the

digester by GC-MS showed, the presence of methane (as the major constituent) and carbondioxide (Fig. 5.1). The analysis also showed the presence of butane. The mass spectrum clearly indicated (M^+) at 58 and ($M-CH_3$) at 43 indicating loss of methyl group (Fig. 5.2).

The samples were further analysed to assess the quantitative yield of methane and carbondioxide as they were the predominant gases (Fig. 5.3).

b. Influence of the addition of cowdung and urea on biogas production

Different proportions of cowdung were added to the effluent after enriching it with urea. Non-enriched samples were also simultaneously maintained. The span of gas production was found to be directly proportional to the quantity of cowdung added. In the case of non-enriched effluent samples mixed with 5%, 10% and 20% cowdung, the biogas production continued for 6, 21 and 68 days respectively.

Since the effluent contained negligible amount of nitrogen as mentioned earlier, urea was added in the samples and its influence was studied. Enrichment of the effluent with urea did not increase the span of gas production except in the case of slurries containing 5% cowdung which has produced the gas for a period of 10 days as against 6 days in non-enriched samples (Table 28). The span of gas production

in the case of non-enriched samples mixed with 10% cowdung was 21 days as against 20 days in the case of enriched effluent samples. There was significant difference in the period of gas production of enriched effluent with 20% cowdung and non-enriched effluent with 20% cowdung which showed a span of 34 days and 68 days respectively.

The proportion of total gas production in both enriched and non-enriched samples increased with the quantity of cowdung (Fig. 5.6). In the case of enriched samples with 5% cowdung the total gas production was 20 l/kg dry matter whereas in the case of effluents mixed with 10% cowdung the gas production was increased to 30 l/kg dry matter. There was a sharp increase in the output of gas to the level of 66.18 l/kg dry matter when the concentration of cowdung was increased to 20%. But the rate of total biogas production was found to be higher in the case of non-enriched effluents mixed with 10% and 20% cowdung (31.46 l/kg dry matter and 130.2 l/kg dry matter respectively) than the enriched (Table 28).

The average methane content in the digester enriched with urea was slightly higher than the non-enriched samples. Methane content of the biogas samples obtained from digester with 5% cowdung and urea was to the tune of 42% and this was actually more than double the methane content of the samples from non-enriched (without urea). Methane content increased by 3% in both the enriched effluent digester containing 10%

and 20% cowdung (Table 28), compared to the non-enriched effluent digester (Fig. 5.6).

c. Reduction of total solids and COD

The total solids and COD in the enriched and non-enriched effluent samples containing different proportions of cowdung decreased during biomethanation (Table 28 and Fig. 5.7). In the case of enriched effluent mixed with 5% cowdung the percentage reduction of total solids and COD was 12.17 and 6.39 respectively whereas the percentage reduction of total solids and COD in non-enriched effluent samples was to the extent of 7.06 and 5.68 respectively. There was no significant difference in the percentage reduction of total solids and COD when the concentration of cowdung was increased to 10% in both enriched and non-enriched samples. The percentage reduction of total solids and COD in the enriched samples was 38.93 and 9.5 respectively, whereas in the non-enriched samples the percentage reduction of total solids and COD was 39.42 and 11.14 respectively. Similar trend in the percentage reduction of total solids was observed in both enriched and non-enriched effluent samples mixed with 20% cowdung (41.24 and 42.58 respectively). But the COD reduction was much higher in the case of non-enriched samples (62.5% as against 21.95% in enriched samples).

4.7.2. Semi-continuous digestion of primary effluent of cassava starch factory in single stage digesters

In semi-continuous biomethanation of cassava starch factory effluent, experiments were conducted to achieve maximum output of biogas (yield) and reduction of total solids with minimum retention time under natural conditions. From the earlier batch digestion experiments it was observed that the rate of methane production was directly proportional to the total gas output under mesophilic condition. Hence in these semi-continuous digestion experiments only the quantitative estimation of the biogas was done. Fresh samples were added periodically which displaced same amount of digested sample to maintain the reactor volume constant. Different feeding rates in terms of COD were tried and the results obtained are given below

a. Effect of feeding 1,300 mg COD on the output of biogas and solids reduction per litre of reactor volume at every 144 hr

When 20 ml of cassava starch factory effluent sample (1300 mg COD) was introduced into the digester, there was an output of 770 ml of biogas during the period of 144 hrs (Fig.5.8 Maximum gas production was obtained during the first 72 hrs (680 ml) with a solids reduction of 48.2%. Another 24 hr of incubation could produce only 60 ml of gas with a corresponding reduction of solids to 56.0%. The gas production was only 20 ml and 10 ml on the fifth and sixth days respectively.

The cumulative solids reduction on fifth and sixth days was 56.7% and 58.1% respectively (Fig. 5.9). There was no significant change in the pH of the digested materials during the period of gas production (Table 29) and the average pH was measured as 8.65.

b. Effect of feeding 2,600 mg COD on the output of biogas and solids reduction per litre of reactor volume at every 96 hr

In this experiment the digester was fed with 40 ml (2,600 mg COD) of cassava starch factory effluent and data on total biogas production, changes in pH and reduction of total solids were collected (Table 30). Observations made during 24 hrs of incubation showed that there was 400 ml gas production with 30.1% solids reduction. But the gas output came down to 320 ml during the next 24 hrs of incubation. The corresponding reduction of total solids was 38.4%. The gas output increased to 400 ml with a corresponding solids reduction of 49% between 48 and 72 hrs. The rate of gas production was found to be 160 ml on the fourth day of incubation and reduction of total solids was 55.6%. The pH variation of the samples of digester during the experiment was in the range of 8.45 to 8.38.

c. Effect of feeding 6,500 mg COD on the output of biogas and solids reduction per litre of reactor volume at every 144 hr

The gas production was greatly influenced by the increase in the quantity of cassava starch factory effluent (Table 31) . When 100 ml (6,500 mg COD) of material was fed to the digester at room temperature, the gas production was one litre during 24 hrs and the same trend in gas production continued during the next 24 hrs also. There was a drastic reduction in the biogas output between 48 and 72 hrs of incubation (460 ml) and it reached to 40 ml after 120 hrs of incubation.

During the first 24 hrs of anaerobic digestion, the total solids reduction was only 15.0%. But slowly it has increased to 24.0% during the next 24 hrs, between 48 hrs and 72 hrs 47.5%, between 72 hrs and 96 hrs 51.2% and between 96 hr and 120 hr 54.0%. There was a slight reduction in pH in the beginning (7.67) of digestion and it gradually increased to 8.35 after 120hr.

d. Effect of feeding 7,800 mg COD on the output of biogas and solids reduction per litre of reactor volume at every 96 hr

The digester was fed with 120 ml (7,800 mg COD) of effluent and the gas output, pH and total solids reduction in the digested material were determined simultaneously (Table 32 and Figs. 5.8 & 5.9). The total gas production during the 24 hrs of incubation was 1,080 ml and it increased to 1,160 ml during the next 24 hrs. The gas output on the third and fourth days was recorded as 460 ml and 500 ml respectively. The corresponding reduction of solids in the

effluent was accounted as 12.8%, 22.2%, 34.7% and 46.0% during 24 , 48, 72 and 96 hrs respectively. The pH variation was in the range of 6.83 to 7.6 during the digestion.

e. Effect of feeding 9,100 mg COD on the output of biogas and solids reduction per litre of reactor volume at every 96 hr

The experiment was started by feeding the digester with 140 ml (9,100 mg COD) of starch factory effluent. In contrast to the earlier results obtained from the semi-continuous digestion of cassava starch factory effluent, the increase in the amount of feeding material, actually reduced the gas production (Table 33). During the first 24 hrs there was an average gas production of 900 ml and between 24 hr and 48 hr it was reduced to 720 ml. A gradual reduction was observed on the subsequent period of incubation (610 ml and 400 ml between 48 and 72 & 72 and 96 hrs respectively).

During the first 24 hrs of incubation the solids reduction was 11.5% and it increased to 16.3% during the next 24 hrs and by 72 hr the reduction of solids was 32.6%. When the experiment was stopped at 96 hr there was a reduction of 43.2% in the total solids. The pH was in the acidic range throughout the experiment (5.92 - 6.20).

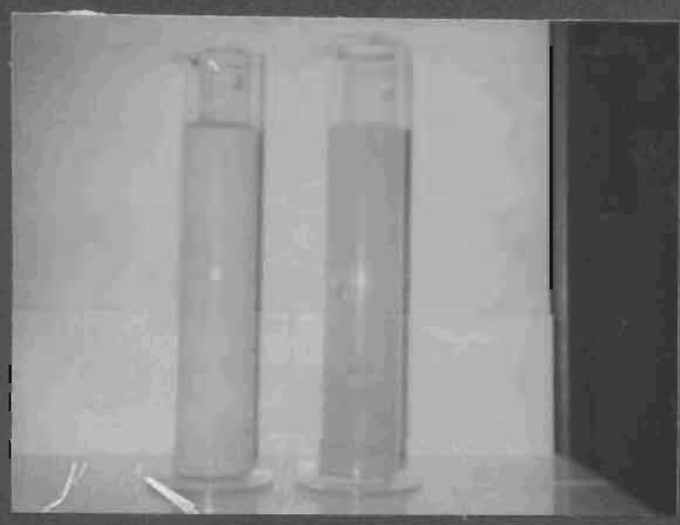
The values of treated samples were considered to be significant at 5% level if the calculated values are greater than the standard value, 2.571. The analysed results are given in the tables presented in Appendix.

Fig. 2A Ashtamudi lake, Kerala

Fig. 2B Polluted lake

Fig. 2C Polluted lake

Fig. 2D Polluted lake



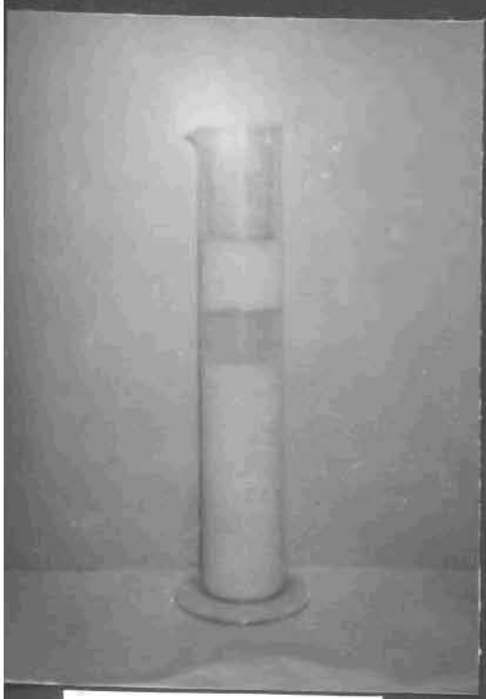


Fig. 2.2
Primary effluent during settling

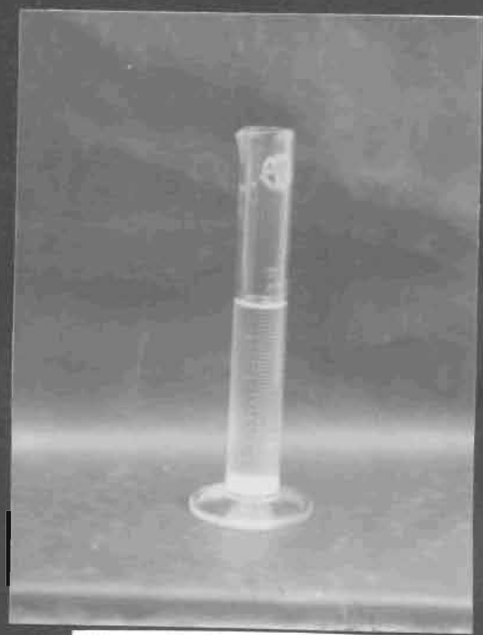


Fig. 2.3
Secondary effluent after settling

Fig. 2.4 Microbial proliferation in primary effluent under ambient conditions

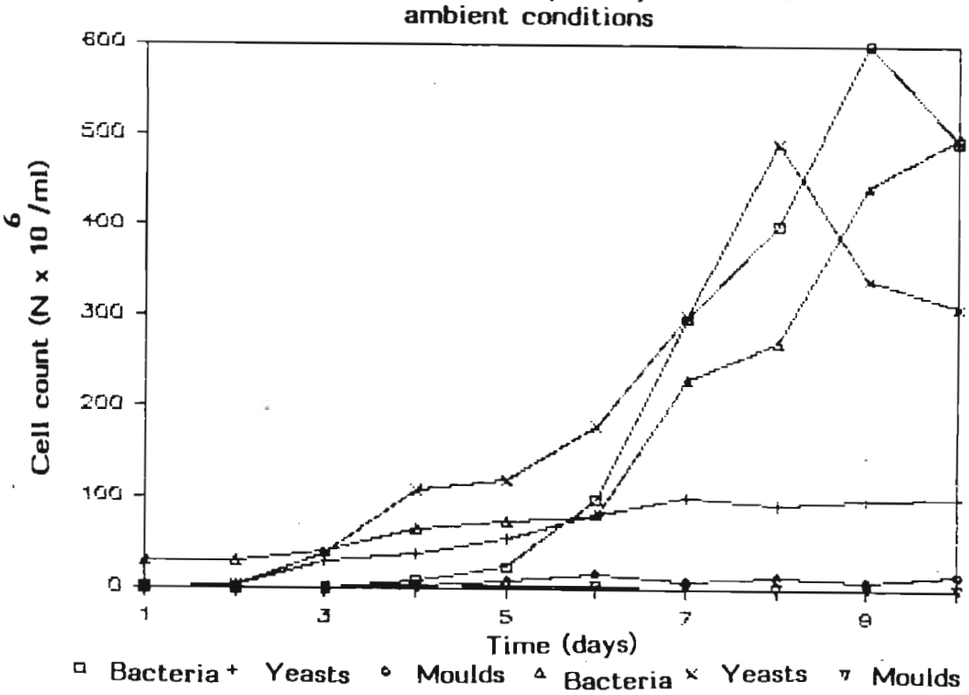


Fig. 2.5 Biochemical changes in cassava starch factory effluents under ambient conditions

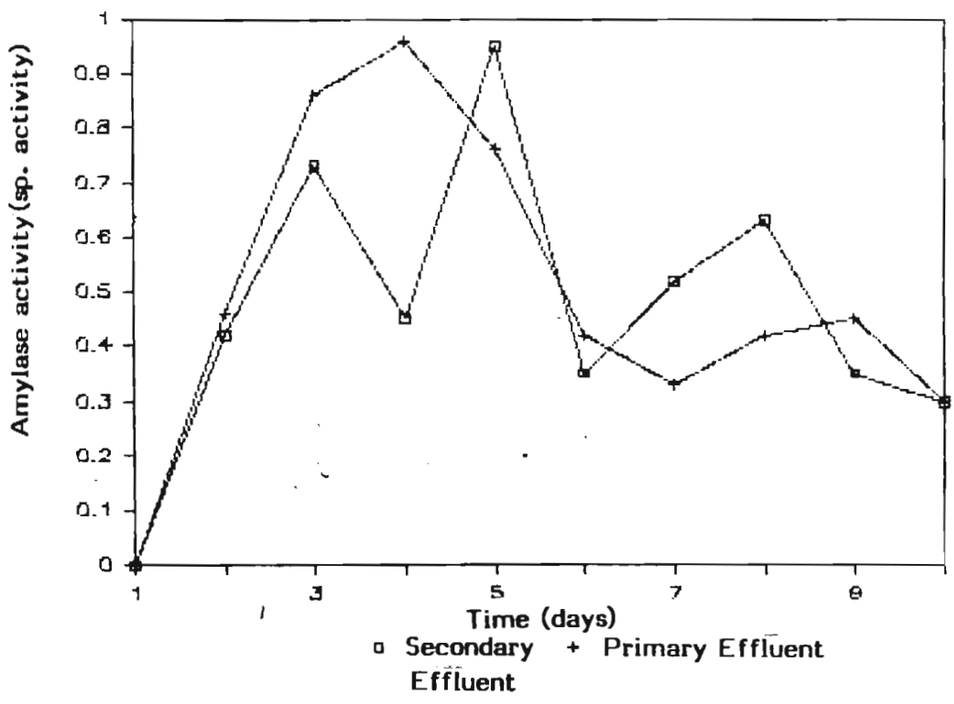


Fig. 2.6 Biochemical changes in cassava starch factory effluents under ambient conditions

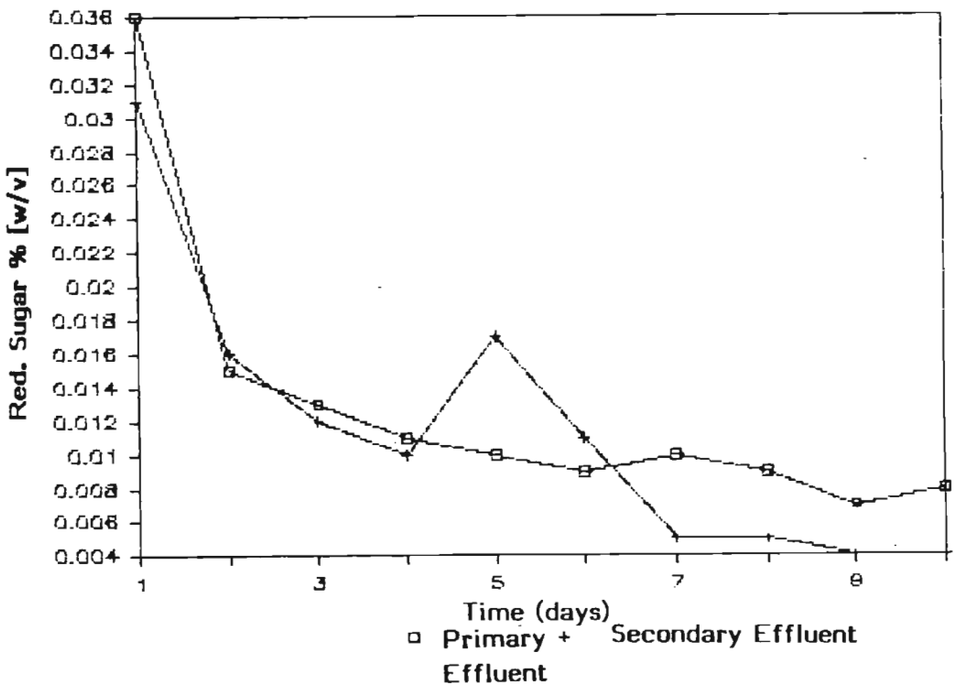


Fig. 2.7 Biochemical changes in cassava starch factory effluents under ambient conditions

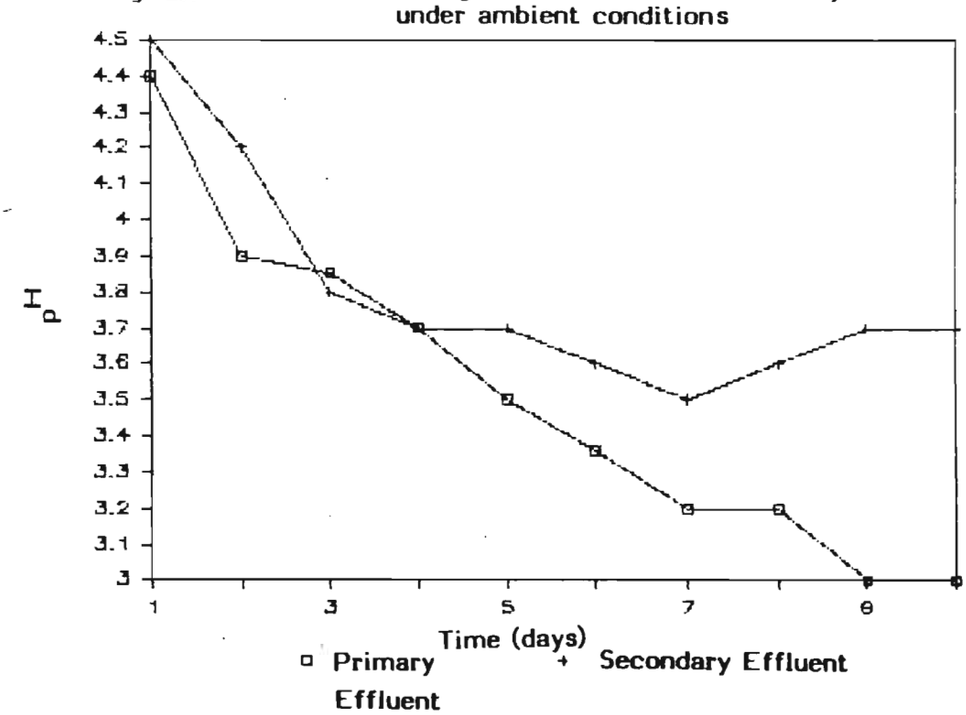
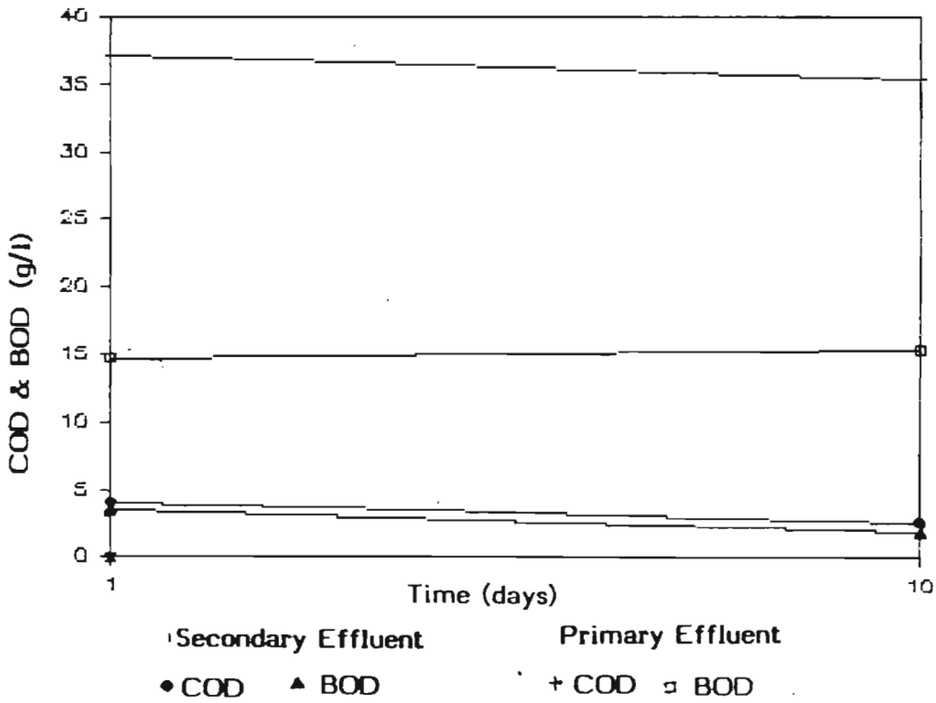


Fig. 2.8 Biochemical changes in cassava starch factory effluents under ambient conditions



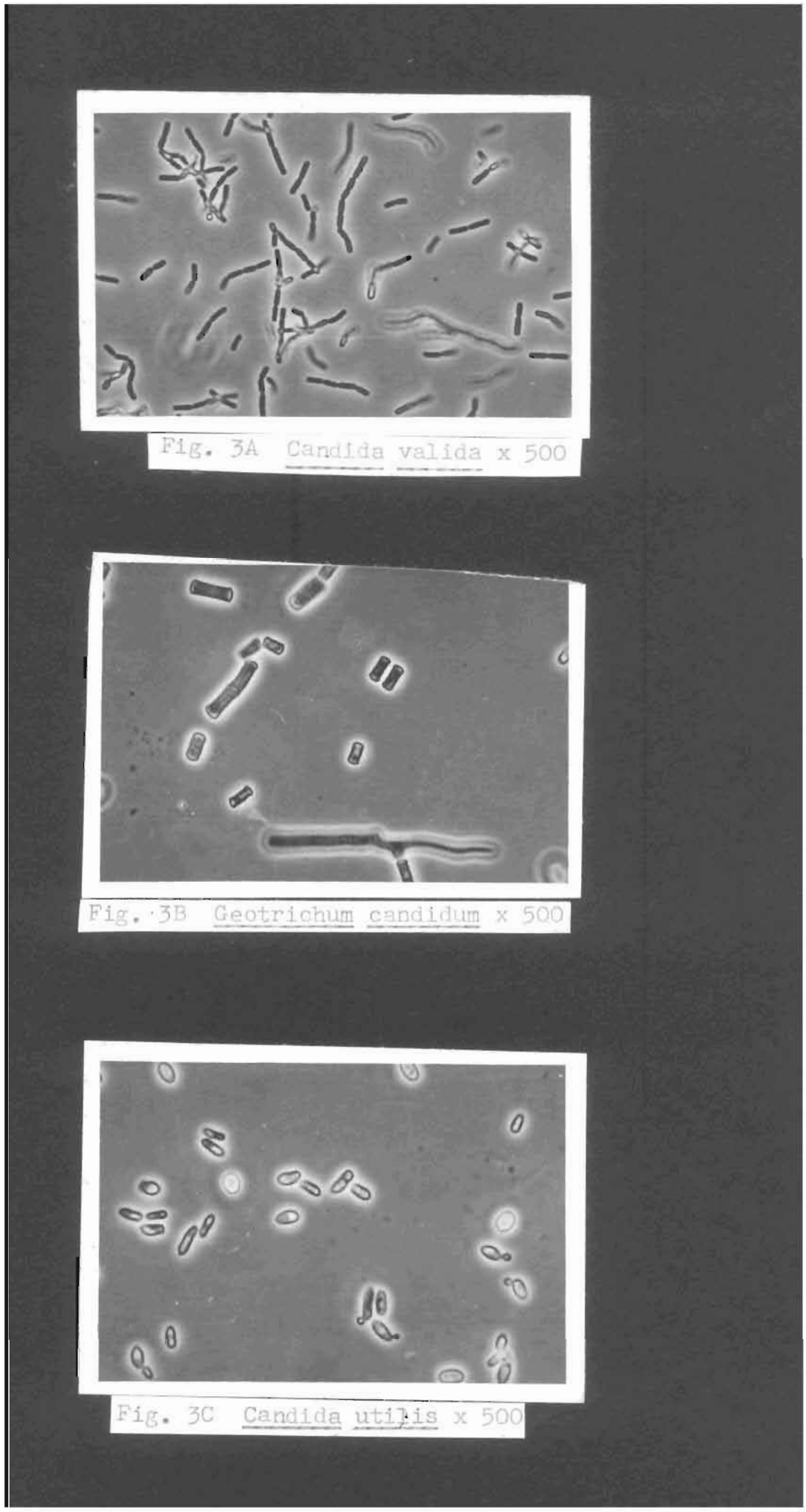


Fig. 3A Candida valida x 500

Fig. 3B Geotrichum candidum x 500

Fig. 3C Candida utilis x 500

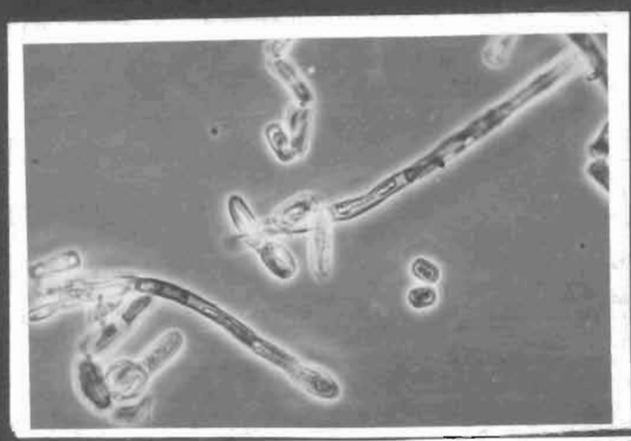


Fig. 3D Endomycopsis magnusi x 500

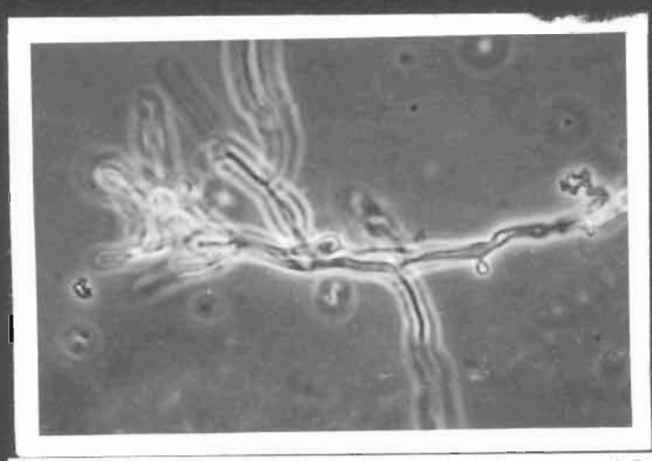


Fig. 3E Endomycopsis fibuliger x 500

Fig. 3F

Fig. 3.1 Performance of C. valida, G. candidum, C. utilis, E. magnusi & E. fibuliger in Primary Effluent

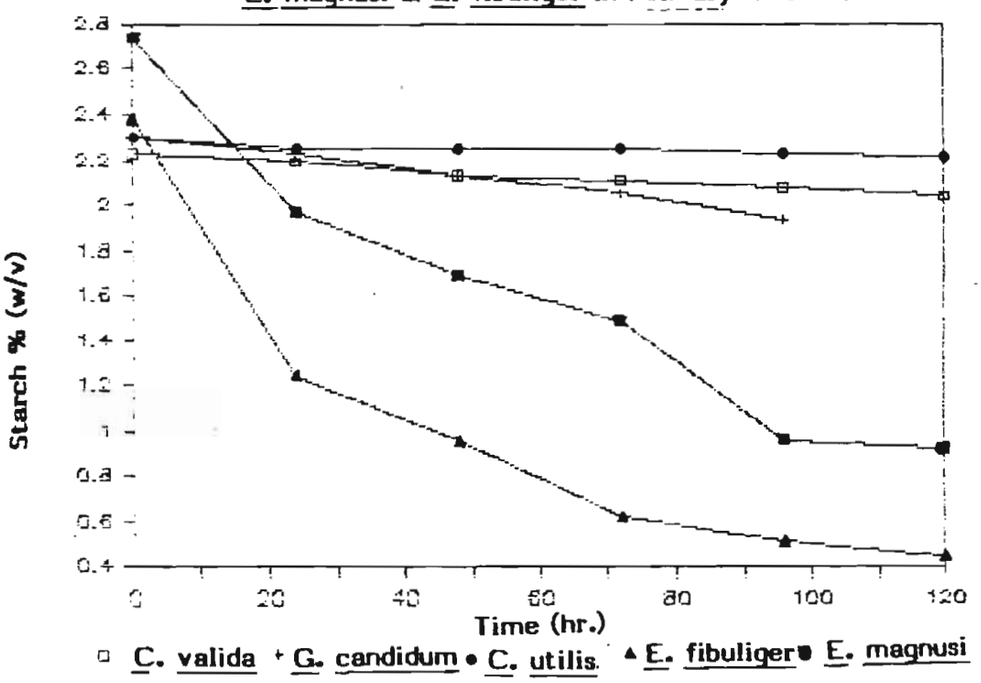


Fig. 3.2 Performance of C. valida, G. candidum, C. utilis, E. magnusi & E. fibuliger in Primary Effluent

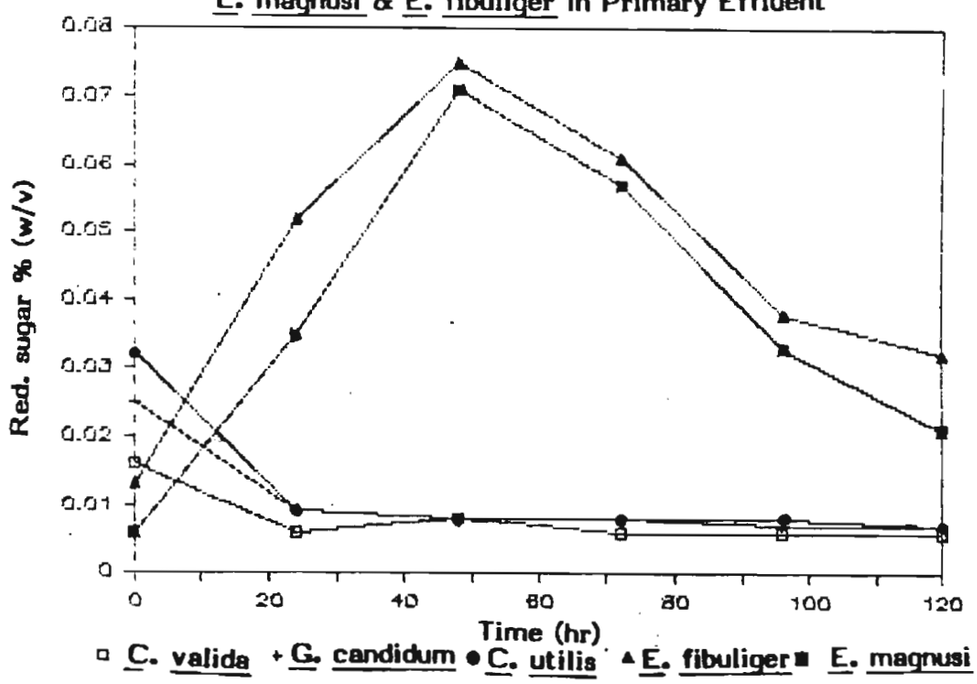


Fig. 3.3 Performance of C. valida, G. candidum, C. utilis, E. magnusi & E. fibuliger in Primary Effluent

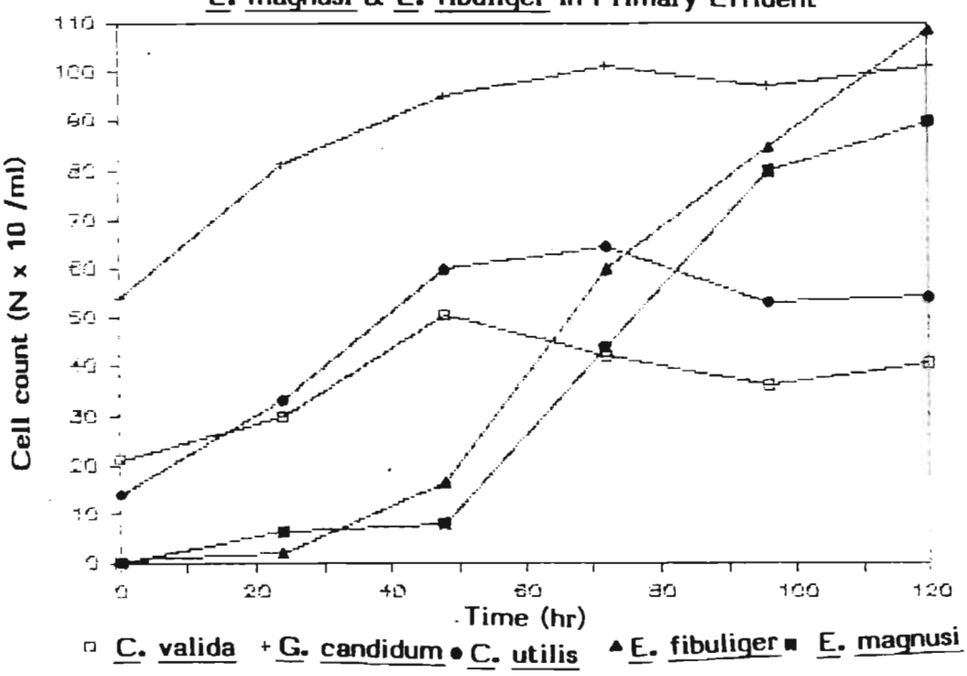


Fig. 3.4 Performance of C. valida, G. candidum, C. utilis, E. magnusi & E. fibuliger in Primary Effluent

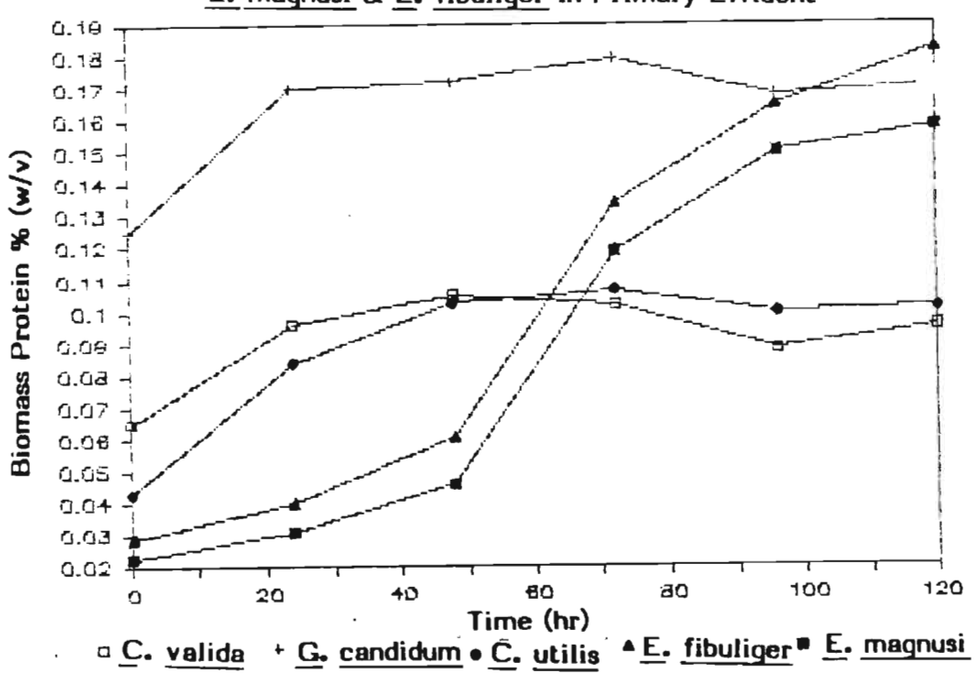


Fig. 3.5 Performance of C. utilis, & E. fibuliger in Secondary Effluent

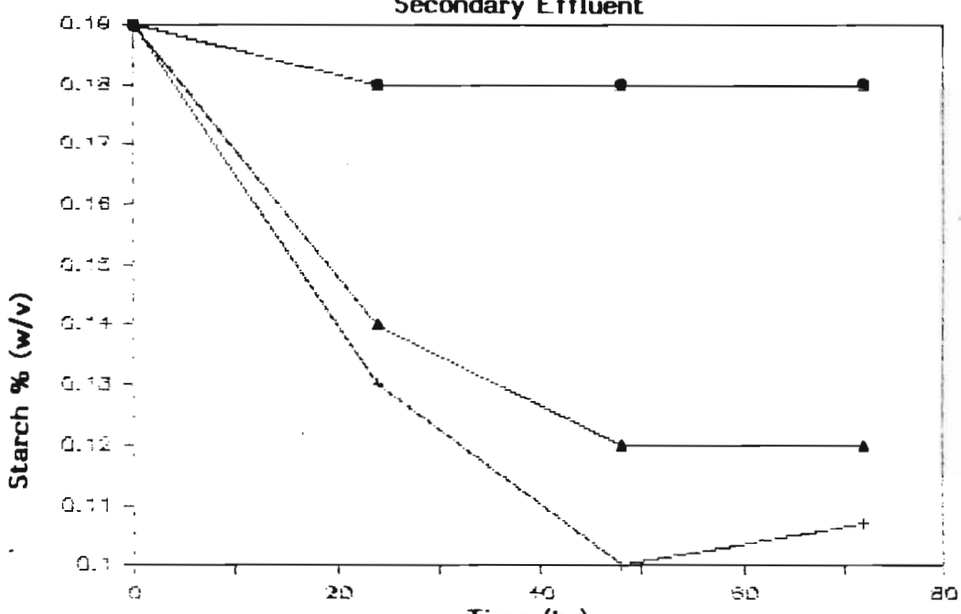


Fig. 3.6 Performance of C. utilis, & E. fibuliger in Secondary Effluent

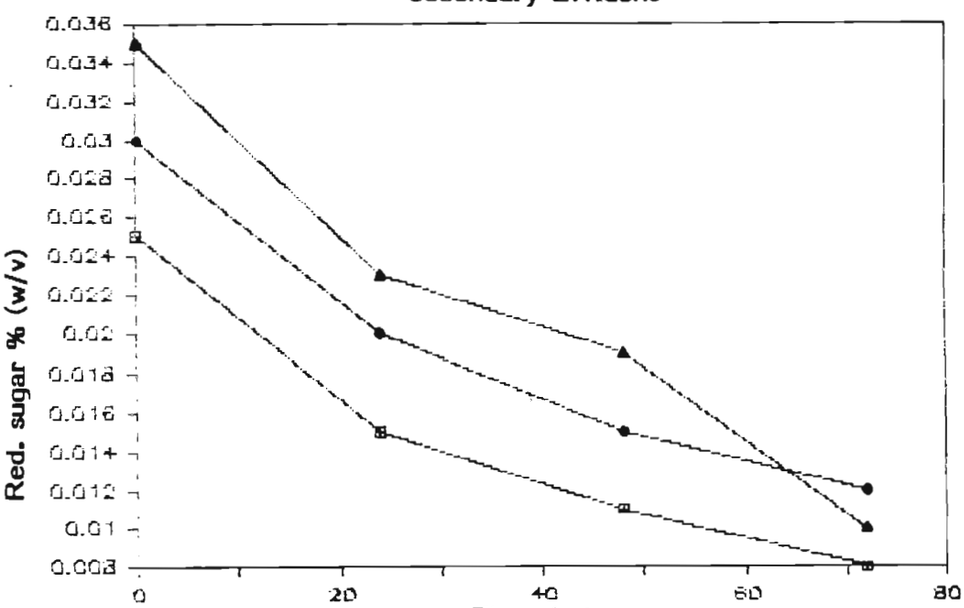


Fig. 3.7 Performance of C. utilis, & E. fibuliger in Secondary Effluent

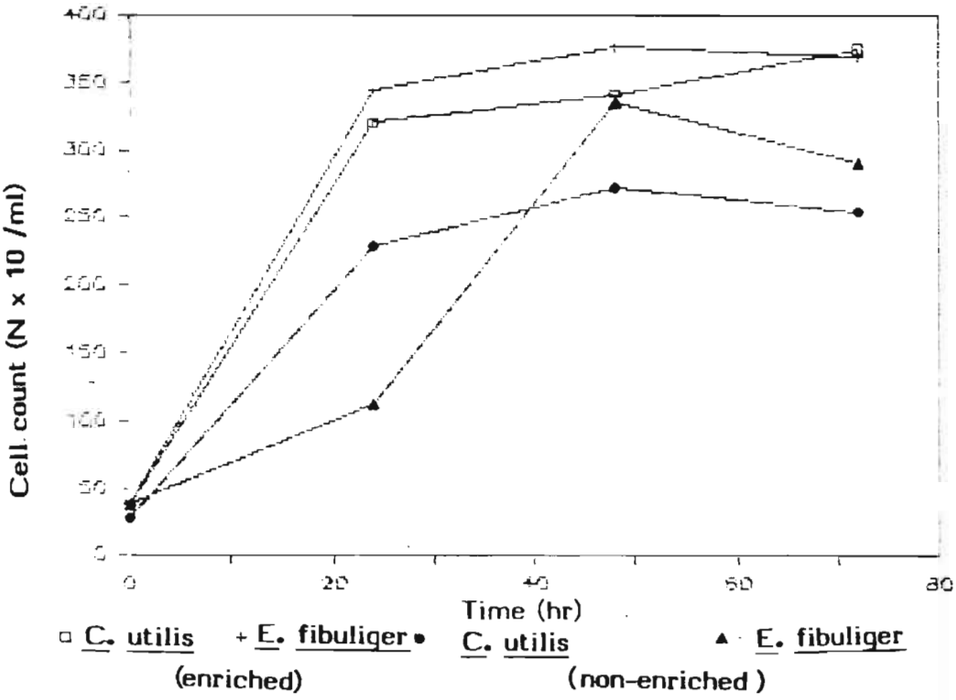


Fig. 3.8 Performance of C. utilis, & E. fibuliger in Secondary Effluent

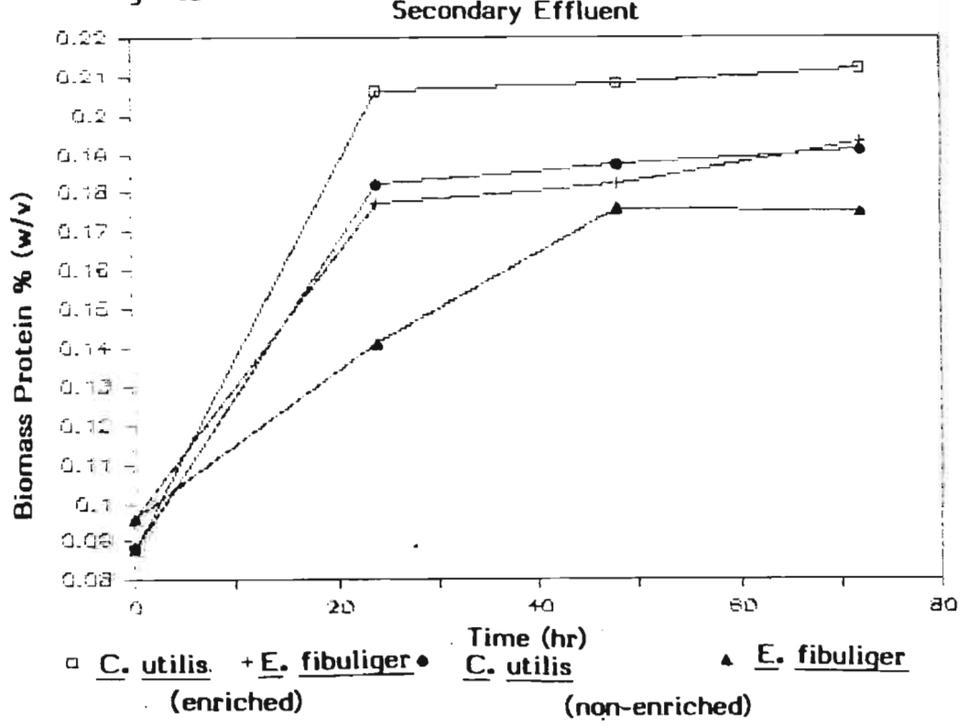


Fig. 3.9 Co-culturing of C. utilis and E. fibuliger & C. utilis and E. magnusi in Primary Effluent

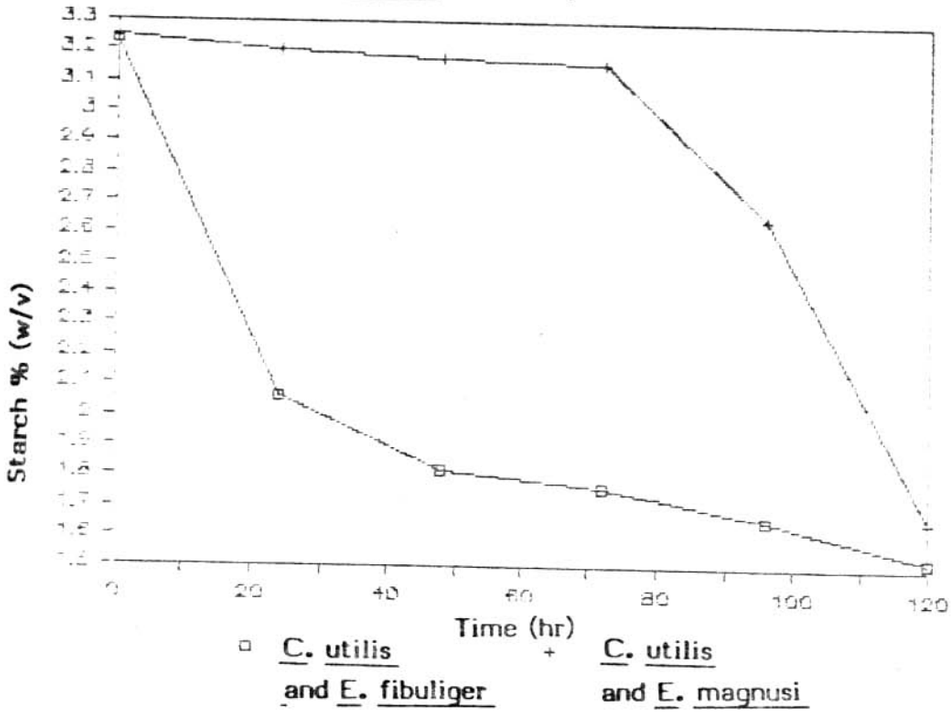


Fig. 3.10 Co-culturing of C. utilis and E. fibuliger & C. utilis and E. magnusi in Primary Effluent

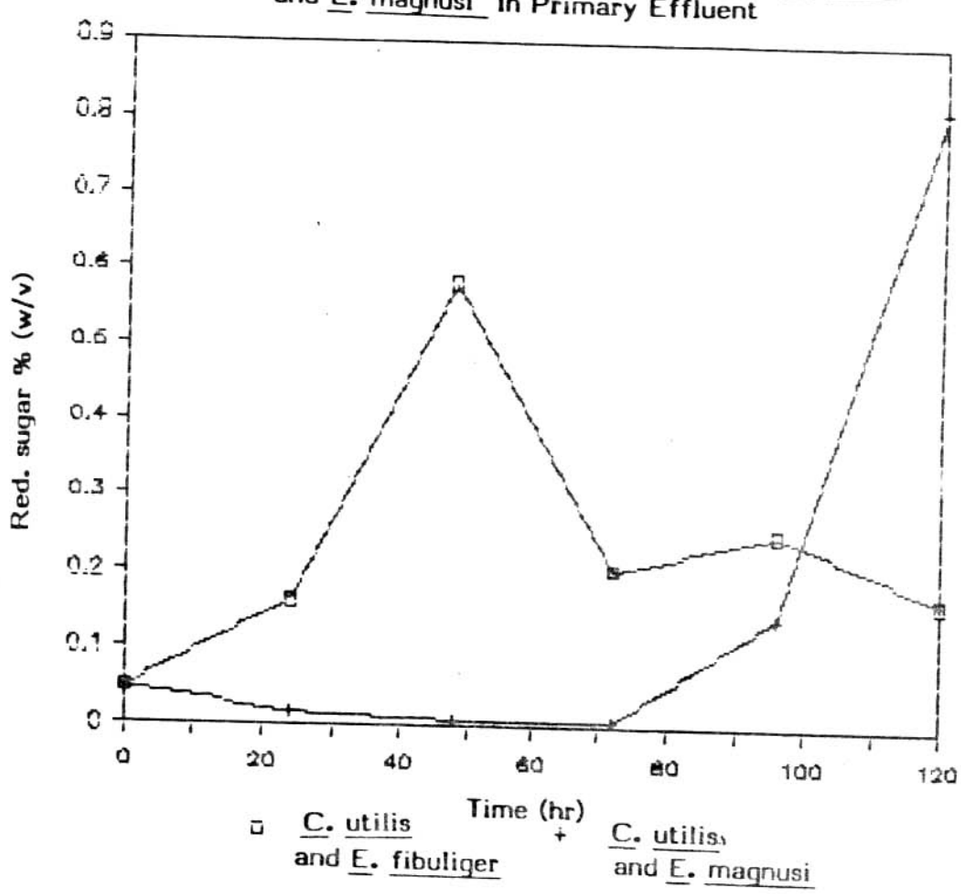


Fig. 3.11 Co-culturing of C. utilis and E. fibuliger in Primary Effluent

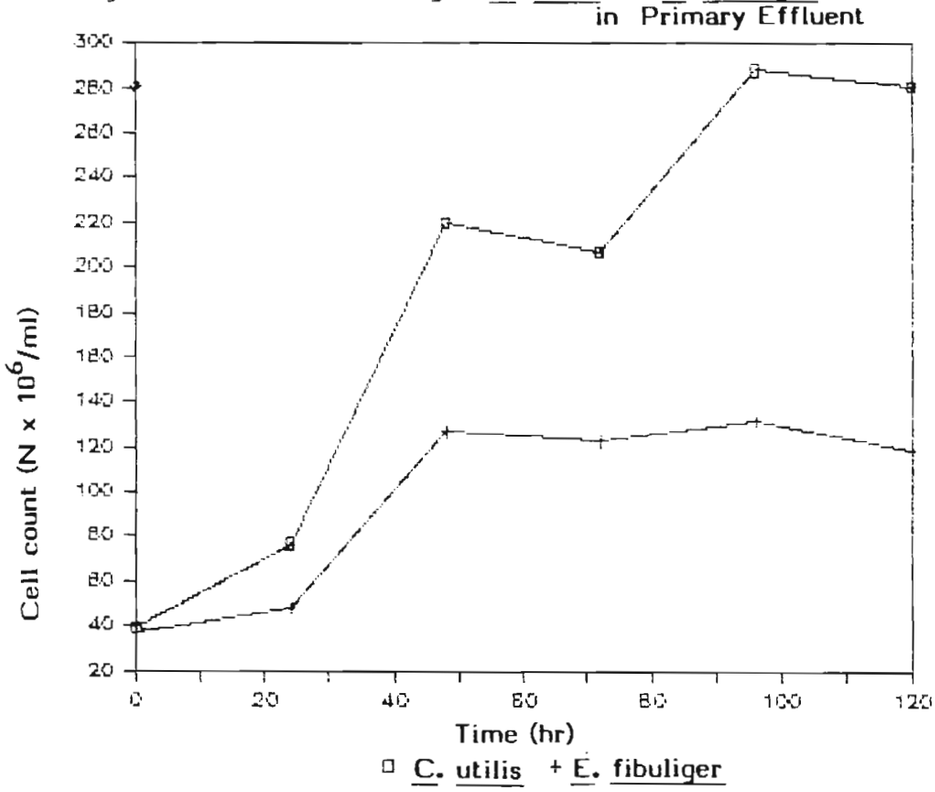


Fig. 3.12 Co-culturing of C. utilis and E. magnusi in Primary Effluent

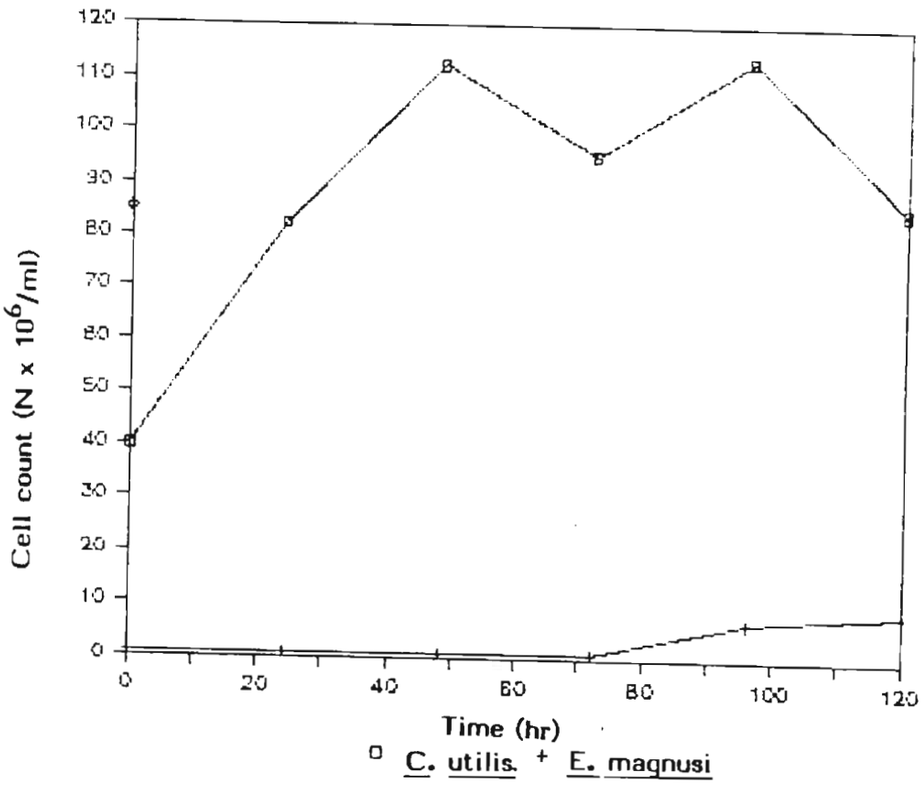


Fig. 3.13 Co-culturing of C. utilis and E. fibuliger & C. utilis and E. magnusi in Primary Effluent

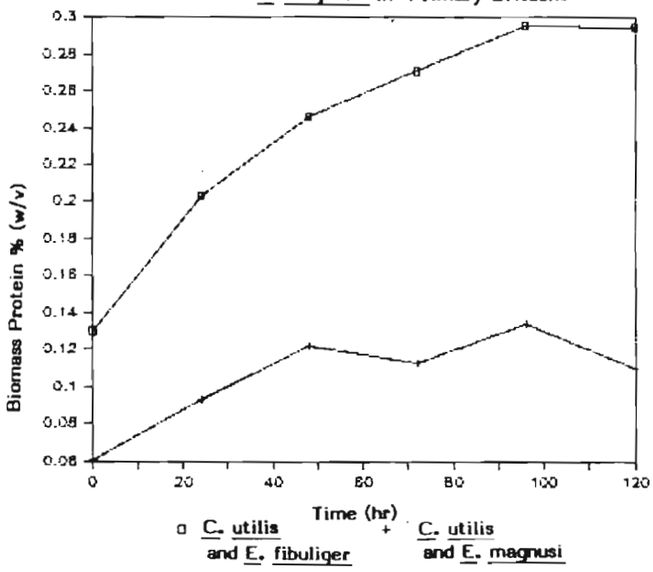


Fig. 3.14 Co-culturing of C. utilis and E. fibuliger & C. utilis and E. magnusi in Primary Effluent

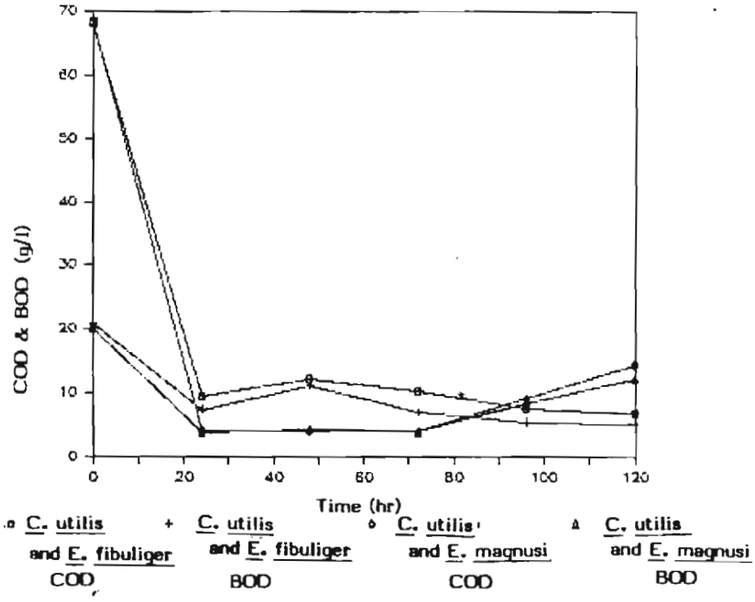


Fig. 3.15 Co-culturing of C. utilis and E.fibuliger in non-enriched and enriched Secondary Effluent

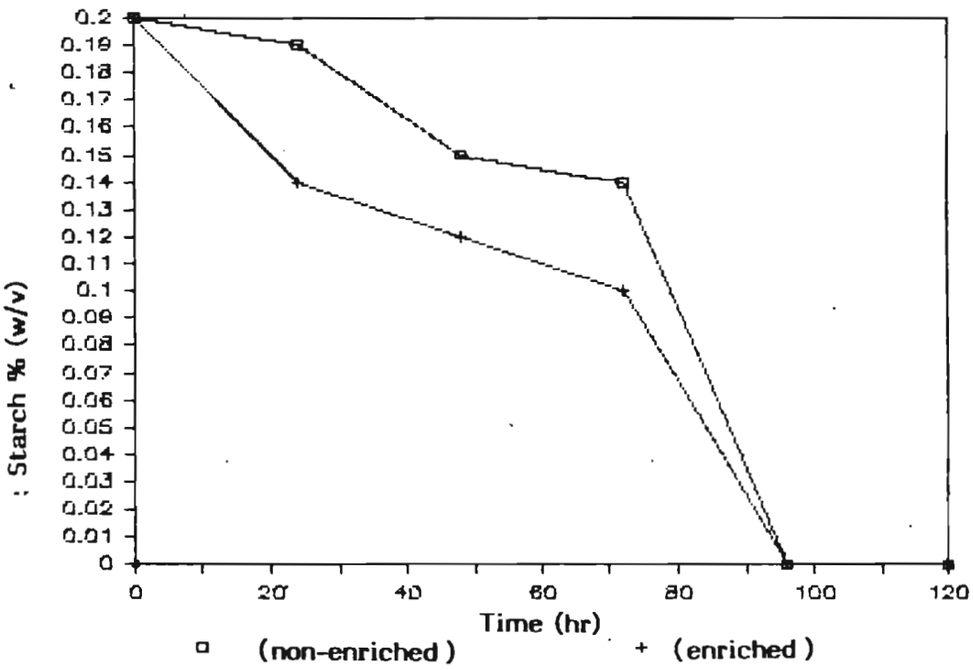


Fig. 3.16 Co-culturing of C. utilis and E. fibuliger in non-enriched and enriched Secondary Effluent

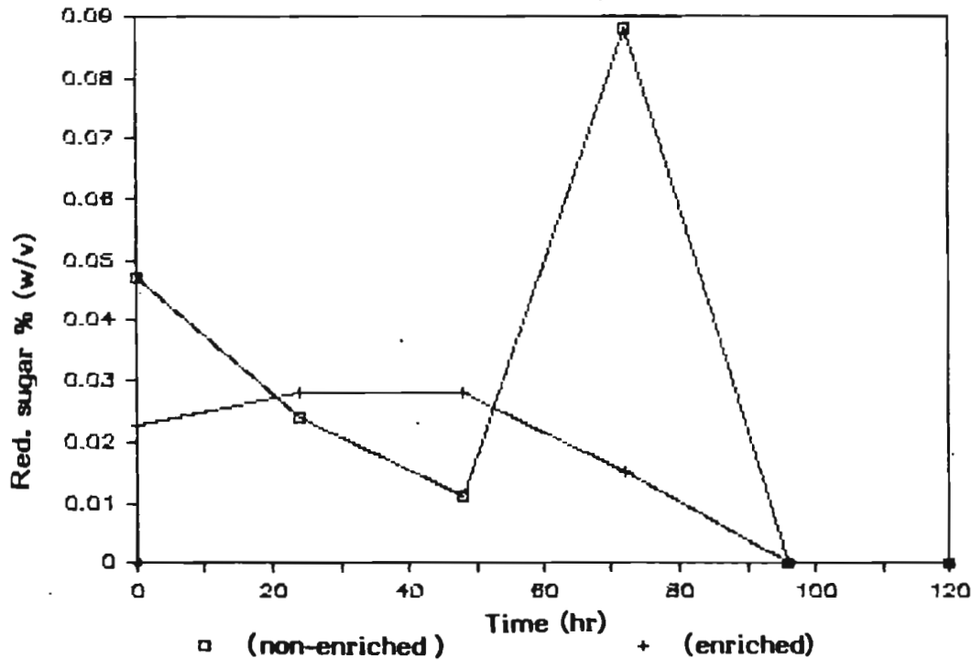


Fig.3.17 Co-culturing of C. utilis and E.fibuliger in non-enriched and enriched Secondary Effluent

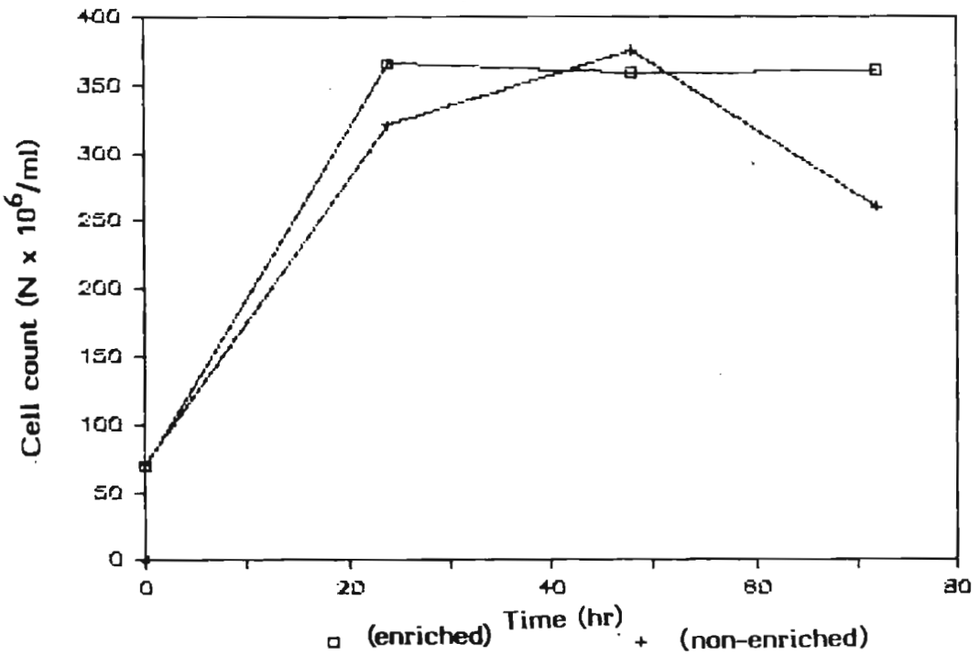


Fig. 3.19 Co-culturing of C. utilis and E. fibuliger in non-enriched and enriched Secondary Effluent

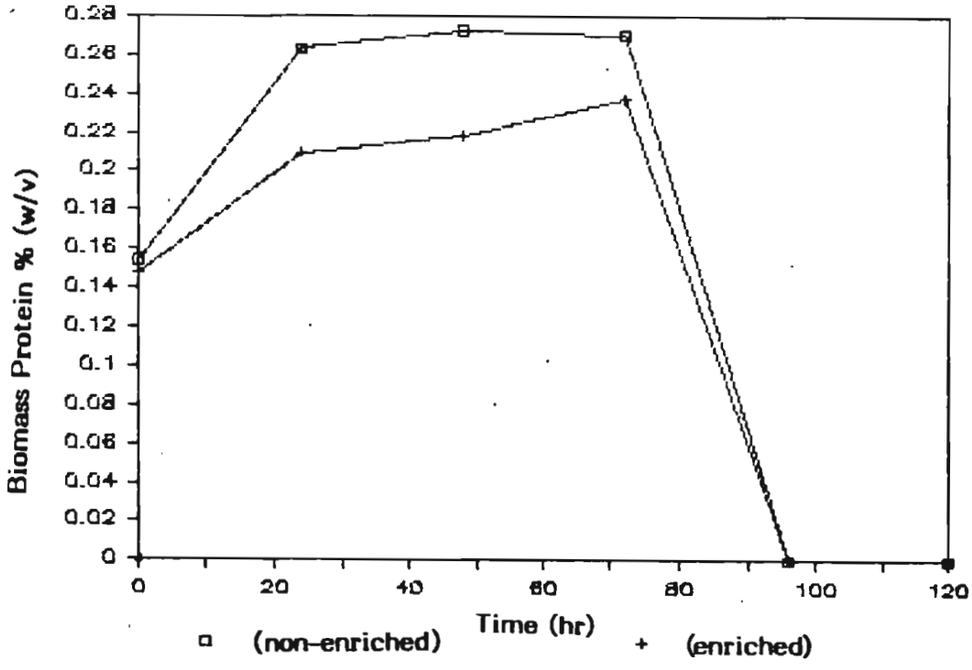


Fig. 3.20 Co-culturing of C. utilis and E.fibuliger in non-enriched and enriched Secondary Effluent

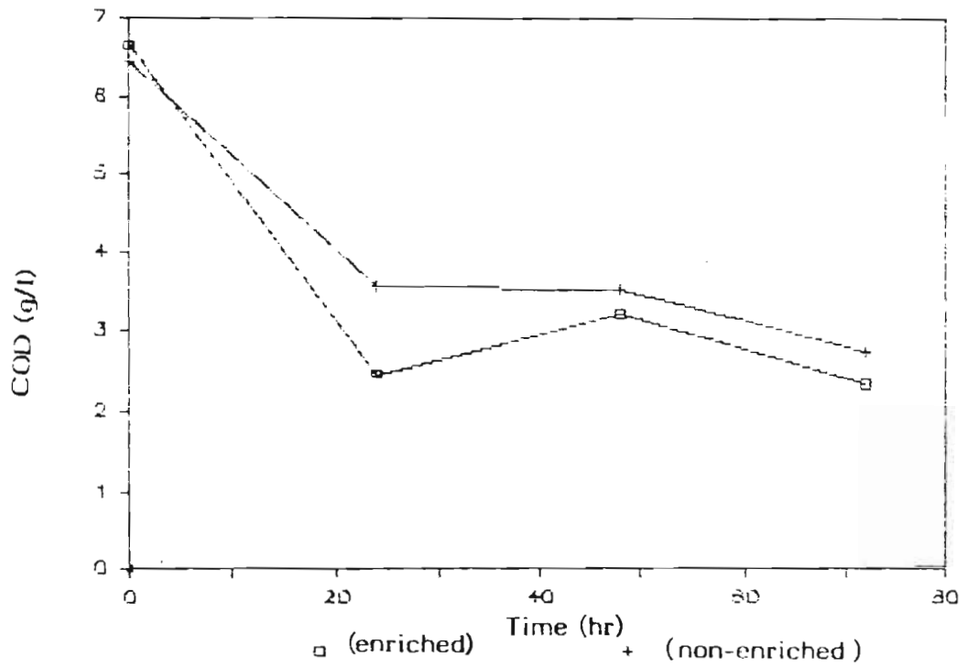


Fig. 3.21 Co-culturing of C. utilis and E. fibuliger in Primary Effluent under monitored conditions

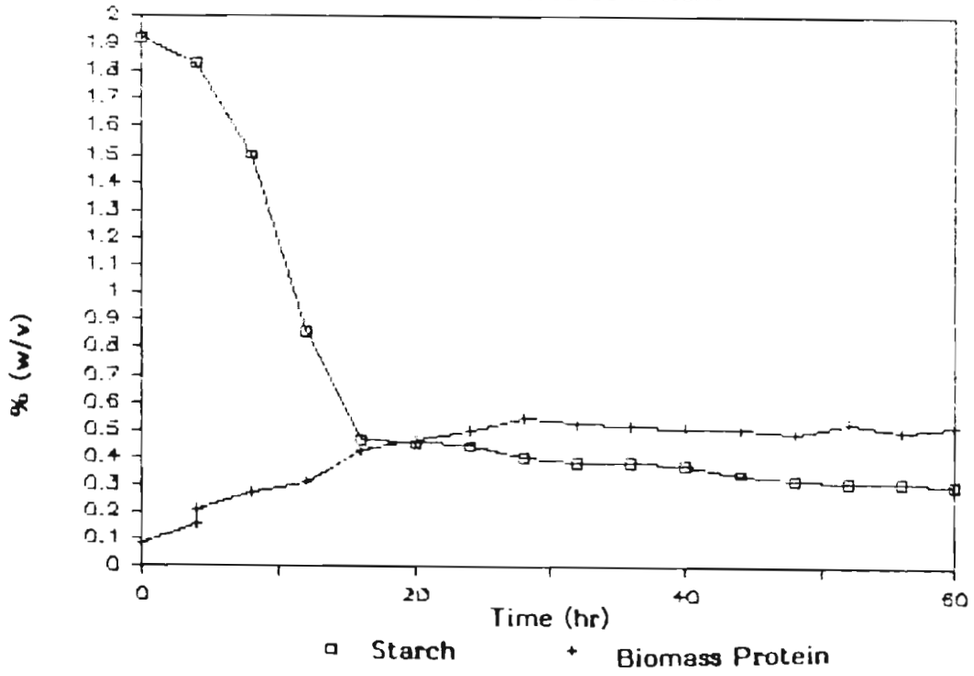


Fig. 3.22 Co-culturing of C. utilis and E. fibuliger in Primary Effluent under monitored conditions

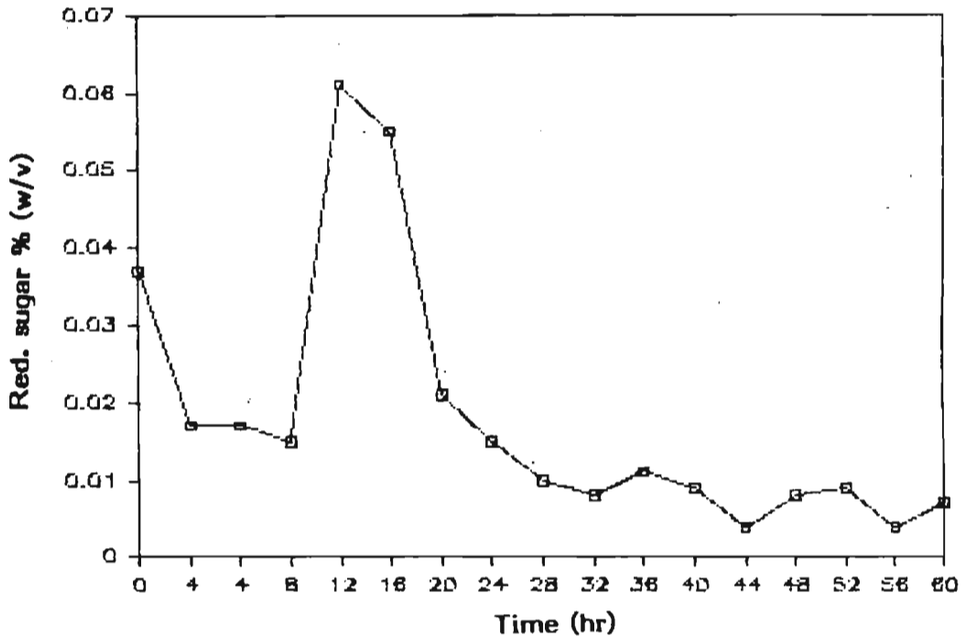


Fig. 3.23 Co-culturing of C. utilis and E. fibuliger in Primary Effluent under monitored conditions

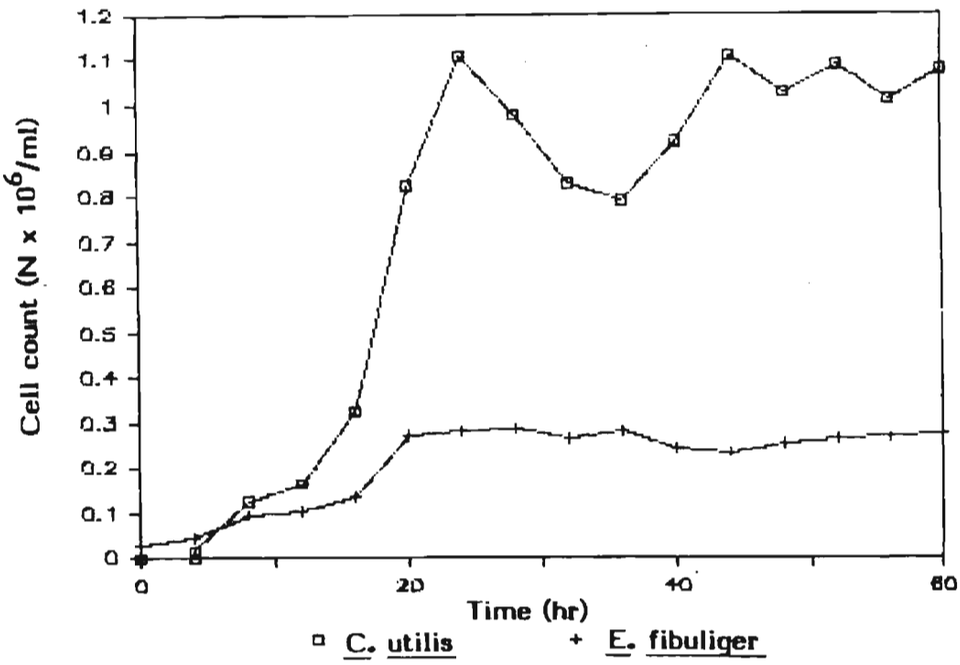


Fig. 3.24 Co-culturing of C. utilis and E. fibuliger in Primary Effluent under monitored conditions

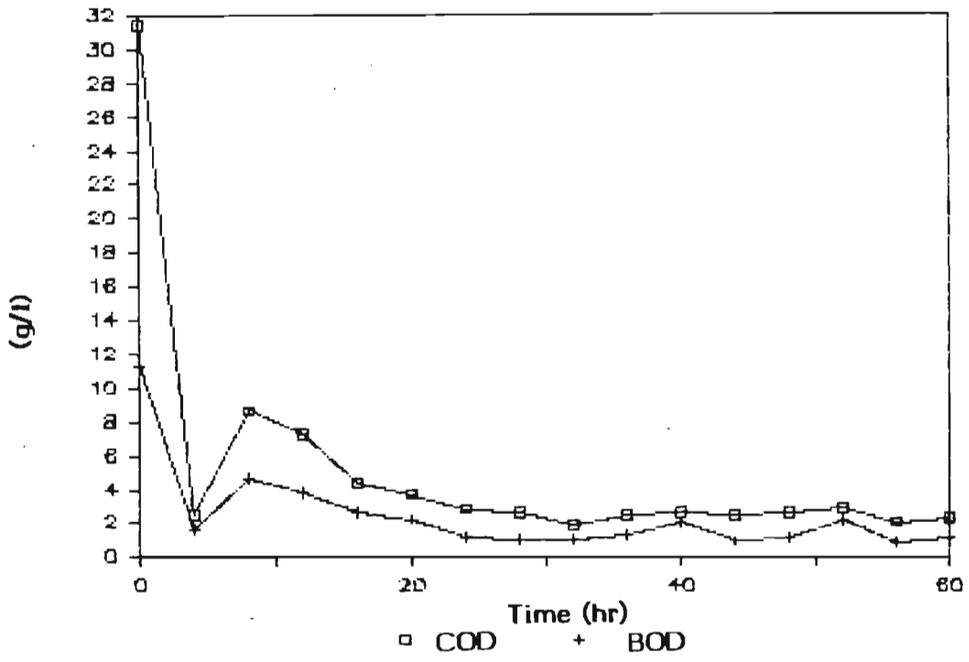


Fig. 4.1 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger

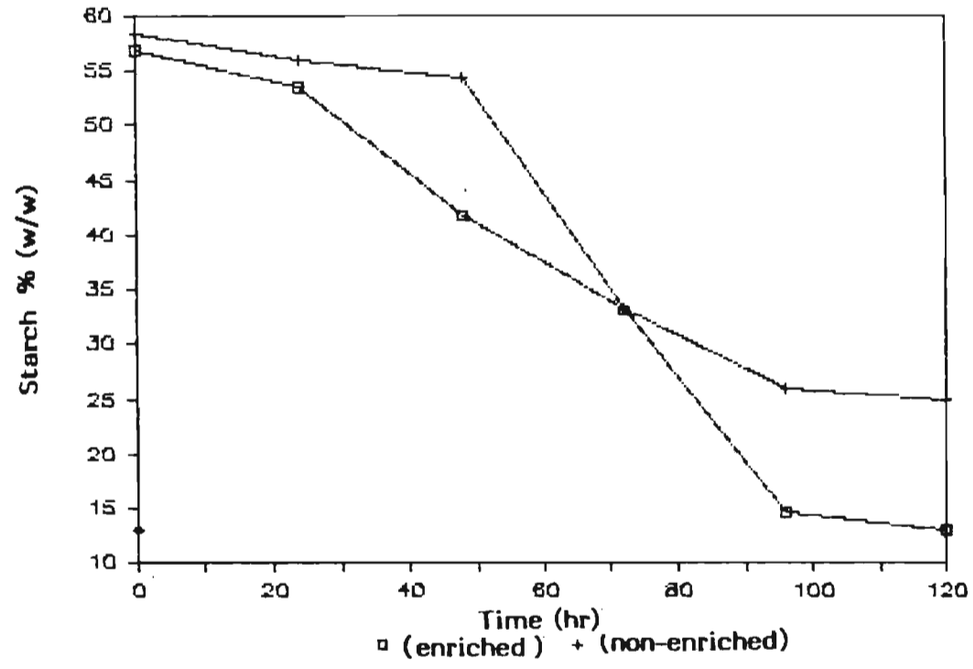


Fig. 4.2 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger

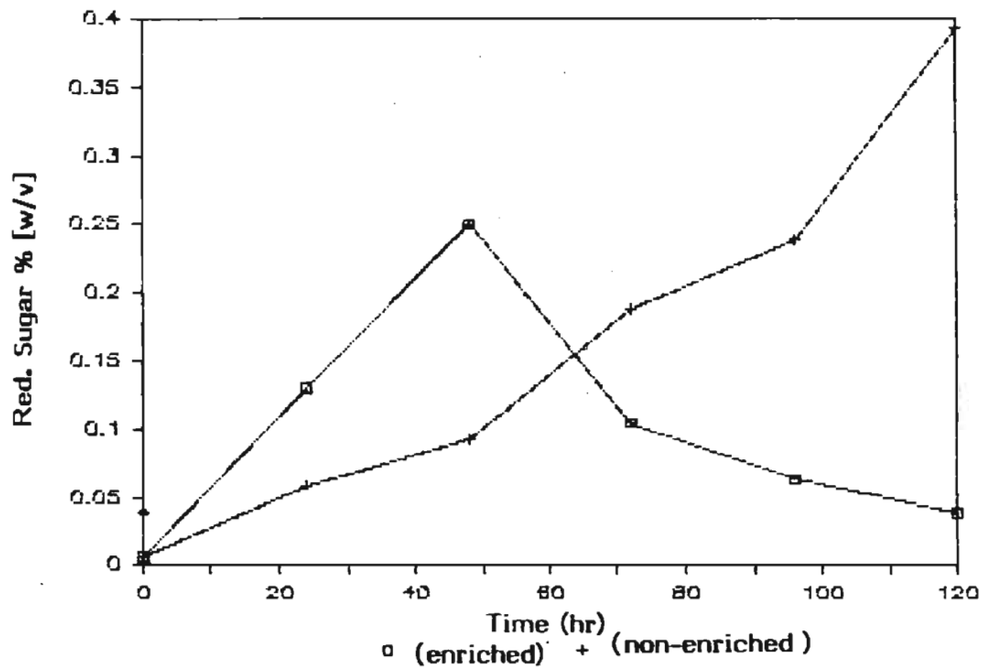


Fig. 4.3 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger

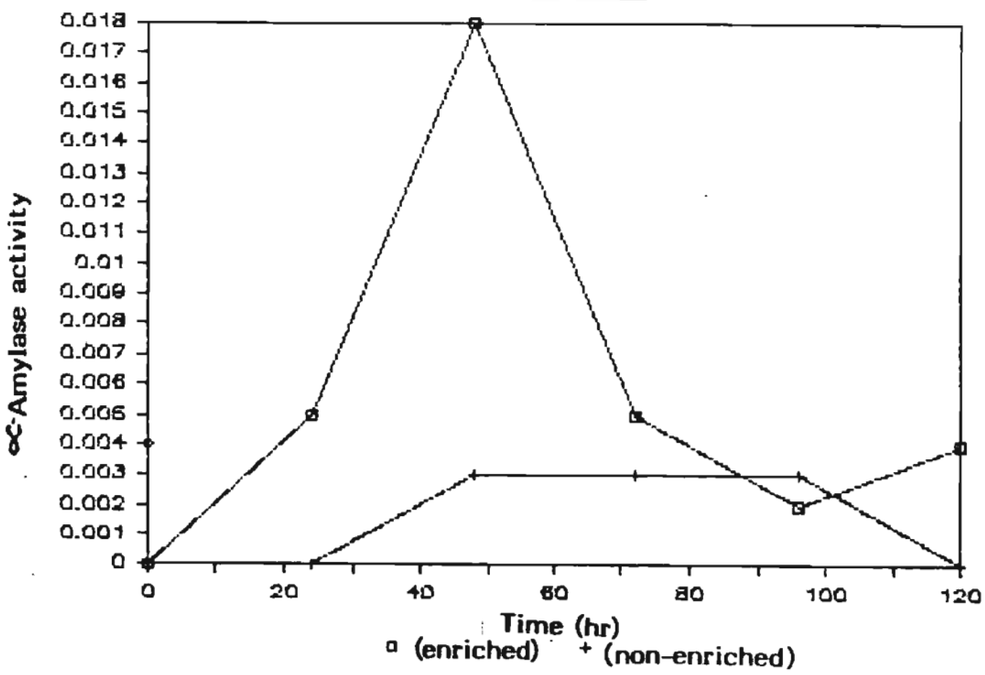


Fig. 4.4 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger

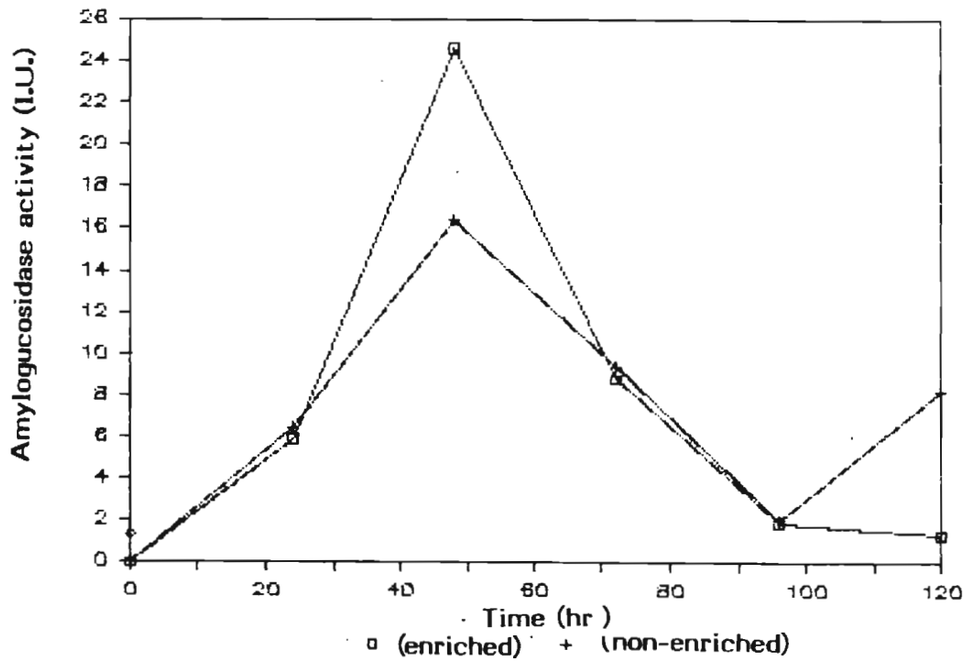


Fig. 4.5 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger

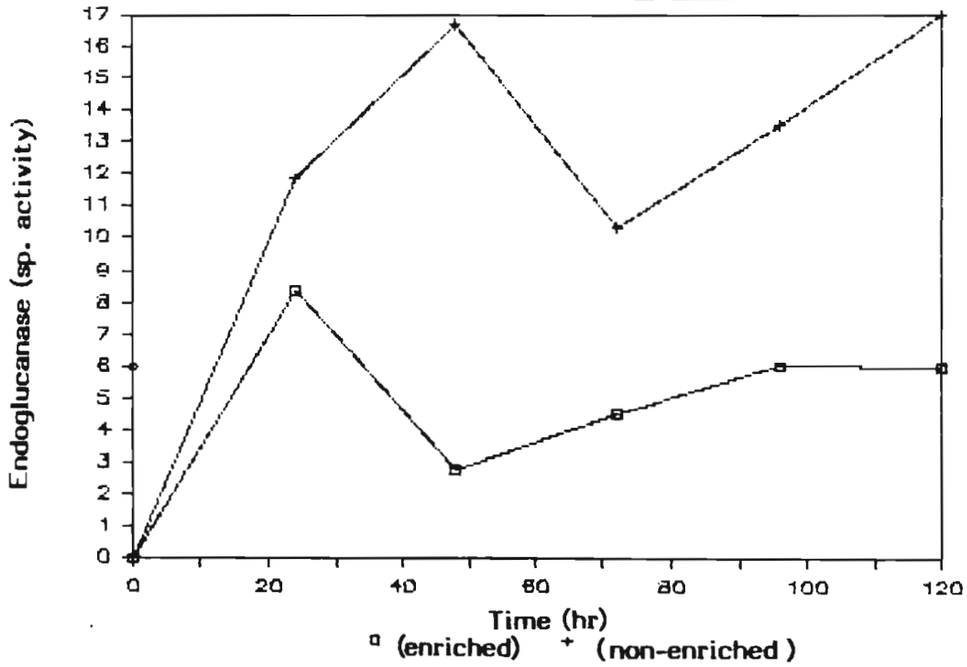


Fig. 4.6 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger

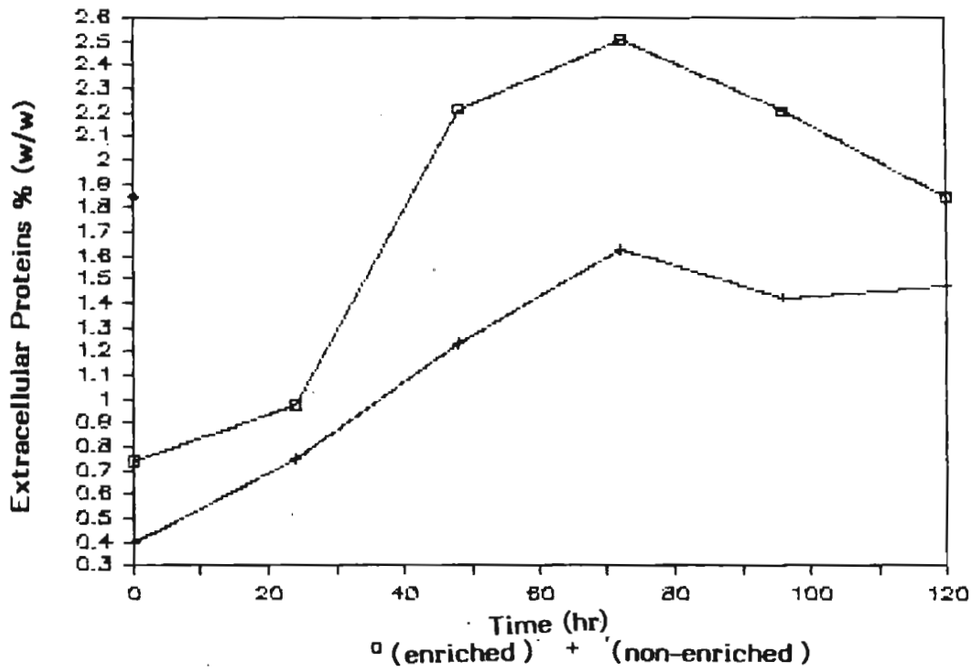
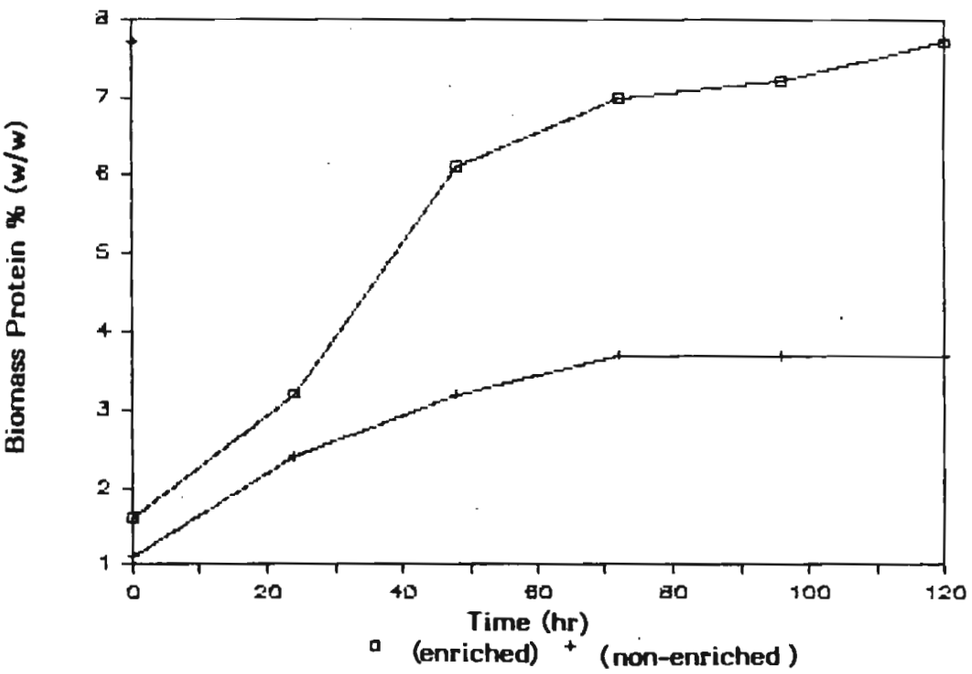


Fig. 4.7 Fermentation of enriched and non-enriched cassava starch factory waste with A. niger



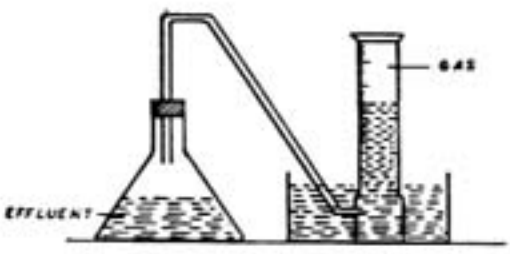


Fig. 5A Batch digester for cassava starch factory effluent (Capacity 1 litre)

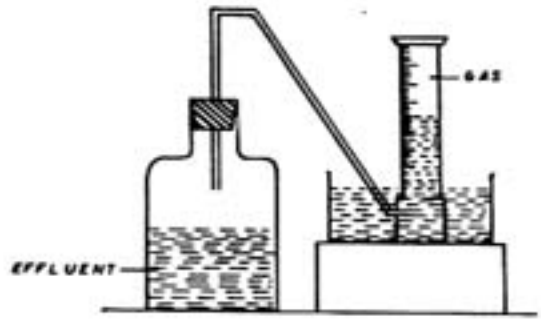


Fig. 5B Batch digester for cassava starch factory effluent (Capacity 7 litres)

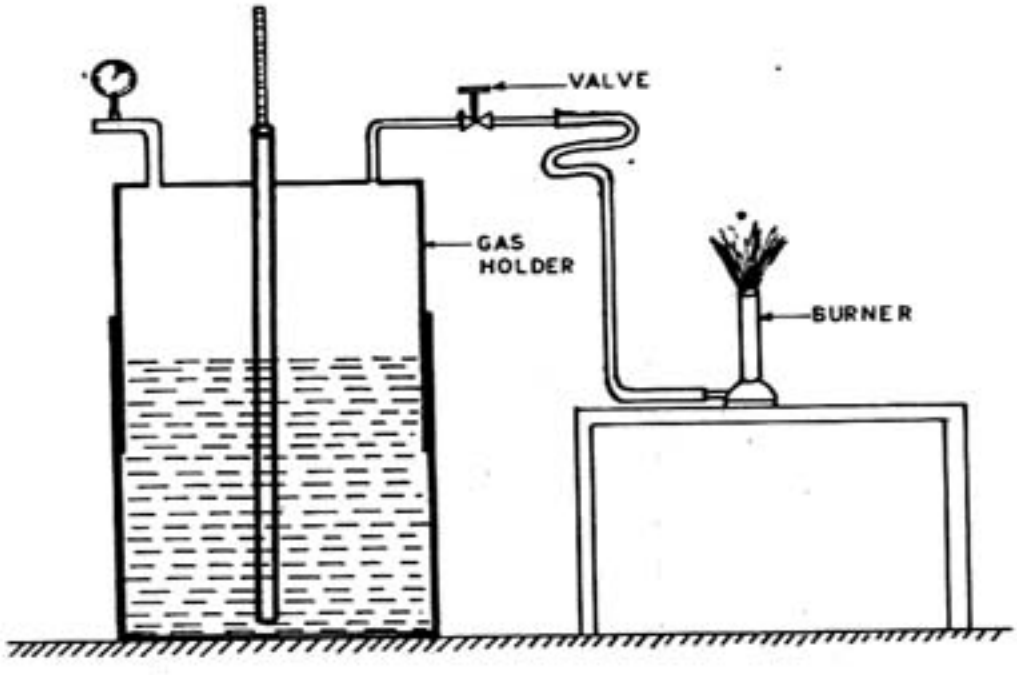


Fig. 5C Digester for biogasification of Cassava starch factory effluent (Capacity 200 litres)

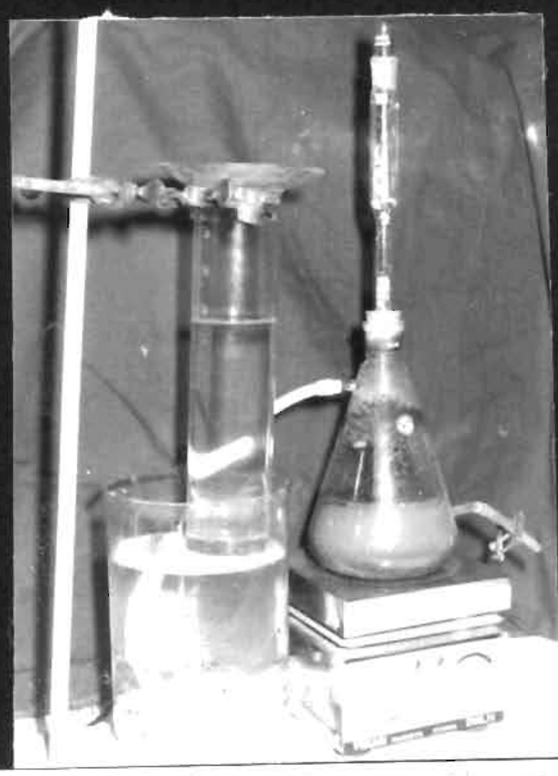


Fig. 50 Semi-continuous digester

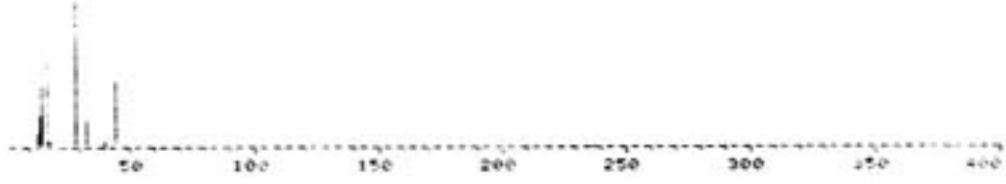


Fig. 5.1 MS of Methane and Carbon dioxide

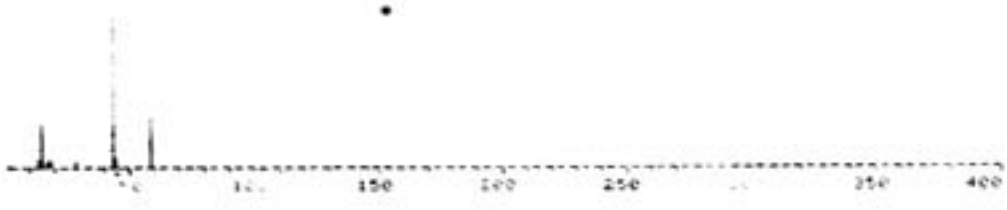


Fig. 5.2 MS of Butane

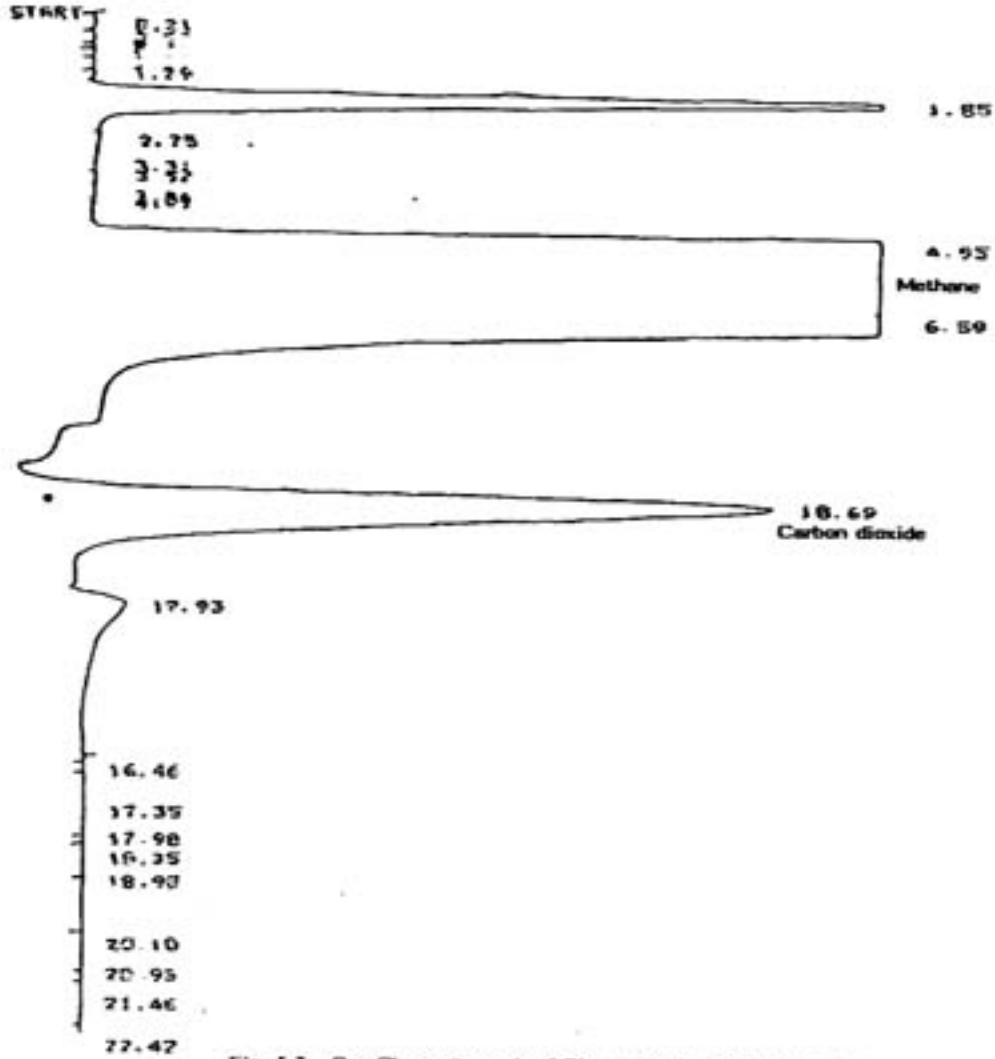


Fig. 5.3 Gas Chromatograph of Biogas obtained from Cassava Starch Factory Effluent Digester

Fig. 5.4 Biomethanation of cassava starch factory effluent in batch digesters

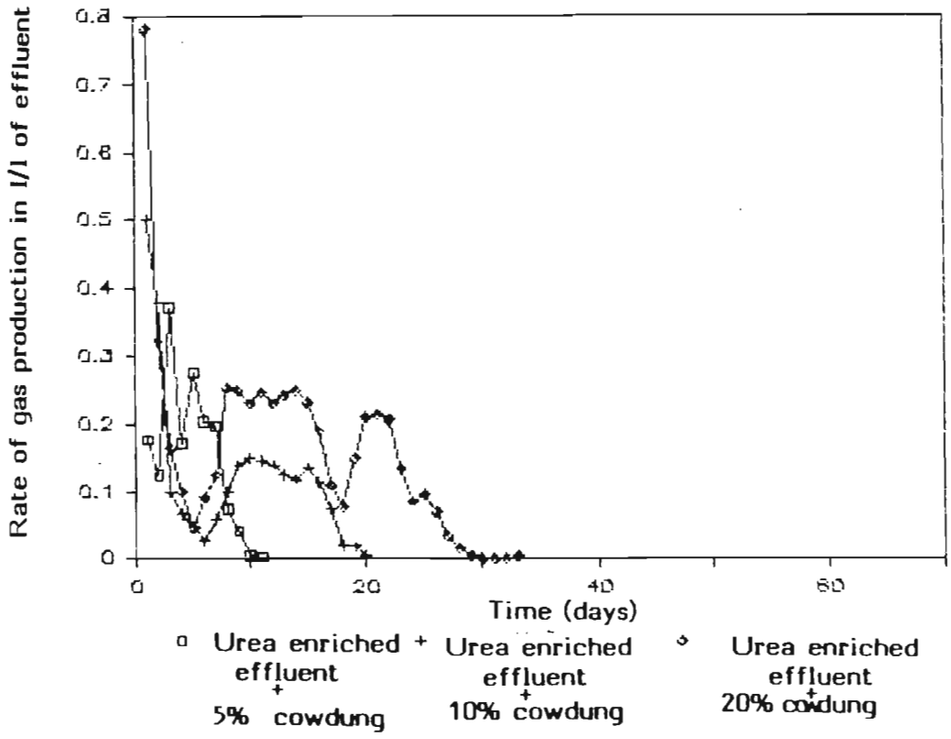


Fig. 5.5 Biomethanation of cassava starch factory effluent in batch digesters

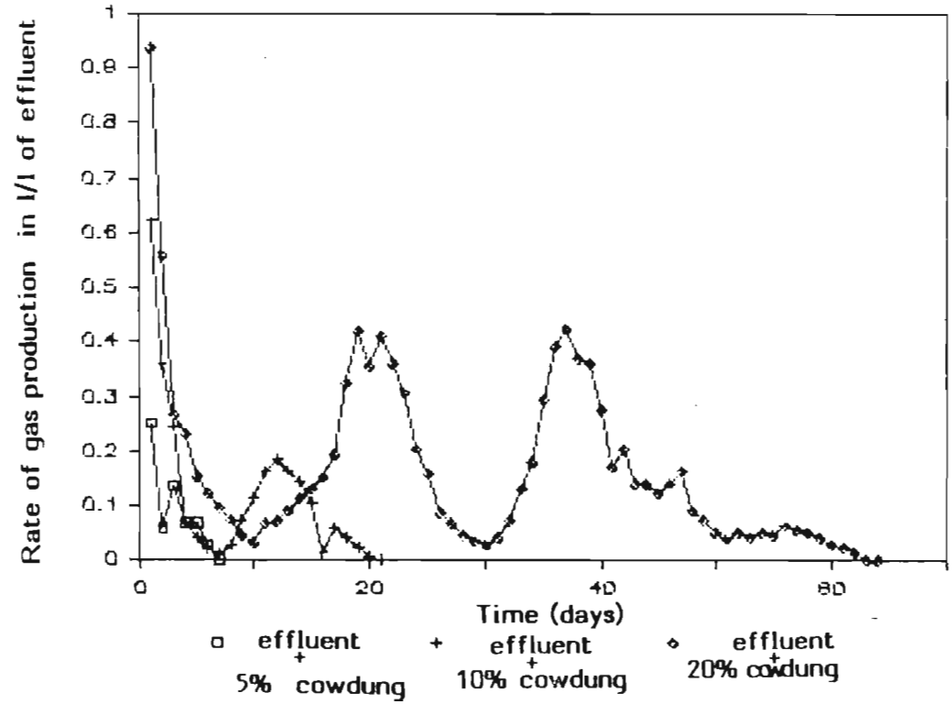


Fig. 5.6 Biomethanation of cassava starch factory effluent in batch digesters

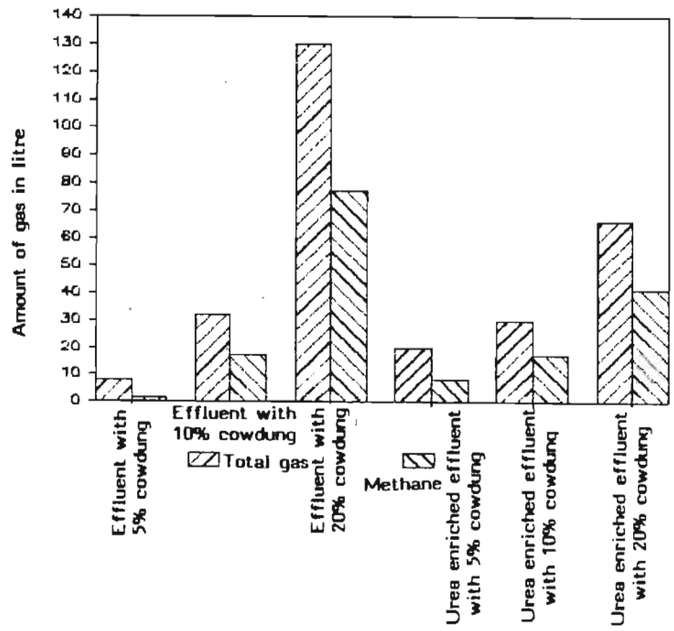
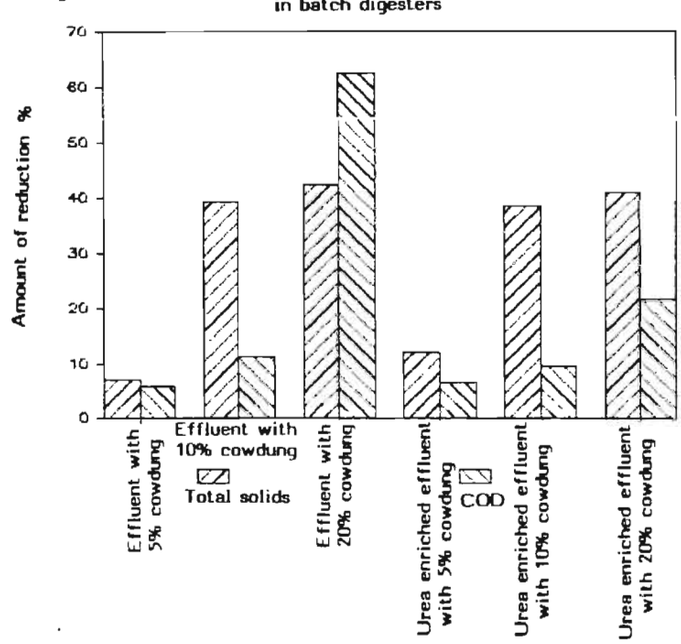
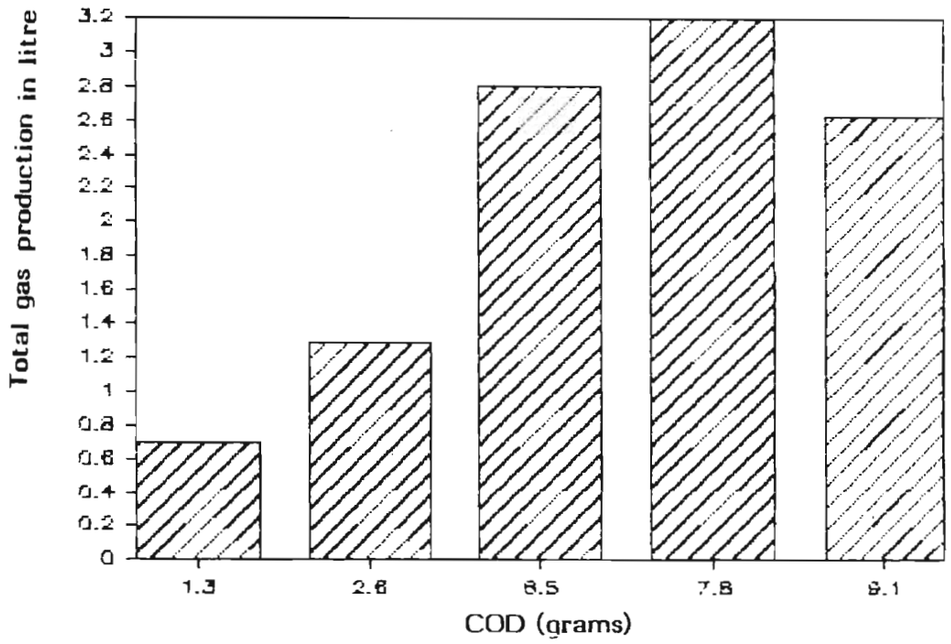


Fig. 5.7 Biomethanation of cassava starch factory effluent in batch digesters



effluent

Fig. 5.8 Biomethanation of cassava starch factory in semi-continuous digesters



effluent

Fig. 5.9 Biomethanation of cassava starch factory in semi-continuous digesters

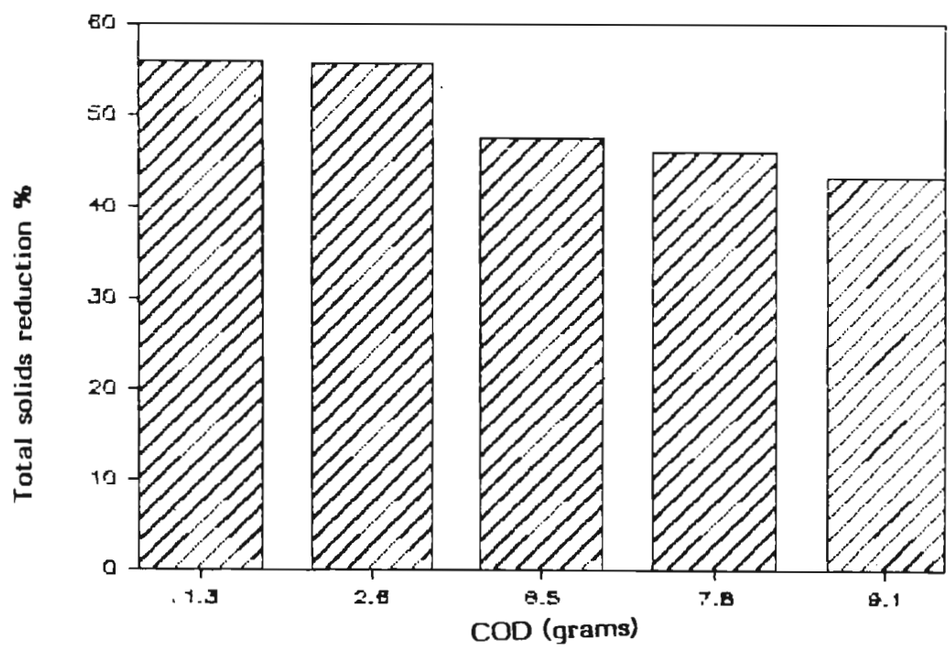


Table 1 PHYSICO-CHEMICAL CHARACTERISTICS OF CASSAVA STARCH FACTORY EFFLUENTS

	EFFLUENTS OF LARGE SCALE STARCH FACTORY							
	PRIMARY EFFLUENTS				SECONDARY EFFLUENTS			
	1	2	3	4	1	2	3	4
pH(+OR-0.2)	4.5	4.6	4.5	4.7	4.5	4.6	4.7	4.7
TOTAL SOLIDS (g/l)	35.64	33.2	40	42	3.9	3.2	5.5	9.6
SUSPENDED SOLIDS (g/l)	33.25	31.2	35.1	37	1.234	.978	2.26	4.078
HYDROLYSABLE SUGAR AS GLUCOSE (g/l)	22.99	22.61	24.61	29.28	1.46	1.12	1.92	2.761
FREE REDUCING SUGAR AS GLUCOSE (g/l)	.525	.425	.78	1.85	.978	.735	1.347	2.06
TOTAL NITROGEN (g/l)	.108	.097	.013	.182	.07	.065	.077	.086
COD (g/l)	40.61	38.22	45.64	48.82	4.75	3.8	6.03	12.05
BOD (g/l)	13.6	13.2	14.05	14.3	4.2	3.6	6	7.05

1,2,3,4 - Samples collected at different timings

Table 2

PHYSICO-CHEMICAL CHARACTERISTICS OF CASSAVA STARCH FACTORY EFFLUENTS

SECONDARY EFFLUENTS OF SMALL-SCALE STARCH FACTORY			
	1	2	3
pH(+OR-0.2)	4.5	4.6	4.7
TOTAL SOLIDS (g/l)	4	5.2	6.6
SUSPENDED SOLIDS (g/l)	1.87	2.32	2.96
HYDROLYSABLE SUGAR AS GLUCOSE (g/l)	1.59	2.34	3.02
FREE REDUCING SUGAR AS GLUCOSE (g/l)	.64	.97	2.075
TOTAL NITROGEN (g/l)	.065	.068	.074
COD (g/l)	4.87	6.8	9.6
BOD (g/l)	3.87	4.76	6.02

1,2,3- samples collected at different timings

Table 3
MICROBIAL LOAD OF THE CASSAVA STARCH FACTORY EFFLUENTS

PRIMARY EFFLUENTS- LARGE SCALE STARCH FACTORY

SAMPLES	BACTERIAL COUNT (NX10^5 /ml)	ACTINO MYCETES COUNT (NX10^3 /ml)	MOULDS COUNT (NX10^5 /ml)	YEAST COUNT (NX10^3 /ml)
1	5	3	20	1.2
2	10	10	17.5	14.73
3	.7	.9	3.6	10
4	3.45	1	20	3.1

1,2,3,4-Samples collected at different timings

Table 4
MICROBIAL LOAD OF THE CASSAVA STARCH FACTORY EFFLUENTS

SECONDARY EFFLUENTS-LARGE SCALE STARCH FACTORY

SAMPLES	BACTERIAL COUNT (NX10^5 /ml)	ACTINO MYCETES COUNT (NX10^3 /ml)	MOULDS COUNT (NX10^5 /ml)	YEAST COUNT (NX10^3 /ml)
1	199.5	2	1.7	28.88
2	400	3	1.6	13.13
3	365	1.7	2.7	30
4	273.88	1.56	2.5	19.08

1,2,3,4-Samples collected at different timings

Table 5
MICROBIAL LOAD OF THE CASSAVA STARCH FACTORY EFFLUENTS

SECONDARY EFFLUENT-SMALL SCALE STARCH FACTORY

SAMPLES	BACTERIA COUNT (NX10^5 /ml)	ACTINO MYCETES COUNT (NX10^3 /ml)	MOULDS (NX10^5 /ml)	YEASTS (NX10^3 /ml)
1	10.5	2	15	35
2	15.45	1.5	4	26.3
3	5.5	2	29	12
4	12.3	1.2	33	29

1,2,3,4-samples collected at different timings

Table 6 Morphological grouping of bacteria, yeasts & fungi

Microorganisms	Primary (large-scale)	Secondary (large-scale)	Secondary (small-scale)
Bacteria			
Gram-positive cocci	-	+	-
Gram-positive rods	+	+	+
Gram-negative coccobacilli	+	+	+
Gram-negative rods	-	+	+
Spore formers	+	+	+
Non-spore formers	+	+	+
Fungi			
Aspergillus	+	+	+
Penicillium	+	+	+
Monocillium	-	+	-
Humicola	+	-	-
Unidentified	+	+	+
Yeasts			
Candida valida	+	+	+
Geotrichum candidum	+	+	+
Unidentified	+	+	+

+ Present - Absent

Table 7
BIOCHEMICAL CHANGES OF CASSAVA STARCH FACTORY

PRIMARY EFFLUENT AT AMBIENT CONDITIONS

INCUBATN TIME	REDUCING SUGARS (%W/V)	AMYLASE ACTIVITY (Sp.activity)	COD (g/litre)	BOD (g/litre)	pH (+/-.2)
1	.036	0.00	14.71	37.20	4.40
2	.015	.46			3.90
3	.013	.86			3.85
4	.011	.96			3.70
5	.01	.76			3.50
6	.009	.42			3.30
7	.01	.33			3.20
8	.009	.42			3.20
9	.007	.45			3.00
10	.008	.30	15.36	35.30	3.00

Table 8
BIOCHEMICAL CHANGES OF CASSAVA STARCH FACTORY

SECONDARY EFFLUENT AT AMBIENT CONDITIONS

INCUBATN TIME (DAY)	REDUCING SUGARS (%W/V)	ANYLASE ACTIVITY (sp.activity)	COD (g/litre)	BOD (g/litre)	pH (+/-.2)
1	.031	0	3.95	3.57	4.5
2	.016	.42			4.2
3	.012	.73			3.8
4	.01	.45			3.7
5	.017	.95			3.7
6	.011	.35			3.6
7	.005	.52			3.5
8	.005	.62			3.6
9	.004	.35			3.7
10	.004	.30	2.58	1.94	3.7

Table 9 EFFECT OF GRAVITY SETTLING ON SECONDARY EFFLUENT

	Solids (% w/v)	BOD (g/lit.)	COD (g/lit.)
Initial	0.46	3.80	4.26
After settling	0.27	1.96	2.15

Table 10
MICROBIAL PROLIFERATION IN THE C.S.F.PRIMARY EFFLUENT UNDER AMBIENT CONDITIONS

INCUBATN TIME (DAY)	BACTERIAL COUNT (NX10^6 /ml)	YEASTS COUNT (NX10^6 /ml)	MOULDS COUNT (NX10^6 /ml)
1	.09	.60	.01
2	.12	4.00	.30
3	.90	30.00	2.10
4	9.10	39.40	4.60
5	24.30	54.40	8.70
6	97.80	82.00	18.55
7	296.60	100.10	10.20
8	400.00	93.64	15.00
9	600.05	97.00	10.80
10	495.60	99.80	18.00

Table 11
MICROBIAL PROLIFERATION IN THE C.S.F.
SECONDARY EFFLUENT UNDER AMBIENT CONDITIONS

INCUBTN TIME (DAY)	BACTERIAL COUNT (NX10^6 /ml)	YEASTS COUNT (NX10^6 /ml)	MOULDS COUNT (NX10^6 /ml)
1	30.08	2.40	.04
2	30.00	4.50	.30
3	40.50	40.00	.39
4	65.00	110.00	2.02
5	75.00	120.50	4.00
6	30.50	180.00	3.96
7	230.00	299.98	2.30
8	270.00	491.79	1.00
9	442.36	339.80	2.00
10	500.00	310.00	1.82

Table 12
PERFORMANCE OF C.valida IN THE C.S.F. PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	2.23	.016	21.2	.065
24	2.19	.006	30	.096
48	2.13	.008	50.7	.105
72	2.11	.006	42.3	.102
96	2.08	.006	36.2	.088
120	2.04	.006	40.88	.095

Table 13
PERFORMANCE OF G. candidum IN THE C.S.F.PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	2.34	.03	54.35	.13
24	2.23	.01	81.10	.17
48	2.13	.01	95.38	.17
72	2.05	.01	101.00	.18
96	1.94	.01	97.00	.17
120		.01	101.25	.17

Table 14
PERFORMANCE OF C. utilis IN THE C.S.F.PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	2.3	.032	13.96	.043
24	2.25	.009	33.29	.084
48	2.25	.008	59.9	.103
72	2.25	.008	64.58	.107
96	2.23	.008	53.24	.1
120	2.22	.007	54.6	.101

Table 15
PERFORMANCE OF E.fibuliger IN THE C.S.F. PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	2.38	.013	.55	.03
24	1.24	.052	2.03	.04
48	.95	.075	16.50	.06
72	.62	.061	60.00	.13
96	.51	.038	84.54	.17
120	.45	.032	108.62	.18

Table 16

PERFORMANCE OF *E. magnusi* IN THE C.S.F. PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	2.74	.006	.40	.02
24	1.97	.035	6.52	.03
48	1.69	.071	8.30	.05
72	1.48	.057	44.25	.12
96	.96	.033	80.00	.15
120	.92	.021	89.94	.16

Table 17

PERFORMANCE OF *C.utilis* IN THE C.S.F.SECONDARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	.19	.025	27.36	.088
24	.18	.015	320.06	.206
48	.18	.011	341.25	.208
72	.18	.008	375.00	.213

Table 18

PERFORMANCE OF *C.utilis* IN THE C.S.F.SECONDARY EFFLUENT(Non-enriched)

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
0	.19	.03	27.5	.088	6.55
24	.18	.02	230	.182	3.16
48	.18	.015	272.88	.187	2.85
72	.18	.012	255	.191	2.55

Table 19
PERFORMANCE OF E.fibuliger IN THE C.S.F.SECONDARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
0	.19	.025	38.37	.096
24	.13	.015	345	.177
48	.1	.011	375.72	.182
72	.107	.008	369.55	.193

Table 20
PERFORMANCE OF E.fibuliger IN THE C.S.F.SECONDARY EFFLUENT(Non-enriched)

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
0	.19	.035	38.42	.096	6.54
24	.14	.023	112.2	.141	3.41
48	.12	.019	335	.176	2.85
72	.12	.01	290	.175	2.54

Table 21
CO-CULTURING OF C.utilis and E.fibuliger IN THE C.S.F.PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml) C.utilis	CELL NO (NX10^6 /ml) E.fibuliger	BIOMASS PROTEIN (%w/v)	COD (g/l)	BOD (g/l)	pH (+/-.2)
0	3.23	.05	39.54	37.01	.13	68.29	20.82	4.80
24	2.06	.166	76.66	48.05	.203	9.46	7.35	4.10
48	1.82	.58	219.80	127.00	.246	12.20	11.03	3.10
72	1.76	.204	206.50	122.50	.271	10.28	7.01	3.0
96	1.66	.255	288.00	132.00	.295	7.42	5.47	3.45
120	1.53	.168	281.08	118.75	.295	6.71	5.12	3.20

Table 22
CO-CULTURING OF C.utilis and E.magnusi IN THE C.S.F.PRIMARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml) C.utilis	CELL NO (NX10^6 /ml) E.magnusi	BIOMASS PROTEIN (%w/v)	COD (g/l)	BOD (g/l)	pH (+/-.2)
0	3.25	.051	39.95	.59	.061	68.35	20.15	4.80
24	3.20	.018	82.25	.75	.093	3.86	3.64	4.55
48	3.18	.008	112.53	.83	.122	3.92	4.25	4.15
72	3.16	.008	95.50	.90	.113	4.04	3.86	4.50
96	2.65	.145	133.74	6.69	.134	9.25	8.40	3.90
120	1.66	.811	85.40	8.67	.11	14.45	12.20	3.35

Table 23
CO-CULTURING OF C.utilis AND E.fibuliger IN THE C.S.F.SECONDARY EFFLUENT

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
0	.20	.047	69.36	.15	6.64
24	.14	.024	365.00	.26	2.45
48	.12	.011	358.00	.27	3.22
72	.10	.008	360.03	.27	2.35

Table 24
CO-CULTURING OF C.utilis AND E.fibuliger IN THE C.S.F.SECONDARY EFFLUENT (Non-enriched)

TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
0	.2	.023	70.68	.148	6.44
24	.19	.028	320	.21	3.58
48	.15	.028	375	.218	3.53
72	.14	.015	260.5	.237	3.74

Table 25
CO-CULTURING OF C.utilis AND E.fibuliger IN THE C.S.F.PRIMARY EFFLUENT UNDER CONTROLLED CONDITIONS

TIME (HR)	STARCH (IW/U)	RED.SUGAR (IW/U)	COD (g/litre)	BOD (g/litre)	E.f CELL COUNT (NX10^6 /ml)	C.u CELL COUNT (NX10^6 /ml)	BIOMASS PROTEIN (Iw/u)	BIOMASS PROTEIN (Iw/ml
00							.0144	.51
0	1.92	.037	31.42	11.25	28.25		.0876	3.13
4	1.83	.035	2.52	1.6	46.25		.1555	5.55
04	1.83	.017	2.52	1.6	46.25	16.25	.2134	7.62
8	1.5	.015	8.63	4.7	96.75	127.25	.2737	10.33
12	.86	.061	7.25	3.8	105.62	166.37	.313	11.81
16	.46	.055	4.39	2.7	137.5	324	.4247	16.20
20	.45	.021	3.72	2.28	270.62	822.62	.4619	18.85
24	.44	.015	2.63	1.22	281	976.87	.53	21.63
28	.4	.01	2.63	1.06	288	976.87	.5407	22.07
32	.38	.008	1.95	1.08	266.75	828	.521	22.00
36	.38	.008	2.55	1.32	283.12	789.25	.5134	21.95
40	.37	.011	2.63	2.03	245.62	918.5	.5109	21.51
44	.34	.009	2.55	1	236.87	1106	.503	21.86
48	.32	.004	2.7	1.26	254.37	1027.5	.4904	21.92
52	.31	.008	2.9	2.25	265	1085.37	.5274	21.85
56	.31	.009	2.14	.95	269.37	1012.75	.4999	21.78
60	.3	.004	2.32	1.18	278.5	1075.75	.515	22.02

Table 26
BIO CHEMICAL CHANGES IN THE ENRICHED-CASSAVA STARCH FACTORY WASTES
DURING THE GROWTH OF *Aspergillus niger*

INCUBATN TIME (HR)	STARCH AS GLU. (ZW/V)	RED.SUGAR AS GLUCOS (ZW/V)	AMYLO GLU.ACT (I.V)	ENDOGLU. ACTIVITY (Sp.activity	EXTRA CELLULAR (ZW/V)	BIOMASS PROTEIN (ZW/V)	TOTAL AMYLASE	pH +/-0.2
0	56.85	.006	0.00	0.00	.742	1.6	0	5.4
24	53.5	.13	5.9	8.35	.975	3.2	.005	4.5
48	41.85	.252	24.62	2.8	2.211	6.1	.018	3.1
72	33.15	.104	8.77	4.53	2.505	7	.005	3.1
96	14.55	.063	1.88	6.04	2.202	7.2	.002	3
120	13.05	.039	1.3	6	1.844	7.7	.004	3

Table 27
BIO CHEMICAL CHANGES IN THE NON-ENRICHED CASSAVA STARCH
FACTORY WASTES DURING THE GROWTH OF *Aspergillus niger*

INCUBATN TIME (hr)	STARCH AS GLUCOSE (Zw/w)	RED.SUGAR AS GLUCOSE (Zw/w)	AMYLO GLUCOSIDASE ACTIVITY(I.V)	ENDOGLU ACTIVITY	EXTRA CELLULAR PROTEIN (Zw/w)	BIOMASS PROTEIN (Zw/w)	TOTAL AMYLASE	pH (+OR-0.2)
0	58.4	.006	0.00	0.00	.395	1.1	0	5.4
24	56	.059	6.51	11.84	.749	2.4	0	3.1
48	54.45	.093	16.45	16.67	1.234	3.2	.003	2.9
72	33.3	.188	9.4	10.33	1.627	3.7	.003	2.85
96	26.1	.238	2.05	13.56	1.426	3.68	.003	2.85
120	25	.393	8.19	17	1.468	3.7	0	2.85

Table 28

BIOMETHENATION OF CASSAVA STARCH FACTORY EFFLUENTS (C.S.F.E) IN BATCH DIGESTERS

FEEDING CONDITIONS	SPAN OF GAS PRODUCTN (DAYS)	GAS PRODUCED (l/kg dry matter)	AVERAGE METHANE CONTENT %	INITIAL TOTAL SOLIDS %	FINAL TOTAL SOLIDS %	TOTAL SOLIDS REDN %	INITIAL COD (g/l)	FINAL COD (g/l)	COD REDN (g/l)
C.S.F.E+5% COWDUNG+UREA	10	20	42	8.22	7.22	12.17	65.12	60.96	6.39
C.S.F.E+5% COWDUNG WITHOUT UREA	6	7.58	20	8.22	7.64	7.06	64.8	61.12	5.68
C.S.F.E+10% COWDUNG+UREA	20	30	58	8.22	5.02	38.93	64	57.92	9.5
C.S.F.E+10% COWDUNG WITHOUT UREA	21	31.46	55	8.22	4.98	39.42	63.2	56.16	11.14
C.S.F.E+20% COWDUNG+UREA	34	66.18	62	8.22	4.83	41.24	65.6	51.2	21.95
C.S.F.E+20% COWDUNG	68	130.2	59	8.22	4.72	42.58	64	24	62.5
COWDUNG		75.75							
COWDUNG+UREA		59.64							

Table 29 Digestion of cassava starch factory effluents in a semi-continuous digester

Incubation time (hr)	Gas out put (l/l of reactor volume)	pH (± 0.2)	Total solids reduction (%)
24	0.26	8.70	28.00
48	0.28	8.60	35.90
72	0.14	8.60	48.20
96	0.06	8.58	56.00
120	0.02	8.60	56.70
144	0.01	8.62	58.10

Feeding rate of 1,300 mg COD per 144 hrs

Table 30 Digestion of cassava starch factory effluents in a semi-continuous digester

Incubation time (hr)	Gas out put (l/l of reactor volume)	pH (± 0.2)	Total solids reduction (%)
24	0.40	8.45	30.10
48	0.32	8.43	38.40
72	0.40	8.40	49.00
96	0.16	8.38	55.60

Feeding rate of 2,600 mg COD per 96 hrs

Table 31 Digestion of cassava starch factory effluents in a semi-continuous digester

Incubation time (hr)	Gas out put (l/l of reactor volume	pH (± 0.2)	Total solids reduction (%)
24	1.00	7.67	15.00
48	1.00	8.16	24.00
72	0.46	8.18	36.50
96	0.44	8.33	47.50
120	0.12	8.35	51.20
144	0.04	8.30	54.00

Feeding rate of 6,500 mg COD per 144 hrs

Table 32 Digestion of cassava starch factory effluents in a semi-continuous digester

Incubation time (hr)	Gas out put (l/l of reactor volume	pH (± 0.2)	Total solids reduction (%)
24	1.08	6.83	12.80
48	1.16	7.13	22.20
72	0.46	7.20	34.70
96	0.50	7.60	46.00

Feeding rate of 7,800 mg COD per 96 hrs

Table 33 Digestion of cassava starch factory effluents in a semi-continuous digester

Incubation time (hr)	Gas out out (l/l of reactor volume	pH (± 0.2)	Total solids reduction (%)
24	0.90	5.92	11.50
48	0.72	5.84	16.30
72	0.61	6.00	32.60
120	0.40	6.20	43.20

Feeding rate of 9,100 mg COD per 96 hrs

CHAPTER 5

DISCUSSION

5. DISCUSSION

Discharge of effluents from cassava starch factories will lead to perpetual pollution of the environment unless effective methods are developed. The conventional methods of organic waste treatments are energy intensive and hence economically unattractive. There are many approaches to reclaim energy from waste materials. The present approach is a study of how the energy conserved in the organic waste is transformed into valuable protein and methane by micro-organisms. Initially, experiments were carried out to understand the physico-chemical nature of primary and seconday effluents from a large-scale starch factory (Figs. 2.1A and 2.1B) and secondary effluent from a small-scale starch factory since these are the major kinds of effluents constantly polluting the environment (Figs. 2A, 2B, 2C and 2D). Observations were made to assess the rate of degradation of the organic matter under ambient conditions. Studies were further undertaken to convert the effluents into microbial biomass and methane. The results obtained after systematic study of the effluents and its aerobic and anaerobic treatments are discussed in the present chapter.

5.i. Physico-Chemical and Microbiological Characteristics of Cassava Starch Factory Effluents

Analytical data shown in Table 1 and 2 of the cassava starch factory effluents indicate the presence of high pollu-

tion load (COD and BOD). The large amount of starch (more than 50 percent of the total solids) shows the possibility of its degradation/utilization with amylolytic micro-organisms. Subramanyan et al. (1956) have also reported the presence of 56% (w/w) starch in the dried waste of tapioca starch factory. Free reducing sugar present in all primary and secondary effluents of a small-scale starch factory can serve as the immediate carbon source for micro-organisms to favour their growth in the effluents during the course of discharge. The content of reducing sugar in these effluents analysed ranged from 0.043 to 0.208% (w/v). This is considerably lower than the values reported by Thanh and Wu (1975) who found 0.08 - 0.4% (w/v) reducing sugar in the effluents of S. R. Tapioca Ltd., Thailand. Extremely low content of nitrogen observed in both primary and secondary effluents indicates the necessity of enrichment during the microbial treatment. The effluents were also found to be acidic. The presence of prussic acid (hydrocyanic acid) released during the process of extraction of starch has been reported (Knight, 1969). The test for hydrocyanic acid in the fresh samples of effluents using sodium picrate indicator paper was positive. Remarkable variations in the characteristics of the effluent samples at different times of collection were also observed in both primary and secondary effluents of large-scale and secondary effluent of small-scale starch factories.

The tables 3, 4 and 5 on the microbial characteristics reveal that the effluents harboured a heavy load of bacteria,

yeasts, moulds and actinomycetes right from the beginning of its discharge (Manilal et al., 1983). In the secondary effluent, yeast population (13.13 x 10^3-30.0 x 10^3/ml in large-scale and 12 x 10^3-35 x 10^3/ml in small-scale factory effluents) and bacterial population (199.5 x 10^5-400 x 10^5/ml in large-scale and 5.5 x 10^5-15.45 x 10^5/ml in small-scale factory effluents) were found to be higher when compared to the primary effluent (yeasts 1.2 x 10^3-14.75 x 10^3/ml and bacteria 0.7 x 10^5-10.0 x 10^5/ml). Availability of more free reducing sugar in the secondary effluent and large retention time (more than 24 hrs) in the settling tanks might have promoted the growth of yeasts and bacteria before discharge to the environment. High coliform bacterial count was observed in all the samples tested ($\geq$ 1600/100 ml). Compared to gram positive, the gram negative bacteria were fewer in the case of all the samples. Both spore forming and non-spore forming bacteria were found (Table 6). During the isolation of microorganisms, various organisms were identified viz. Candida (Fig. 3A) Geotrichum (Fig. 3B) Saccharomyces, Aspergillus, Penicillium, Fusarium, Rhizopus, Monocillium and Humicola in the primary and secondary effluents of cassava starch factory. Candida valida was found to be the predominant yeast in these effluents.

5.ii. Microbial Proliferation and Biochemical Changes in the Cassava Starch Factory Effluents Under Ambient Conditions

Microbiological and biochemical changes of cassava

starch factory effluents were studied at different days of incubation at ambient conditions. The presence of large number of microorganisms in the effluents was discussed earlier. The establishment of heterotrophic organisms has been reported in the agricultural and carbohydrate wastes (Loehr, 1974). A gradual increase in the microbial count was observed in the cassava starch factory effluents (Tables 10 and 11 & Fig. 2.4) during incubation at ambient conditions. Presence of assimilable carbohydrates and degradable organic matter in the effluents promoted the growth of microorganisms. Suihko and Dragic (1983) have reported the growth of wide spectrum of microorganisms in water containing the organic matter. The genetic quality of heterotrophs to liberate extracellular enzymes and their ability to subsist on organic compounds have been reported (Hamer et. al., 1985).

In the primary effluent, a decline in the growth of yeasts and moulds appeared by 7th and 8th day respectively. But the maximum number of bacteria was found on the 9th day of incubation (600 x 10^6/ml), and on the 10th day it had declined to 495.6 x 10^6/ml. Normally shifts in the microbial species and activities in waste treatment system occur due to the changes in the environmental conditions such as temperature, pH, available dissolved oxygen, ultimate hydrogen acceptor or available food (Loehr, 1974). Results obtained showed that some of these environmental factors might have profoundly influenced the growth and thereby degradation of organic matter

in the primary effluent. The easily assimilable free reducing sugar was readily utilized and it had reduced to a negligible amount by the 8th day (Table 7 and Fig. 2.6). Depletion of free reducing sugar perhaps favours the hydrolysis of starch and cellulose present in the effluents. The absence of cellulase activity till the end of incubation precluded the possibility of cellulose breakdown to glucose. In the primary effluent, the amylase activity slowly increased from the beginning of the first day to 0.96 (specific activity) on the 4th day, and thereafter the activity decreased (Fig. 2.5). This again showed that in the primary effluent the production of sugars from starch was diminished after 4th day of incubation. Lack of sufficient nitrogen in the effluent might be another factor retarding the organic matter degradation by the organisms. Rapid utilization of available dissolved oxygen by the large number of heterotrophs and keeping the effluent without aeration and agitation (under ambient conditions) might have easily led to anaerobic conditions for the development of acidity as indicated by the low pH of 3.5 by the 5th day, and the decreasing trend continued thereafter (Fig. 2.7). Under anaerobic conditions, the formation of organic acids such as formic, acetic, propionic and butyric acids, in addition to some alcohols, by the incomplete oxidation of glucose, was reported by Turton et al. (1983) and Hartmanis and Gatenbeck (1984). The lowering of pH of the effluent demonstrated the development of acidity and at the acidic pH range prevailing in the

effluent towards the latter periods, the activities of enzymes like amylase, amyloglucosidase, etc. will be retarded.

The negligible reduction of COD (5%) during 10 days showed the poor degradation of organic matter under ambient conditions. According to Joubert and Britz (1986), the pH itself has a profound influence on the hydrolysis of substrates as evidenced by a reduction in the bacterial activity to the extent of 85% within 3 days at low pH (less than 3.2). There was a slight increase in the BOD (4.4%) of primary effluent during incubation under ambient conditions (Fig. 2.8). This might be due to the accumulation of more dissolved compounds by the metabolic activity of microorganisms.

Significant reduction in the BOD and COD (46% and 35% respectively) was found in the secondary effluent (Table 8) which contained less organic solids during 10 days of incubation in contrast to the primary effluent. Degradation of organics as indicated by the accelerated growth of bacteria and yeasts could be the cause of the reduction in BOD and COD. Appreciable growth of moulds could not be observed in the secondary effluents perhaps because of their slow growing characteristics, and the possible suppression of their growth by the overgrowth of bacteria and yeasts. There was prolonged amylase activity which again supported the increased microbial activity. A similar effluent, tapioca starch separator waste, when subjected to treatment in the facultative ponds after

enrichment (COD:N:P =100:20:1) removed 40% and 47% BOD during 5.52 and 9.25 days respectively (Udin, 1970 and Yothin, 1975).

The slow digestion of the effluents under ambient conditions emphasises the requirement of artificial means of treatments and enrichment of this carbohydrate-rich waste having low nitrogen. Nemerow (1978) has discussed the retardation of microbial growth under inadequate quantity of nutrients such as nitrogen and phosphorus during waste treatment.

The gravity settling experiments of the solids in the secondary effluent were found effective to separate 41.3% total solids, and thereby to cause a reduction of 48.42% BOD and 49.53% COD of the effluent (Table 9 and Fig. 2.3). The figure 2.2 shows the poor settling of solids in the primary effluent of cassava starch factory.

5.iii. Submerged Fermentation of Cassava Starch Factory Effluents with Yeasts and Yeast-like Organisms for the Production of Single-Cell Protein

Utilization of inexpensive carbon sources, especially agro-industrial wastes, for single-cell protein production has been the interest of many investigators recently. Senez (1984) has stressed the obvious importance of protein enrichment of starchy and lignocellulosic wastes for the developing countries. The present study was started with a view to

convert easily decomposable carbohydrates of cassava starch factory effluents into microbial biomass and for the subsequent reduction of pollution load of the effluents. Only yeasts and yeast-like organisms were selected for these studies (Figs. 3A, 3B,3C,3D & 3E) because of their established acceptance in the food and feed industry. Growth pattern of the cultures was studied by measuring free sugars, starch, cell numbers and total biomass protein. Co-culturing of C. utilis and E. fibuliger was found to be efficient in removing COD and BOD with appreciable production of valuable SCP.

The performance of C. valida in the primary effluent showed its ability to utilize the free reducing sugar at a faster rate (Fig. 3.2). During 120 hrs of incubation there was a negligible amount of starch assimilation (0.19%). Generally the starch assimilation of Candida yeast was not significant except in the case of strains like C. tropicalis (Azoulay, et al., 1980). It is evident from the results given in table 12 and fig. 3.4 that there was no biomass protein production or increase in cell numbers of C. valida after 48 hrs. The depletion of easily assimilable carbon sources could be the main reason for the poor growth of the organism.

Results of the fermentation of primary effluent with G. candidum presented in table 13 shows similar pattern of growth as observed in the case of C. valida. In the case of

G. candidum also there was poor assimilation of starch. Cell multiplication ceased when the reducing sugar content depleted, indicating sole dependence of the organism on the reducing sugar. Quinn and Marchant (1979 and 1980) exploited the same quality of G. candidum in the treatment of distillery wastes rich in sugars.

The fermentation of cassava starch factory effluents with C. utilis indicated the rapid uptake of reducing sugar for their growth and multiplication (Table 14). Observation on the effect of enrichment in the secondary effluent (Tables 17 and 18) illustrated that the sugar intake and protein production increased with enrichment, showing the necessity of enrichment to achieve higher protein yield (Figs. 3.6, 3.7 and 3.8). Thanh and Wu (1975) made similar observations on enrichment during the growth of Torula yeast in tapioca waste waters. Cabib et. al. (1983) have also pointed out the importance of enrichment in sugarcane stillage for SCP production. The C. utilis cell multiplication was found declining as soon as the free reducing sugar content decreased eventhough there was enough quantity of organics in the medium (Fig. 3.3). The poor amylolytic activity as evidenced by the unutilized starch has ultimately restricted the growth of C. utilis. Balasubramanya and Bhatawdekar (1981) have also reported the lack of amylolytic activity of C. utilis in their studies on agricultural waste utilization for SCP production.

The amylolytic activity of E. fibuliger has been well documented (Jarl, 1969; Rattakul, 1976; Sales and Menezes, 1976; Lemmel et al., 1979). Amylolytic activity of E. fibuliger was obviously remarkable when it was cultured in the effluents of cassava starch factory (Table 15). In the primary effluent about 81% starch was hydrolysed during 120 hrs of incubation at room temperature (30 ± 2°C). The increase in reducing sugar content at the early stages (48 hr) and subsequent decrease in reducing sugar with diminishing starch content also indicated the hydrolysis of starch by the organism (Fig. 3.1). The decrease in the glucose production after 48 hrs might be due to end product repression as well as the depletion of starch content. End product repression of amylase activity is reported for many other organisms (Saito and Yamamoto, 1975). The slow growth of the organism and subsequent low biomass production are in agreement with the findings of Jarl (1969). Lesser utilization of starch and glucose in the non-enriched secondary effluents when compared with enriched samples emphasised the need of enrichment for better growth of the organism (Tables 19 and 20 and Figs.3.5 and 3.6).

The primary effluent of cassava starch factory was fermented with another yeast-like organism, E. magnusi and the results (Table 16) showed that about 48% of starch was being hydrolysed during 120 hrs of incubation. The highest concentration of reducing sugar in the culture indicated that

maximum amylase activity could be at 48 hr (Fig. 3.2). E. magnusi accumulated 0.135% biomass protein at 120 hr of incubation, whereas E. fibuliger could accumulate 0.153% protein.

In the present study of fermentation of cassava starch factory effluents, co-culturing techniques of C. utilis and E. fibuliger were used for the production of biomass. Co-culturing of the same organisms has been tried to produce biomass from starchy materials (Jarl, 1969; Sales and Menezes, 1976; Rattakul, 1976; Lemmel et al., 1979).

When the experiments were conducted independently with the individual organisms viz., E. fibuliger and C. utilis in enriched primary effluent, it was found that E. fibuliger was able to saccharify starch of the effluent to the extent of 81% whereas the saccharification rate in the case of C. utilis was negligible. The biomass yield of both organisms was comparatively negligible when the experiments were conducted individually. But there was significant increase in the yield of C. utilis when it was co-cultured with E. fibuliger whose amylolytic activity resulted in the production of reducing sugar required for the multiplication of C. utilis (Table 21). There was stagnancy in the growth of organisms after 96 hrs of incubation in co-culture system (Fig. 3.11). Slater (1981) has discussed the inherent problems associated with the traditional mixed culture fermentations. Cama and Edward (1970) and Paca (1982) suggested that the low pH (below 4.0) developed

as a result of the catabolism of sugars has profound influence on the retardation of growth. Low pH developed during the culturing could be a major reason for the stagnant growth of C. utilis after 96 hrs. The maximum biomass protein production of 0.165% was obtained at the 120th hr of incubation (Fig. 3.13). Non-enriched control experiments in the secondary effluent proved that enrichment had tremendous influence on the conversion of starch to biomass protein and COD removal as shown in tables 23 and 24 and figs.3.15-3.20.

The COD and BOD values were found decreasing at 24 hr, increasing the maximum at 48 hr and again decreasing during further incubation (Fig. 3.14). The drastic decrease in the COD and BOD at the early stage might be due to the separation of solids while centrifuging to remove yeast biomass before estimation. Jesuites (1966) has carried out sedimentation studies on separator waste and found reduction in BOD and COD level by solids removal. Increase in the amount of solubles (sugars and other metabolites) could be the cause for further increase in the COD and BOD values.

When similar co-culturing of C. utilis with E. fibuliger was carried out, there was no starch hydrolysis at the early stages of incubation (Figs. 3.9 and 3.10). But there was significant conversion of starch to sugars when E. magnusi was cultured alone (Table 16). The low multiplication rate of E. magnusi during co-culturing with C. utilis as shown in

table 22 and Fig. 3.12 might be the reason for the poor amylolytic activity.

Eventhough the reducing sugar level was found to be maximum at 120 hr, it was found that the growth of C. utilis was retarded. Formation of inhibitory metabolites as suggested by Slater (1981) and Maiorella et.al(1983) could be the reason for the retardation of growth of C. utilis. It has been found that inhibitory metabolites, produced even in small amounts, have a dramatic effect on microbial growth (Pons et al., 1986). As a result of the poor growth of C. utilis the protein production was also lower when compared to the co-culturing experiments of C. utilis and E. fibuliger (Fig. 3.13). The soluble COD and BOD values were found increasing with incubation again due to the possible contribution of soluble carbohydrates (Moon et al., 1978).

Rattakul (1976) has stressed that the observation of Jarl (1969) that controlled pH, inoculum size, as well as dissolved oxygen conditions of culture media influenced the cell ratio and cell yield of mixed cultures in starch fermentation. The results of the fermentation experiments carried out in the laboratory fermentor showed that under controlled conditions the co-culturing was dramatically fructified (Table 25). Around 79% of starch was hydrolysed and assimilated by the organisms during 28 hrs of incubation. After 28 hrs the reduction in starch content was very slow, probably due to the depletion of assimilable substrate. The highest

biomass (biomass protein) was observed at the 28th hr and thereafter it gradually decreased (Fig. 3.21). Depletion of reducing sugar could be the major reason for the retarded growth of C. utilis after 24 hrs, which influenced the protein yield as evidenced by the cell ratio of E. fibuliger and C. utilis (Jarl, 1969). The longer residence time might have also curbed the net biomass yield after 24 hrs. Many investigators have reported that a lower net cell production with longer residence time might be the result of increased maintenance energy and cell death (Lawrence and McCarty, 1970; Sherrard and Schroeder, 1972; Surucu et al., 1975).

There was a gradual increase in the soluble COD and BOD values in the beginning similar to the observations made in the experiment conducted in shake flasks with C. utilis and E. fibuliger. But the COD and BOD values decreased rapidly after 8 hrs of incubation. Maximum reduction of COD and BOD was observed (94% and 91% respectively) at the 28th hr of incubation under controlled conditions (Fig. 3.24). With adequate aeration and agitation there was rapid multiplication of cells (Fig. 3.23) which might have devoured the soluble organics in the effluents. Mazumder (1984) reported the co-oxidation of non-growth supporting carbon substrates with the growth supporting compounds when the cell density is high. The COD and BOD could not be further

reduced by the prolonged incubation. Moon et al.(1978) have explained such a phenomenon while treating cheese whey and whey permeate with yeasts. The residual COD/BOD would be the total output of soluble proteins and in certain cases the extracellular polysaccharides of yeasts and some other metabolites (Moon et al.,1978). Thanh and Wu (1975) also pointed out the possible contribution of broken cell debris and cell excretions in the media for the residual COD/BOD.

5.iv. Solid State Fermentation of Cassava Starch Factory Wastes with Aspergillus niger

Organic pollution as a result of the development of agro-industries poses serious problems to human health. Agro-industrial waste can be channelled into more useful products with the use of microorganisms which continously degrade the organic wastes accumulated on the earth. The partial recovery of agricultural wastes as single-cell protein is one of the means for the conservation of fixed carbon resources produced on the land . Since the organic wastes constitute various types of compounds, appropriate microorganisms capable of elaborating specific enzymes decompose the organic matter into simpler molecules. Therefore, in the case of SCP production from agricultural waste, it is required to employ microorganisms with relevant enzymatic activity. Solid state fermentation of organic wastes has been attempted as a means of elevating

the total protein content of biomass by many workers (Grant et al., 1978; Balasubramanyan and Bhatawdekar, 1981; Rodriguez et al., 1985). In the present study an attempt was made to convert cassava starch factory wastes into SCP by a strain of Aspergillus niger. The high growth rate and very high β-glucosidase activity distinguish A. niger as a potential candidate for the production of SCP (Sukan and Yasin, 1986). Results given in the tables 26 & 27 and figure 4.1 show that there was a gradual reduction in the starch content of the wastes inoculated with A. niger from 0 hr to 120 hr of incubation in both enriched and non-enriched wastes. The reduction in the starch content of the substrate could be correlated with the amyloglucosidase activity during the active growth phase of the organism as evidenced by the protein build-up at 48 hr (Figs. 4.6 and 4.7). The subsequent reduction in the amyloglucosidase activity could be due to changes in pH as well as reduction of starch content. The pH variation was found to be in the range of 5.4 to 3.0 in the case of enriched substrate. Highest amount of enzymatic activity was observed when the substrate was enriched with the basal media containing ammonium sulfate (Figs. 4.3 and 4.4). The influences of ammonium sulfate (Ferriksova, 1957) and salts like potassium dihydrogen phosphate, magnesium sulfate, calcium chloride,(Manonmani et al., 1983) etc., on amylase production by Aspergillus oryzae and Aspergillus niger were reported. The changes in the endoglucanase activity were found to be the maximum in the case of non-enriched wastes (Fig. 4.5). The low amylolytic activity has reduced the

production of glucose in the media (Fig. 4.2) and this might have derepressed the endoglucanase activity in the substrate. Nokrans (1967), Mandels (1975) and Takagi (1984) have reported the repression phenomena in the presence of glucose during cellulase production.

Fermentation of cassava starch factory waste with A. niger could bring the protein content to 7.0% at the end of 72 hr of incubation from a negligibly low level (1.60%). After 72 hrs the protein production or the biomass formation was insignificant because of the exhaustion of starch substrate and development of unfavourable conditions like highly acidic pH. Lack of nutrients seriously affected the growth of A. niger and thereby the formation of fungal biomass, as evidenced by the low protein content in the non-enriched waste.

5.v. Biomethanation of Cassava Starch Factory Effluents

Biogas from the agricultural waste usually contains 60 to 70 percent methane and 30 to 40 percent carbon dioxide as reported by Fry and Merrill (1973). Reports are also available on higher yield of methane with different feed stocks and technology (Messing, 1982). In the present study, biomethanation of cassava starch factory effluents was undertaken. Biomethanation of wastes from cassava starch factories has not been reported so far. The biogas obtained in the batch digestion on analysis by gas chromatography-mass spectrometry (GC-MS) has shown the presence of methane, carbon

dioxide and butane (Figs .5.1 and 5.2). The presence of butane in the biogas is probably reported for the first time. Cowley and Wase (1981) reviewed the presence of traces of gases such as carbondioxide, hydrogen, nitrogen, oxygen and hydrogen sulfide in addition to methane and carbondioxide in the biogas. The quantitative estimation of the methane was carried out using the gas chromatograph (Fig. 5.3) and the average value, of samples obtained at different conditions are accounted in table 28.

In the present study only 20% methane was present in the non-enriched cassava starch factory waste containing 5% cow-dung. But 59% methane could be obtained in the digester when the percentage of cow-dung was increased to 20%. Oi *et al.* (1985) emphasised the influence of inoculum ratio on the production of total gas, methane content of the gas and span of gas production during the methanogenesis of ramie-refining waste water. During the methanogenesis of primary effluent samples containing cow-dung in the ratio 5:95, 10:90 and 20:80, there was a great enhancement in the total gas production in proportion with the quantitative increase in cow-dung which was mainly intended to use as a source of inoculum (Fig. 5.6). Presence of large number of methanogenic bacteria in the cow-dung as already reported by Smith and Hungate (1958) and Paynter and Hungate (1968) could be the reason for the enhancement of biogas production when the proportion of cow-dung

was increased in the effluent digester. Maximum gas production of 130.20 l/kg dry matter was obtained when cow-dung was added at the rate of 20% whereas in the case of digesters containing 5% cow-dung, the gas output was only 7.58 l/kg dry matter. The span of gas production was also enhanced with the quantitative increase of cow-dung (Figs.5.4 and 5.5) and it was directly proportional to the amount of gas produced in these batch experiments.

There was no methane production when starch factory waste was subjected to anaerobic digestion in the absence of cow-dung or other seeding materials. The constant reduction in pH of the digestion fluid indicated the presence of acid forming bacteria in the digester.

High carbohydrate waste usually promotes the growth of acid formers whose activity results in acidic and toxic environment for the methane producing bacteria (Cowley and Wase, 1981). Cassava starch factory waste being a carbohydrate material with negligible amount of protein might have promoted the growth of acid formers. Normally, ammonium ions from the proteins react with carbondioxide and water to form ammonium bicarbonate which will act as the primary buffer and maintain the pH in the neutral range (Cowley and Wase, 1981; Perry, 1984). The role of C:N ratio also cannot be ruled out here since there is a possibility of attaining a C:N ratio of 30:1 by the addition of cow-dung having a C:N ratio of 20:1.

Cowley and Wase (1981) have also reported that the C:N ratio of 30:1 favourably supports biomethanation. Ammonium ions resulting from degradation of nitrogenous compounds, such as proteins and urea, become inhibitive above a threshold concentration (McCarty, 1964).

The introduced acid formers of the microbial flora mainly from the cow-dung (McInerney and Bryant, 1981) might have degraded the insoluble polysaccharides of the cassava starch factory effluent into simpler soluble forms. The degradation of polysaccharides into acetate, CO_2, H_2, propionate, butyrate, ethanol,etc., has been extensively discussed (Bryant, 1967; Loehr, 1974). Perhaps the 5% cow-dung was not sufficient to supply enough microbial load to solubilize the polysaccharides to the extent of solids solubilization attained in 10% and 20% cow-dung mixed effluent, and as a result, the solids reduction was not significant (Fig. 5.7). In the case of non-enriched effluents mixed with 20% cow-dung maximum COD removal occurred in the digester (62.5%). As discussed earlier maximum methane production was also observed in the same digester. Young and McCarty (1968) and Nyns (1980) have indicated that efficient removal of pollution load has resulted in significantly higher volume of gas formation.

Anaerobic digestion of cassava starch factory effluent in semi-continuous digesters

The gas production is related to loading rate which in

turn depends on retention time and solids concentration (Horton and Hawkes, 1979). An increased gas production was reported with higher loading rates of sewage sludges and other organics. Similar results were obtained when the loading rate of cassava starch factory effluent was enhanced in the semi-continous digesters (Tables 29,30,31 and 32). Thus, an increased gas production was achieved with increased rate of feeding until 7.8 g COD/l . of reactor volume/96 hr was reached. But further increase in the rate of addition did not improve the gas production but reduced it (Table 33). Loehr (1974) has reported the possibility of diminishing gas production at a higher loading rate than optimum. Decreasing pH, decreasing alkalinity, increasing volatile acids and increasing content of CO_2 in biogas are indications of failure of anaerobic process (Loehr, 1974). Loehr (1974) has also reported that increased addition of easily digestible materials like potato waste will change the digester sour (pH even less than 6.5). As and when the cassava starch factory effluent was fed at a rate of 9.5 g COD/l . of reactor volume/96 hr, the pH of the digester liquid got lowered to acidic side (5.92-6.20). A reduction in the gas production observed simultaneously supports the view that the process of biomethanation moved to unfavourable conditions at the above rate of loading and the subsequent change in pH.

If the gas production is correlated to pH it is evident

that the maximum output of biogas was achieved in the neutral pH range with optimum loading rate. This is in accordance with reports by earlier workers.

Cowley and Wase (1981) have reviewed the importance of detention time of the contents of the digester, irrespective of digester design especially for gas yield and solids reduction (Hawkes and Horton, 1981). Usually the farm waste digesters are run between 7 and 30 days (Meynell, 1976; Summers and Bonsfield, 1978). In the experiments of anaerobic digestion with cassava starch factory effluent digestion at 3[illegible].32 days hydraulic detention time (7.8 g COD/l . /96 hr), maximum gas production could be achieved, with fairly good reduction of solids (Figs.5.8 and 5.9) at room temperature (30 $\pm$ 3^{o}C) and minimum stirring (3 times daily for 2 min).

It has been reported that at thermophilic conditions in completely mixed digesters the retention time could be reduced to 5 days with some other organic materials (Cooney and Wise,1975; Varel et.al.,1977; Temper et al., 1982). Meynell (1976) has suggested that the time required to degrade all the organic matter in an untreated digester is almost six months.

The rate of degradation of solids is not appreciable by further extension of hydraulic detention time (40 days with 47.5% or 300 days with 58.1% solids reduction). As mentioned earlier the primary effluent samples contained about 50% starch.

In this short hydraulic detention time only the easily hydrolysable starch might have been degraded. The rapid degradation of starch was also suggested by Noike et al. (1985) in connection with the anaerobic digestion of carbohydrates. Assuming that the easily hydrolysable starch has been utilised for biogas generation, calculations were carried out according to the method of Chittenden et al. (1980). It was shown that an average value of 92% starch got degraded at 33.32 hydraulic detention time and produced 724 l. of biogas per kg starch, the theoretical value being 830 l. of biogas per kg of starch.

The acidic discharges of starch factories carry large amounts of organic matter such as free reducing sugar, easily degradable starch and slow decomposable fibrous matter, etc. This carbonaceous pabulum is being contaminated with microflora comprising bacteria, moulds and actinomycetes. The primary effluent was resistant to stabilization under natural conditions even though fairly good reduction in the BOD and COD could be achieved in the secondary effluent by keeping it at ambient conditions or by plain sedimentations to remove solids. Aerobic fermentations of the effluents with C. valida, G. candidum, C. utilis, E. fibuliger and E. magnusi, individually and in different combinations, showed a possibility to convert the effluents into yeast biomass. But the co-culturing of amylolytic yeast E. fibuliger and well known fodder yeast C. utilis was found useful in obtaining a dried product (Fig. 3F) containing 22.07% (w/w) protein with significant reduction of

pollution load (83% COD). A. niger luxuriantly grew on this waste and produced a protein rich residue. Anaerobic fermentation of the effluents for methane production could convert the soluble sugars and about 92% starch into biogas.

An integrated scientific approach to solve the problem of pollution of environment by cassava starch factory effluents for productions of SCP and biogas, described in this work, is hoped to have a tremendous impact on the management of effluent problems of agro-based industries.

CHAPTER 6

SUMMARY

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SUMMARY

Serious pollution problems from cassava starch factories indicated the necessity for developing remedial measures. Investigations on the aerobic and anaerobic treatment of cassava starch factory effluents were therefore made. The stabilization of the waste was achieved with valuable by-products like single-cell proteins and methane.

The following significant observations were made during the study.

1. Two kinds of effluents, the primary and secondary effluents from the large-scale starch factory and the secondary effluent from the small-scale starch factory were the main sources of pollution.

2. The primary effluent was a thick slurry with a high pollution load as evidenced by a BOD content of 13,200-14,300 mg/l and a COD content of 38,220-48,820 mg/l.

3. The secondary effluent was rather a diluted one, with a comparatively low BOD of 3,600-7,050 mg/l and a COD of 3,800-12,050 mg/l.

4. The analysis of cassava starch factory effluent showed the presence of soluble sugars, starch, celluloses, and traces of

nitrogenous matter.

5. At the early stages of discharge the effluents showed the presence of the toxic compound, hydrocyanic acid.

6. Various kinds of microorganisms including bacteria, yeasts, moulds, and actinomycetes were present in the effluent.

7. Gram-positive bacteria, spore formers, non-spore formers, and coliforms were found in large numbers.

8. Candida valida was the predominant yeast identified in the effluents.

9. Large number of Geotrichum candidum, Saccharomyces species, common saprophytic fungi like Rhizopus, Aspergillus, Penicillium, Fusarium, Monocillium and Humicola were observed.

10. Removal of solids to the extent of 43.3% by sedimentation resulted in reducing 50% of COD from the secondary effluents. But in the primary effluent there was no reduction in COD by sedimentation.

11. Under ambient conditions the degradation of organics by the natural flora was comparatively slow. A reduction of only 5% COD was observed in the primary effluent of cassava starch factory during 10 days of incubation. At the same time 35% COD removal was observed in the secondary effluent.

12. Rapid utilization of free reducing sugar was possible with Candida valida, Geotrichum candidum and Candida utilis.

13. Among the various yeasts and yeast-like organisms chosen for the aerobic treatment of cassava starch factory effluents, Endomycopsis magnusi and Endomycopsis fibuliger were found to be efficient in utilizing starch present in the effluent, though they had a comparatively slow growth.

14. Co-culturing of Candida utilis and the Endomycopsis fibuliger was found to be a better method for the hydrolysis of starch and assimilation of the resultant free reducing sugar.

15. The conversion of carbohydrate waste into SCP was rapid when fermentation was carried out under monitored conditions i.e. in a laboratory fermentor with proper mixing, aeration, pH and temperature control.

16. About 94% of the COD and 91% of the BOD were removed by aerobic treatment.

17. The protein content of the dried residue obtained from the fermentor at 28 hr of incubation was 22%, which was found to be maximum during the incubation of 0 hr to 60 hr.

18. Solid state fermentation of cassava starch factory waste with Aspergillus niger could increase the total protein content to 7.7% from the initial level of 1.6%.

19. Amyloglucosidase enzyme activity during the fermentation of cassava starch factory waste with Aspergillus niger was lower than that in other substrates reported in the literature.

20. In both submerged and solid state fermentation, enrichment of the substrate with nutrients promoted the growth of the organisms and production of protein.

21. Anaerobic digestion of cassava starch factory effluent in batch digesters produced 130.2 l. of biogas per kg dry matter with an average methane content of 59%.

22. The anaerobic process could reduce 63% COD during two months of incubation.

23. Semi-continuous digestion of cassava starch factory effluents produced about 325 l. of biogas per kg dry matter.

24. During the hydraulic retention time of two days in the anaerobic digester 46% solids were digested under ambient temperature.

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APPENDIX

Table 1 PHYSICO-CHEMICAL CHARACTERISTICS OF CASSAVA STARCH FACTORY EFFLUENTS

		EFFLUENTS OF LARGE SCALE STARCH FACTORY							
		PRIMARY EFFLUENTS				SECONDARY EFFLUENTS			
		1	2	3	4	1	2	3	4
pH(+OR-0.2)		4.5	4.6	4.5	4.7	4.5	4.6	4.7	4.7
TOTAL SOLIDS	XBAR	35.64	33.2	40	42	3.9	3.2	5.5	9.6
(g/l)	SIGMA	1.766	.709	1.223	.758	.15	.117	.203	.89
	C.V.	4.95	2.13	3.06	1.81	3.85	3.67	3.7	9.27
SUSPENDED SOLIDS	XBAR	33.25	31.2	35.1	37	1.234	.978	2.26	4.078
(g/l)	SIGMA	1.038	.117	.136	.189	.112	.079	.16	.112
	C.V.	3.12	.37	.39	.51	8.99	8.08	7.06	2.74
HYDROLYSABLE SUGAR	XBAR	22.99	22.61	24.61	29.28	1.46	1.12	1.92	2.761
AS GLUCOSE	SIGMA	.612	.181	.074	.84	.064	.057	.063	.097
(g/l)	C.V.	2.66	.8	.3	2.93	4.4	5.1	3.26	3.52
FREE REDUCING	XBAR	.525	.425	.78	1.85	.978	.735	1.347	2.06
SUGAR AS GLUCOSE	SIGMA	.028	.029	.054	.067	.061	.079	.08	.13
(g/l)	C.V.	5.3	6.79	6.94	3.65	6.19	10.69	5.96	6.32
TOTAL NITROGEN	XBAR	.108	.097	.013	.182	.07	.065	.077	.086
(g/l)	SIGMA	.01	.004	.005	.005	.003	.002	.007	.005
	C.V.	9.17	4.28	3.57	2.79	4.27	3.39	8.55	6.35
COD	XBAR	40.61	38.22	45.64	48.82	4.75	3.8	6.03	12.05
(g/l)	SIGMA	.338	2.325	2.646	.656	.224	.162	.333	.873
	C.V.	.83	6.08	5.8	1.34	4.72	4.27	5.53	7.25
BOD	XBAR	13.6	13.2	14.05	14.3	4.2	3.6	6	7.05
(g/l)	SIGMA	.637	.985	.681	.813	.38	.371	.333	.032
	C.V.	4.69	7.46	4.85	5.68	9.04	10.3	5.53	.46

XBAR -Average
SIGMA -Standard Deviation
C.V. -Co-efficient of Variation

1,2,3,4-Samples collected at different timings

Table 2.

PHYSICO-CHEMICAL CHARACTERISTICS OF CASSAVA STARCH FACTORY EFFLUENTS

SECONDARY EFFLUENTS OF SMALL-SCALE STARCH FACTORY

		1	2	3
pH(+OR-0.2)		4.5	4.6	4.7
TOTAL SOLIDS (g/l)	XBAR	4	5.2	6.6
	SIGMA	.121	.286	.317
	C.V.	3.03	5.5	4.81
SUSPENDED SOLIDS (g/l)	XBAR	1.87	2.32	2.96
	SIGMA	.169	.185	.241
	C.V.	9.04	7.97	8.13
HYDROLYSABLE SUGAR AS GLUCOSE (g/l)	XBAR	1.59	2.34	3.02
	SIGMA	.129	.089	.197
	C.V.	8.11	3.79	6.54
FREE REDUCING SUGAR AS GLUCOSE (g/l)	XBAR	.64	.97	2.075
	SIGMA	.032	.102	.174
	C.V.	5.01	10.49	8.37
TOTAL NITROGEN (g/l)	XBAR	.065	.068	.074
	SIGMA	.002	.004	.004
	C.V.	3.08	5.89	5.58
COD (g/l)	XBAR	4.87	6.8	9.6
	SIGMA	.259	.196	.134
	C.V.	5.33	2.88	1.39
BOD (g/l)	XBAR	3.87	4.76	6.02
	SIGMA	.222	.183	.511
	C.V.	5.73	3.85	8.49

Table 3

MICROBIAL LOAD OF THE CASSAVA STARCH FACTORY EFFLUENTS

PRIMARY EFFLUENTS- LARGE SCALE STARCH FACTORY

SAMPLES		BACTERIAL COUNT (NX10^5 /ml)	ACTINO MYCETES COUNT (NX10^3 /ml)	MOULDS COUNT (NX10^5 /ml)	YEAST COUNT (NX10^3 /ml)
XBAR	1	5	3	20	1.2
SIGMA		.28	.25	1.3	.06
C.V.		5.77	8.4	6.5	5
XBAR	2	10	10	17.5	14.73
SIGMA		.81	.63	1.1	1.8
C.V.		8.1	6.3	6.29	12.2
XBAR	3	.7	.9	3.6	10
SIGMA		.09	.11	.35	.42
C.V.		12.86	12.22	9.72	4.2
XBAR	4	3.45	1	20	3.1
SIGMA		.26	.1	.7	.25
C.V.		7.54	10.2	7.29	8.13

Table 4

MICROBIAL LOAD OF THE CASSAVA STARCH FACTORY EFFLUENTS

SECONDARY EFFLUENTS-LARGE SCALE STARCH FACTORY

SAMPLES		BACTERIAL COUNT (NX10^5 /ml)	ACTINO MYCETES COUNT (NX10^3 /ml)	MOULDS COUNT (NX10^5 /ml)	YEAST COUNT (NX10^3 /ml)
XBAR	1	199.5	2	1.7	28.88
SIGMA		8.43	.15	.11	2.84
C.V.		4.22	7.5	6.47	9.83
XBAR	2	400	3	1.6	13.13
SIGMA		9.32	.26	.14	.52
C.V.		2.33	8.6	8.5	3.97
XBAR	3	365	1.7	2.7	30
SIGMA		17.18	.18	.24	3.51
C.V.		4.71	10.59	8.89	11.71
XBAR	4	273.88	1.56	2.5	19.08
SIGMA		20.96	.07	.09	1.6
C.V.		7.65	4.26	3.6	8.39

Table 5

MICROBIAL LOAD IN THE CASSAVA STARCH FACTORY EFFLUENTS

SECONDARY EFFLUENT-SMALL SCALE STARCH FACTORY

SAMPLES		BACTERIA COUNT (NX10^5 /ml)	ACTINO MYCETES COUNT (NX10^3 /ml)	MOULDS (NX10^5 /ml)	YEASTS (NX10^3 /ml)
XBAR	1	10.5	2	15	35
SIGMA		.5	.17	.7	2.2
C.V.		4.88	8.5	4.71	6.17
XBAR	2	15.45	1.5	4	26.3
SIGMA		.83	.09	.43	1.4
C.V.		5.4	6	10.75	5.5
XBAR	3	5.5	2	29	12
SIGMA		.67	.18	3.4	1.2
C.V.		12.31	9	11.61	10
XBAR	4	12.3	1.2	33	29
SIGMA		1.2	.09	2.9	2.1
C.V.		9.76	7.5	7.27	6.36

Table 7

BIOCHEMICAL CHANGES OF CASSAVA STARCH FACTORY

PRIMARY EFFLUENT AT AMBIENT CONDITIONS

	INCUBATN TIME	REDUCING SUGARS (%W/V)	AMYLASE ACTIVITY (Sp.activity)	COD (g/litre)	BOD (g/litre)
XBAR	1	.04	0.00	14.71	37.20
SIGMA		.00		1.22	1.80
C.V		4.73		8.28	4.59
XBAR	2	.02	.46		
SIGMA		.00	.04		
C.V		28.28	8.12		
T-VALUE		-31.03			
Significance level		*	*		
XBAR	3	.01	.86		
SIGMA		.00	.02		
C.V		10.08	3.26		
T-VALUE		-33.99	135.98		
Significance level		*	*		
XBAR	4	.01	.96		
SIGMA		.00	.02		
C.V		5.17	2.25		
T-VALUE		-44.61	151.79		
Significance level		*	*		
XBAR	5	.01	.76		
SIGMA		.00	.03		
C.V		8.16	4.36		
T-VALUE		-43.76	80.11		
Significance level		*	*		

(Contd)

XBAR	6	.01	.42		
SIGMA		.00	.02		
C.V		7.95	4.35		
T-VALUE		-46.44	66.41		
Significance level		*	*		
XBAR	7	.01	.33		
SIGMA		.00	.03		
C.V		5.89	9.79		
T-VALUE		-45.61	34.79		
Significance level		*	*		
XBAR	8	.01	.42		
SIGMA		.00	.02		
C.V		6.60	4.94		
T-VALUE		-47.36	66.41		
Significance level		*	*		
XBAR	9	.01	.45		
SIGMA		.00	.03		
C.V		7.14	6.67		
T-VALUE		-51.75	47.43		
Significance level		*	*		
XBAR	10	.01	.30	15.36	35.30
SIGMA		.00	.02	.67	2.27
C.V		7.50	6.67	4.34	6.43
T-VALUE		-49.12	47.43	1.48	-2.07
Significance level		*	*	**	**

Table 8

BIOCHEMICAL CHANGES OF CASSAVA STARCH FACTORY

SECONDARY EFFLUENT AT AMBIENT CONDITIONS

	INCUBATN TIME (DAY)	REDUCING SUGARS (%W/V)	ANYLASE ACTIVITY (sp.activity)	COD (g/litre)	BOD (g/litre)
XBAR	1	.03	0.00	3.95	3.57
SIGMA		.00		.19	.10
C.V.		4.27		4.81	2.67
XBAR	2	.02	.42		
SIGMA		.00	.03		
C.V.		4.66	6.38		
T-VALUE		-32.13			
level		*			
XBAR	3	.01	.73		
SIGMA		.00	.02		
C.V.		7.05	3.03		
T-VALUE		-39.36	27.19		
Significance level		*	*		
XBAR	4	.01	.45		
SIGMA		.00	.03		
C.V.		4.21	6.09		
T-VALUE		-48.82	2.24		
Significance level		*	**		
XBAR	5	.02	.95		
SIGMA		.00	.03		
C.V.		5.33	2.89		
T-VALUE		-28.00	39.50		
Significance level		*	*		

(Contd)

XBAR	6	.01	.35		
SIGMA		.00	.03		
C.V.		9.18	8.41		
T-VALUE		-38.56	-5.22		
Significance level		*	*		
XBAR	7	.01	.52		
SIGMA		.00	.03		
C.V.		8.51	5.08		
T-VALUE		-60.45	7.45		
Significance level		*	*		
XBAR	8	.01	.52		
SIGMA		.00	.03		
C.V.		4.32	4.23		
T-VALUE		-62.51	7.45		
Significance level		*	*		
XBAR	9	.00	.35		
SIGMA		.00	.04		
C.V.		7.50	11.43		
T-VALUE		-64.00	-4.43		
Significance level		*	*		
XBAR	10	.00	.30	2.58	1.94
SIGMA		.00	.03	.22	.14
C.V.		5.00	10.00	8.49	7.13
T-VALUE		-64.91	-8.94	-14.90	-30.00
Significance level		*	*	*	*

Table 10
MICROBIAL PROLIFERATION IN THE C.S.F.PRIMARY EFFLUENT UNDER AMBIENT CONDITIONS

	INCUBATN TIME (DAY)	BACTERIAL COUNT (NX10^6 /ml)	YEASTS COUNT (NX10^6 /ml)	MOULDS COUNT (NX10^6 /ml)
XBAR	1	.09	.60	.01
SIGMA		.01	.05	.00
C.V		8.89	7.50	7.50
XBAR	2	.12	4.00	.30
SIGMA		.01	.30	.04
C.V		8.33	7.45	12.16
T-VALUE		7.41	35.35	25.29
Significance level		*	*	*
XBAR	3	.90	30.00	2.10
SIGMA		.05	2.69	.16
C.V		5.56	9.00	7.82
T-VALUE		50.59	34.56	40.26
Significance level		*	*	*
XBAR	4	9.10	39.40	4.60
SIGMA		.72	3.73	.37
C.V		7.91	9.47	7.96
T-VALUE		39.57	32.89	39.64
Significance level		*	*	*

(Contd)

XBAR	5	24.30	54.40	8.70
SIGMA		1.96	5.03	.60
C.V		8.05	9.25	6.93
T-VALUE		39.06	33.82	45.56
Significance level		*	*	*
XBAR	6	97.80	82.00	18.55
SIGMA		5.58	6.52	1.46
C.V		5.71	7.95	7.89
T-VALUE		55.37	39.48	40.04
Significance level		*	*	*
XBAR	7	296.60	100.10	10.20
SIGMA		29.19	7.36	7.04
C.V		9.84	7.35	6.90
T-VALUE		32.12	42.75	4.58
Significance level		*	*	*
XBAR	8	400.00	93.64	15.00
SIGMA		12.19	9.11	.96
C.V		3.05	9.73	6.38
T-VALUE		103.74	32.30	49.53
Significance level		*	*	*
XBAR	9	600.05	97.00	10.80
SIGMA		28.23	8.21	1.08
C.V		4.71	8.46	10.00
T-VALUE		67.21	37.13	31.59
Significance level		*	*	*
XBAR	10	495.60	99.80	18.00
SIGMA		36.17	4.47	2.00
C.V		7.30	9.49	11.11
T-VALUE		43.32	70.17	28.44
Significance level		*	*	*

* Significant at 5% level
** Not significant

Table 11

MICROBIAL PROLIFERATION IF THE C.S.F.
SECONDARY EFFLUENT UNDER AMBIENT CONDITIONS

	INCUBTN TIME (DAY)	BACTERIAL COUNT (NX10^6 /ml)	YEASTS COUNT (NX10^6 /ml)	MOULDS COUNT (NX10^6 /ml)
XBAR	1	30.08	2.40	.04
SIGMA		3.83	.20	.00
C.V.		12.77	7.50	5.23
XBAR	2	30.00	4.50	.30
SIGMA		3.30	.20	.02
C.V.		11.00	5.50	7.83
T-VALUE		-.05	23.48	35.61
Significance level		**	*	*
XBAR	3	40.50	40.00	.39
SIGMA		2.24	2.30	.22
C.V.		5.53	5.78	5.73
T-VALUE		7.43	51.50	5.03
Significance level		*	*	*
XBAR	4	65.00	110.00	2.02
SIGMA		3.46	12.45	.14
C.V.		5.32	7.05	7.00
T-VALUE		21.39	27.33	44.72
Significance level		*	*	*

(Contd)

XBAR	5	75.00	120.50	4.00
SIGMA		3.41	8.18	.41
C.V.		4.55	6.79	10.22
T-VALUE		27.70	45.64	30.54
Significance level		*	*	*
XBAR	6	80.50	180.00	3.96
SIGMA		7.34	9.49	.27
C.V.		9.12	5.27	6.82
T-VALUE		19.26	59.17	45.91
Significance level		*	*	*
XBAR	7	230.00	299.98	2.30
SIGMA		9.00	9.49	.27
C.V.		3.91	5.27	11.90
T-VALUE		64.64	99.14	26.47
Significance level		*	*	*
XBAR	8	270.00	491.79	1.00
SIGMA		10.41	39.47	.60
C.V.		3.86	8.03	6.00
T-VALUE		68.40	39.21	5.06
Significance level		*	*	*
XBAR	9	442.36	339.80	2.00
SIGMA		12.45	19.00	1.88
C.V.		2.81	5.59	9.41
T-VALUE		100.09	56.15	3.30
Significance level		*	*	*
XBAR	10	500.00	310.00	1.82
SIGMA		24.89	26.73	.19
C.V.		4.98	8.62	10.45
T-VALUE		59.01	36.39	29.62
Significance level		*	*	*

Table 12

PERFORMANCE OF *Candida valida* IN THE C.S.F.PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	2.23	.016	21.2	.065
SIGMA		.08	.0007	1.3	.003
C.V		3.63	4.57	6.15	5.6
XBAR	24	2.19	.006	30	.096
SIGMA		.07	.0003	1.33	.002
C.V		3.28	5.35	4.44	1.57
T-VALUE		-1.06430	-37.1391	13.38316	24.31840
Significance level		**	*	*	*
XBAR	48	2.13	.008	50.7	.105
SIGMA		.05	.001	2.49	.003
C.V		2.2	8.33	4.91	2.6
T-VALUE		-2.99813	-18.5371	29.70474	26.66667
Significance level		**	*	*	*
XBAR	72	2.11	.006	42.3	.102
SIGMA		.08	.0004	3.11	.004
C.V		3.84	5.72	7.36	3.43
T-VALUE		-3.00000	-35.0823	17.70509	20.93036
Significance level		**	*	*	*
XBAR	96	2.08	.006	36.2	.088
SIGMA		.06	.0003	4.76	.002
C.V		3.1	4.05	13.16	2.38
T-VALUE		-4.24264	-37.1391	8.598212	18.04268
Significance level		**	*	*	*
XBAR	120	2.04	.006	40.88	.095
SIGMA		.08	.0006	3.09	.002
C.V		3.92	9.52	7.55	2.17
T-VALUE		-4.75000	-30.6786	16.60442	23.53394
Significance level		**	*	*	*

* Significant at 5% level ** Not significant

Table 13
PERFORMANCE OF *Geotrichum candidum* IN THE C.S.F.PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	2.34	.025	54.35	.125
SIGMA		.04	.002	3.18	.005
C.V		1.74	7.38	5.86	3.71
XBAR	24	2.23	.009	81.1	.17
SIGMA		.03	.0005	4.31	.004
C.V		1.16	5.12	5.31	2.3
T-VALUE		-6.22254	-21.9518	14.12586	19.87767
Significance level		**	*	*	*
XBAR	48	2.13	.008	95.38	.172
SIGMA		.04	.0003	1.75	.004
C.V		1.81	4.35	1.83	2.06
T-VALUE		-10.5000	-23.7756	31.97222	20.76113
Significance level		*	*	*	*
XBAR	72	2.05	.008	101	.179
SIGMA		.04	.0003	4.97	.005
C.V		2	3.45	4.91	2.54
T-VALUE		-14.5000	-23.7756	22.36269	21.60000
Significance level		*	*	*	*
XBAR	96	1.94	.007	97	.168
SIGMA		.04	.0004	5.48	.005
C.V		2.32	6.06	5.65	3.19
T-VALUE		-20.0000	-24.9615	19.03970	17.20000
Significance level		*	*	*	*
XBAR	120		.007	101.25	.171
SIGMA			.0004	6.29	.004
C.V			5.4	6.21	2.63
T-VALUE		-165.463	-24.9615	18.82098	20.31940
Significance level		*	*	*	*

Table 14
PERFORMANCE OF *Candida utilis* IN THE C.S.F. PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	2.3	.032	13.96	.043
SIGMA		.04	.001	1.03	.0007
C.V		1.93	3.72	7.36	1.54
XBAR	24	2.25	.009	33.29	.084
SIGMA		.04	.0004	1.95	.001
C.V		1.92	4.61	5.87	1.21
T-VALUE		-2.50000	-60.4010	24.79173	95.00265
Significance level		**	*	*	*
XBAR	48	2.25	.008	59.9	.103
SIGMA		.04	.0003	2.97	.002
C.V		1.89	3.63	4.95	1.72
T-VALUE		-2.50000	-65.0194	41.33500	80.08904
Significance level		**	*	*	*
XBAR	72	2.25	.008	64.58	.10
SIGMA		.02	.0003	1.98	.00
C.V		.86	3.55	3.06	1.8
T-VALUE		-3.16228	-65.0194	64.14987	85.4283
Significance level		**	*	*	*
XBAR	96	2.23	.008	53.24	.1
SIGMA		.04	.0005	1.91	.002
C.V		1.89	5.92	3.59	1.82
T-VALUE		-3.50000	-60.7157	51.19790	76.08459
Significance level		**	*	*	*
XBAR	120	2.22	.007	54.6	.101
SIGMA		.03	.0003	3.05	.004
C.V		1.39	3.51	5.59	3.8
T-VALUE		-4.52548	-67.7285	35.70652	40.39826
Significance level		**	*	*	*

Table 15
PERFORMANCE OF *Endomycopsis fibuliger* IN THE C.S.F. PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	2.38	.013	.55	.029
SIGMA		.05	.001	.04	.001
C.V		2.3	6.51	6.89	3.41
XBAR	24	1.24	.052	2.03	.04
SIGMA		.04	.004	.15	.003
C.V		3.18	6.78	7.55	7.03
T-VALUE		-50.3568	26.75378	26.96486	9.838699
Significance level		*	*	*	*
XBAR	48	.95	.075	16.5	.061
SIGMA		.02	.004	1.23	.003
C.V		2.42	5.44	7.48	4.25
T-VALUE		-75.1073	42.53165	36.65819	28.62167
Significance level		*	*	*	*
XBAR	72	.62	.061	60	.134
SIGMA		.01	.003	3.37	.007
C.V		2.19	5.25	5.61	5.4
T-VALUE		-97.6272	42.93251	49.89263	42.00000
Significance level		*	*	*	*
XBAR	96	.51	.038	84.54	.165
SIGMA		.01	.002	4.05	.008
C.V		1.89	6.09	4.79	4.67
T-VALUE		-103.729	31.62278	58.65383	47.71196
Significance level		*	*	*	*
XBAR	120	.45	.032	108.62	.182
SIGMA		.01	.002	7.7	.01
C.V		2.15	7.4	7.09	5.5
T-VALUE		-107.057	24.03331	39.69662	43.06017
Significance level		*	*	*	*

Table 16

PERFORMANCE OF *Endomycopsis magnusi* IN THE C.S.F.PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	2.74	.006	.4	.023
SIGMA		.06	.0004	.03	.001
C.V		2.2	7.32	8.51	5.26
XBAR	24	1.97	.035	6.52	.031
SIGMA		.09	.0005	.59	.001
C.V		4.5	1.53	9.07	4.09
T-VALUE		-20.1346	128.1006	29.30108	16.00000
Significance level		*	*	*	*
XBAR	48	1.69	.071	8.3	.046
SIGMA		.04	.002	.54	.001
C.V		2.33	2.28	6.62	1.8
T-VALUE		-41.1844	90.13878	41.31513	46.00000
Significance level		*	*	*	*
XBAR	72	1.48	.057	44.25	.119
SIGMA		.04	.003	3.13	.005
C.V		2.47	5.48	7.08	4.59
T-VALUE		-49.4213	47.66147	39.62327	53.25122
Significance level		*	*	*	*
XBAR	96	.96	.033	80	.15
SIGMA		.04	.003	4.43	.014
C.V		4.19	7.65	5.54	5.4
T-VALUE		-69.8173	25.23254	50.82114	25.59267
Significance level		*	*	*	*
XBAR	120	.92	.021	89.94	.158
SIGMA		.01	.001	5.84	.005
C.V		1.11	5.55	6.49	2.88
T-VALUE		-84.6283	39.39193	43.36541	74.88453
Significance level		*	*	*	*

Table 17
PERFORMANCE OF *Candida utils* IN THE C.S.F.SECONDARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	.19	.025	27.36	.088
SIGMA		.008	.002	.82	.002
C.V		3.99	8.79	3.01	2.02
XBAR	24	.18	.015	320.06	.206
SIGMA		.009	.001	20.92	.003
C.V		4.78	7.72	6.54	1.38
T-VALUE		-2.34888	-12.6491	39.54328	92.56682
Significance level		**	*	*	*
XBAR	48	.18	.011	341.25	.208
SIGMA		.01	.0006	17.79	.004
C.V		7.78	5.66	5.21	1.74
T-VALUE		-2.20863	-18.9640	49.85235	75.89466
Significance level		**	*	*	*
XBAR	72	.18	.008	375	.213
SIGMA		.006	.0004	11.11	.004
C.V		3.31	4.54	2.96	1.69
T-VALUE		-2.82843	-23.5748	88.26346	79.05694
Significance level		**	*	*	*

Table 18

PERFORMANCE OF *Candida utilis* IN THE C.S.F. SECONDARY EFFLUENT (Non-enriched)

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
XBAR	0	.19	.03	27.5	.088	6.55
SIGMA		.088	.001	2.4	.004	.07
C.V		4.08	3.78	8.74	4.26	1.03
XBAR	24	.18	.02	230	.182	3.16
SIGMA		.01	.001	8.61	.007	.2
C.V		5.99	2.21	3.74	3.83	6.47
T-VALUE		-.319357	-20.0000	64.07935	32.97738	-45.2503
Significance level		**	*	*	*	*
XBAR	48	.18	.015	272.88	.187	2.85
SIGMA		.001	.0003	.004	.004	.1
C.V		.62	1.76	2.01	2.01	3.6
T-VALUE		-.321391	-40.6371	289.1827	49.50000	-85.7341
Significance level		**	*	*	*	*
XBAR	72	.18	.012	255	.191	2.55
SIGMA		.002	.0007	13.11	.002	.1
C.V		.83	5.6	5.14	1.09	3.87
T-VALUE		-.321329	-41.7085	48.27982	65.14292	-92.6855
Significance level		**	*	*	*	*

Table 19
PERFORMANCE OF *Endomycopsis fibuliger* IN THE C.S.F.SECONDARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	0	.19	.025	38.37	.096
SIGMA		.01	.002	5.36	.003
C.V		5.14	6.64	13.98	2.65
XBAR	24	.13	.015	345	.177
SIGMA		.006	.001	15.22	.005
C.V		4.91	6.55	4.41	2.71
T-VALUE		-14.5521	-12.6491	53.74740	39.29077
Significance level		*	*	*	*
XBAR	48	.1	.011	375.72	.182
SIGMA		.001	.001	10.84	.005
C.V		1.4	5.99	2.88	2.51
T-VALUE		-25.3295	-17.7088	78.90413	41.71613
Significance level		*	*	*	*
XBAR	72	.107	.008	369.55	.193
SIGMA		.002	.002	22.14	.005
C.V		1.64	6.44	5.99	2.76
T-VALUE		-23.0201	-17.0000	41.12098	47.05191
Significance level		*	*	*	*

Table 20
PERFORMANCE OF *Endomycopsis fibuliger* IN THE C.S.F. SECONDARY EFFLUENT
(Non-enriched)

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
XBAR	0	.19	.035	38.42	.096	6.54
SIGMA		.01	.001	2.39	.001	.1
C.V		6.56	2.81	6.22	1.39	1.58
XBAR	24	.14	.023	112.2	.141	3.41
SIGMA		.004	.001	5.01	.002	.03
C.V		2.77	3.61	4.47	1.39	.88
T-VALUE		-13.1306	-24.0000	37.59432	56.92100	-84.7901
Significance level		*	*	*	*	*
XBAR	48	.12	.019	335	.176	2.85
SIGMA		.006	.001	8.21	.003	.13
C.V		5.07	7.2	2.45	1.85	4.48
T-VALUE		-16.9775	-32.0000	98.10249	71.55418	-63.6349
Significance level		*	*	*	*	*
XBAR	72	.12	.01	290	.175	2.54
SIGMA		.005	.001	10.05	.003	.14
C.V		4.52	8.25	3.47	1.46	5.43
T-VALUE		-17.7088	-50.0000	68.88255	70.65975	-65.7596
Significance level		*	*	*	*	*

Table 21

CO-CULTURING OF C.utilis and E.fibuliger IN THE C.S.F.PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml) C.utilis	CELL NO (NX10^6 /ml) E.fibuliger	BIOMASS PROTEIN (%s/v)	COD (g/l)	BOD (g/l)
XBAR	0	3.23	.05	39.54	37.01	.13	68.29	20.82
SIGMA		.04	.004	3.19	3.12	.004	1.62	.77
C.V		1.35	7.24	8.07	8.43	3.11	2.37	3.69
XBAR	24	2.06	.166	76.66	48.05	.203	9.46	7.35
SIGMA		.1	.006	7.59	3.58	.006	.14	.19
C.V		4.75	3.39	9.9	7.45	2.76	1.44	2.56
T-VALUE		-30.7257	45.49894	12.75231	6.575565	28.63296	-102.332	-48.0383
Significance level		*	*	*	*	*	*	*
XBAR	48	1.82	.58	219.8	127	.246	12.2	11.03
SIGMA		.06	.031	9.44	4.45	.007	.63	.15
C.V		3.56	5.39	4.3	3.5	2.8	5.18	1.37
T-VALUE		-55.3048	47.95938	51.16727	46.83352	40.69549	-91.2711	-35.2979
Significance level		*	*	*	*	*	*	*
XBAR	72	1.76	.204	206.5	122.5	.271	10.28	7.01
SIGMA		.1	.012	12.1	6.32	.003	.7	.88
C.V		5.55	5.99	6.15	5.16	1.11	6.82	5.7
T-VALUE		-38.6041	34.43545	37.73817	34.30706	79.76164	-92.9738	-33.4046
Significance level		*	*	*	*	*	*	*
XBAR	96	1.66	.255	288	132	.295	7.42	5.47
SIGMA		.04	.013	14.72	6.26	.006	.43	.15
C.V		2.39	4.95	5.11	4.74	2.03	5.77	2.72
T-VALUE		-78.5000	42.62977	46.65818	38.41234	64.71832	-102.719	-55.3445
Significance level		*	*	*	*	*	*	*
XBAR	120	1.53	.168	281.08	118.75	.295	6.71	5.12
SIGMA		.04	.004	13.78	8.51	.007	.63	.42
C.V		2.85	2.3	4.9	10.11	2.48	9.4	8.29
T-VALUE		-85.0000	59.00000	48.30021	25.50727	57.88583	-100.205	-50.6287
Significance level		*	*	*	*	*	*	*

Table 22

CO-CULTURING OF C.utilis and S.magnusi IN THE C.S.F.PRIMARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (MX10^6 /ml) C.utilis	CELL NO (MX10^6 /ml) E.magnusi	BIOMASS PROTEIN (%w/v)	COD (g/l)	BOD (g/l)
XBAR	0	3.25	.051	39.95	.59	.061	68.35	20.15
SIGMA		.1	.004	3.32	.04	.006	1.28	1.11
C.V		3.09	8.78	8.3	7.48	9.36	1.88	5.5
XBAR	24	3.2	.018	82.25	.75	.093	3.86	3.64
SIGMA		.17	.001	4.21	.03	.004	.07	.17
C.V		5.24	5.46	5.12	3.46	4.32	1.72	4.63
T-VALUE		-.717035	-22.6378	22.31479	9.050967	12.55143	-142.291	-41.5848
Significance level		**	*	*	*	*	*	*
XBAR	48	3.18	.008	112.53	.83	.122	3.92	4.25
SIGMA		.11	.0004	6.32	.04	.004	.07	.18
C.V		3.61	4.67	5.61	4.47	3.32	1.67	4.25
T-VALUE		-1.33182	-30.2547	28.75589	12.00000	23.92617	-142.159	-39.9929
Significance level		**	*	*	*	*	*	*
XBAR	72	3.16	.008	95.5	.9	.113	4.04	3.86
SIGMA		.01	.0003	2.98	.03	.004	.05	.21
C.V		.45	3.42	3.12	3.45	3.75	1.34	5.4
T-VALUE		-2.53295	-30.3204	35.21859	17.53625	20.39608	-141.998	-40.7856
Significance level		**	*	*	*	*	*	*
XBAR	96	2.65	.145	133.74	6.69	.134	9.25	8.4
SIGMA		.13	.005	3.77	.17	.005	.31	.73
C.V		5.06	3.59	2.78	2.51	3.7	3.4	8.71
T-VALUE		-10.3471	41.52225	52.80770	98.79271	26.43644	-126.924	-25.0156
Significance level		*	*	*	*	*	*	*
XBAR	120	1.66	.811	85.4	8.67	.11	14.45	12.2
SIGMA		.05	.02	4.63	.17	.006	.75	.86
C.V		2.7	2.38	5.42	1.97	5.26	5.19	7.03
T-VALUE		-40.2242	105.3930	22.56365	130.8599	16.33333	-102.762	-16.0137
Significance level		*	*	*	*	*	*	*

Table 23
CO-CULTURING OF C.utilis and E.fibuliger IN THE C.S.F.SECONDARY EFFLUENT

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
XBAR	0	.2	.047	69.36	.154	6.64
SIGMA		.01	.003	6.52	.002	.15
C.V		5.91	7.01	9.4	1.03	2.28
XBAR	24	.14	.024	365	.263	2.45
SIGMA		.008	.001	20.59	.006	.15
C.V		5.49	4.54	5.64	1.75	5.99
T-VALUE		-13.2518	-20.5718	38.71700	48.74628	-55.8667
Significance level		*	*	*	*	*
XBAR	48	.12	.011	358	.272	3.22
SIGMA		.007	.0004	12.14	.003	.12
C.V		5.93	3.86	3.39	1.16	3.67
T-VALUE		-18.5371	-33.6434	59.24483	92.56682	-50.3568
Significance level		*	*	*	*	*
XBAR	72	.1	.008	360.03	.27	2.35
SIGMA		.01	.0004	20.17	.003	.09
C.V		9.88	5.45	5.6	1.07	3.79
T-VALUE		-20.0000	-36.4470	38.78448	90.99789	-69.3652
Significance level		*	*	*	*	*

Table 24

CO-CULTURING OF *C.utilis* and *E.fibuliger* IN THE C.S.F.SECONDARY EFFLUENT (Non-enriched)

	TIME (HR)	STARCH (%w/v)	RD.SUGAR (%w/v)	CELL NO (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)	COD (g/l)
XBAR	0	.2	.023	70.68	.148	6.44
SIGMA		.007	.001	5.62	.001	.28
C.V		3.59	5.49	7.95	.67	3.03
XBAR	24	.19	.028	320	.21	3.58
SIGMA		.008	.001	17.41	.001	.35
C.V		4.18	3.67	5.44	.71	9.76
T-VALUE		-2.66076	10.00000	38.54598	124.0000	-18.0477
Significance level		**	*	*	*	*
XBAR	48	.15	.028	375	.218	3.53
SIGMA		.009	.001	7.21	.002	.32
C.V		5.72	3.67	1.92	1.12	8.95
T-VALUE		-12.4035	10.00000	94.15734	88.54377	-19.3570
Significance level		*	*	*	*	*
XBAR	72	.14	.015	260.5	.237	2.74
SIGMA		.008	.001	6.06	.001	.06
C.V		5.72	5.99	2.33	.63	2.33
T-VALUE		-15.9646	-16.0000	64.96077	178.0000	-36.5460
Significance level		*	*	*	*	*

Table 25

CO-CULTURING OF C.utilis AND E.fibuliger IN THE C.S.F.PRIMARY EFFLUENT UNDER CONTROLLED CONDITIONS

	TIME (HR)	STARCH (%W/V)	RED.SUGAR (%W/V)	COD (g/litre)	BOD (g/litre)	E.f CELL COUNT (NX10^6 /ml)	C.u CELL COUNT (NX10^6 /ml)	BIOMASS PROTEIN (%w/v)
XBAR	00							.0144
SIGMA								.0004
C.V.								2.85
XBAR	0	1.92	.037	31.42	11.25	28.25		.0876
SIGMA		.175	.001	.72	.75	1.35		.0084
C.V.		9.2	3.81	2.29	6.7	4.78		9.59
XBAR	4	1.83	.015	2.52	1.6	46.25		.1555
SIGMA		.07	.001	.05	.12	2.61		.0029
C.V		4.04	3.09	2.07	7.23	5.64		1.88
T-VALUE		1.350580	4.000000	113.2572	35.93536	50.12060		151.6622
Significance level		**	**	*	*	*		*
XBAR	04	1.83	.017			46.25	16.25	.2134
SIGMA		.08	.001			2.61	1.44	.0005
C.V		4.34	.11			5.64	8.88	2.3
T-VALUE		1.322939	40.00000			50.12060		1207.173
Significance level		**	*			*		*
XBAR	8	1.5	.015	8.63	4.7	96.75	127.25	.2737
SIGMA		.09	.001	.34	.07	6.56	6.08	.01
C.V		5.84	5.53	3.99	1.5	.07	4.77	3.69
T-VALUE		6.036686	44.00000	80.95519	24.59471	41.71499	50.24734	77.41405
Significance level		*	*	*	*	*	*	*
XBAR	12	.86	.061	7.25	3.8	105.62	166.37	.313
SIGMA		.006	.002	.09	.12	8.26	4.92	.0048
C.V		.72	2.87	1.24	3.13	.08	2.96	1.55
T-VALUE		17.12213	-30.3579	94.21552	27.74285	36.16689	82.82680	184.4370
Significance level		*	*	*	*	*	*	*

(contd)

XBAR	16	.46	.055	4.39	2.7	137.5	324	.4247
SIGMA		.02	.004	.03	.12	6.45	4.32	.0039
C.V		4.42	6.83	.6	4.28	4.69	1.33	.92
T-VALUE		23.44455	-12.3479	106.0918	31.83910	60.29593	191.1528	308.0085
Significance level		*	*	*	*	*	*	*
XBAR	20	.45	.021	3.72	2.28	270.62	822.62	.4619
SIGMA		.02	.001	.04	.11	17.37	9.71	.0084
C.V		3.56	4.42	1.57	4.66	6.42	1.18	1.82
T-VALUE		23.60513	32.00000	108.6483	33.46992	44.06615	232.3465	155.5298
Significance level		*	*	*	*	*	*	*
XBAR	24	.44	.015	2.86	1.22	281	1107.5	.53
SIGMA		.01	.0008	.07	.04	8.83	40.31	.0057
C.V		2.47	5.36	2.49	3.54	3.14	3.64	1.08
T-VALUE		23.88145	48.58987	111.6678	37.77182	90.00997	76.52080	262.9941
Significance level		*	*	*	*	*	*	*
XBAR	28	.4	.01	2.63	1.06	288	976.87	.5407
SIGMA		.02	.0008	.03	.03	10.21	15.99	.0057
C.V		4.56	8.1	1.14	3.18	3.55	1.64	1.05
T-VALUE		24.40803	59.63302	112.9998	38.39819	79.78325	169.2366	268.3036
Significance level		*	*	*	*	*	*	*
XBAR	32	.38	.008	1.95	1.08	266.75	828	.521
SIGMA		.02	.0004	.02	.09	6.88	12.88	.0113
C.V		5.17	4.15	1.03	8.18	2.58	1.56	2.17
T-VALUE		24.72919	76.15773	115.7245	38.08027	109.6632	177.1552	130.4080
Significance level		*	*	*	*	*	*	*
XBAR	36	.38	.008	2.55	1.32	283.12	789.25	.5134
SIGMA		.008	.0004	.1	.11	12.8	10.9	.0058
C.V		2.24	4.92	3.96	8.09	4.52	1.38	1.12
T-VALUE		24.86419	76.15773	112.3338	37.05198	62.56127	198.8570	250.3646
Significance level		*	*	*	*	*	*	*

(contd)

XBAR	40	.37	.011	2.63	2.03	245.62	918.5	.5109
SIGMA		.002	.0004	.2	.11	8.51	7.65	.0097
C.V		6.05	3.39	.84	5.37	3.46	.83	1.9
T-VALUE		25.05015	68.27935	108.9718	34.40275	81.63552	327.8306	148.9735
Significance level		*	*	*	*	*	*	*
XBAR	44	.34	.009	2.55	1	236.87	1106	.503
SIGMA		.01	.0005	.03	.04	14.05	13.56	.0121
C.V		3.31	5.54	1.21	3.65	5.93	1.23	2.41
T-VALUE		25.49507	70.83502	113.3138	38.60031	47.68466	226.0357	117.5784
Significance level		*	*	*	*	*	*	*
XBAR	48	.32	.004	2.7	1.26	254.37	1027.5	.4904
SIGMA		.009	.0004	.04	.06	9.44	10.41	.0064
C.V		2.83	9.45	1.3	5.06	3.71	.96	1.31
T-VALUE		25.82577	86.66225	112.6491	37.55467	76.21473	272.1680	216.7282
Significance level		*	*	*	*	*	*	*
XBAR	52	.31	.008	2.9	2.25	265	1085.37	.5274
SIGMA		.01	.0005	.04	.07	7.07	17.66	.0425
C.V		3.58	6.62	1.26	3.18	2.67	1.63	8.07
T-VALUE		25.97915	73.36484	111.8646	33.79425	106.0160	170.6639	35.09912
Significance level		*	*	*	*	*	*	*
XBAR	56	.31	.009	2.14	.95	269.37	1012.75	.4999
SIGMA		.01	.0007	.11	.05	13.29	5.88	.0095
C.V		3.16	6.58	4.95	5.5	4.93	.55	1.9
T-VALUE		25.97915	64.87986	113.7034	38.75770	57.32832	465.5830	148.8348
Significance level		*	*	*	*	*	*	*
XBAR	60	.3	.004	2.32	1.18	278.5	1075.75	.515
SIGMA		.009	.0003	.04	.1	16.6	6.84	.0086
C.V		3.04	6.99	4.24	8.56	5.96	.64	.86
T-VALUE		26.14860	89.40168	114.1396	37.64322	42.49908	444.8363	100.5577
Significance level		*	*	*	*	*	*	*

Table 26

BIO CHEMICAL CHANGES IN THE ENRICHED CASSAVA STARCH FACTORY WASTES DURING THE GROWTH OF *Aspergillus niger*

	INCUBATN TIME (HR)	STARCH AS GLU. (%W/W)	RED.SUGAR AS GLUCOS (%W/W)	AMYLO GLU.ACT (%W)	ENDOGLU. ACTIVITY (Sp.activity)	EXTRA CELLULAR (%W/W)	BIOMASS PROTEIN (%W/W)
XBAR	0	56.85	.006	0.00	0.00	.742	1.6
SIGMA		3.03	.0006			.07	.05
C.V.		5.32	9.94			9.53	3.35
XBAR	24	53.5	.13	5.9	8.35	.975	3.2
SIGMA		2.92	.003	.42	.09	.04	.05
C.V.		5.4	2.04	7.12	1.14	3.7	1.63
T-VALUE		-2.52	128.17			9.14	71.55
SIGNIFICANCE LEVEL		**	*			*	*
XBAR	48	41.85	.252	24.62	2.8	2.211	6.1
SIGMA		1.99	.02	1.7	.02	.085	.14
C.V.		4.75	8.01	6.92	.7	3.85	2.28
T-VALUE		-13.09	38.88	33.81	-190.36	42.19	95.72
SIGNIFICANCE LEVEL		*	*	*	*	*	*
XBAR	72	33.15	.104	8.77	4.53	2.505	7
SIGMA		1.72	.001	.61	.03	.184	.17
C.V.		5.19	1.17	7	.67	7.18	2.48
T-VALUE		-21.51	265.74	12.25	-127.33	28.32	96.37
SIGNIFICANCE LEVEL		*	*	*	*	*	*
XBAR	96	14.55	.063	1.88	6.04	2.202	7.2
SIGMA		.37	.003	.17	.07	.118	.15
C.V.		2.57	5.35	9.22	1.16	5.38	2.06
T-VALUE		-43.82	58.92	-28.06	-64.07	33.65	112.00
SIGNIFICANCE LEVEL		*	*	*	*	*	*
XBAR	120	13.05	.039	1.3	6	1.844	7.7
SIGMA		.84	.003	.08	.05	.109	.22
C.V.		6.42	7.81	6.15	.83	5.9	2.88
T-VALUE		-44.05	34.11	-34.02	-72.18	26.90	85.50
SIGNIFICANCE LEVEL		*	*	*	*	*	*

Table 27

BIO CHEMICAL CHANGES IN THE NON-ENRICHED CASSAVA STARCH FACTORY WASTES DURING THE GROWTH OF *Aspergillus niger*

	INCUBATN TIME (hr)	STARCH AS GLUCOSE (%w/w)	RED.SUGAR AS GLUCOSE (%w/w)	AMYLO GLUCOSIDASE ACTIVITY(I.U)	ENDOGLU. ACTIVITY (Sp.activity)	EXTRA CELLULAR PROTEIN (%w/w)	BIOMASS PROTEIN (%w/w)
XBAR	0	58.4	.006	0.00	0.00	.395	1.1
SIGMA		2.14	.0005			.02	.06
C.V		3.67	7.21			4.56	5.75
XBAR	24	56	.059	6.51	11.84	.749	2.4
SIGMA		2.49	.005	.34	.07	.01	.06
C.V		4.44	8.85	5.22	.59	1.61	2.61
T-VALUE		-2.31	33.35			50.06	48.45
Significance value		**	*			*	*
XBAR	48	54.45	.093	16.45	16.67	1.234	3.2
SIGMA		3.17	.007	.77	.12	.036	.11
C.V		5.82	7.05	4.69	.72	2.92	3.32
T-VALUE		-3.27	39.20	37.34	109.94	64.42	53.00
Significance value		**	*	*	*	*	*
XBAR	72	33.3	.188	9.4	10.33	1.627	3.7
SIGMA		2.31	.005	.92	.09	.078	.11
C.V		6.95	2.84	9.81	.87	4.79	2.87
T-VALUE		-25.21	114.54	9.32	-41.88	48.38	65.62
Significance value		*	*	*	*	*	*
XBAR	96	26.1	.238	2.05	13.56	1.426	3.68
SIGMA		1.17	.01	.13	.11	.059	.05
C.V		4.48	6	5.85	.81	4.13	1.34
T-VALUE		-41.88	73.27	-38.75	41.72	52.33	104.46
Significance value		*	*	*	*	*	*
XBAR	120	25	.393	8.19	17	1.468	3.7
SIGMA		1.2	.03	.71	.35	.074	.19
C.V		4.8	7.92	8.63	2.06	5.04	5.05
T-VALUE		-43.05	40.79	6.75	45.72	44.26	41.26
Significance value		*	*	*	*	*	*

Table 28
BIOMETHENATION OF CASSAVA STARCH FACTORY EFFLUENTS (C.S.F.E) IN BATCH DIGESTERS

	FEEDING CONDITIONS	SPAN OF GAS PRODUCTN (DAYS)	GAS PRODUCED (l/kg dry matter)	AVERAGE METHANE CONTENT %	INITIAL TOTAL SOLIDS %	FINAL TOTAL SOLIDS %	TOTAL SOLIDS REDN %	INITIAL COD (g/l)	FINAL COD (g/l)	COD REDN (g/l)
XBAR	C.S.F.E+5%	10	20	42	8.22	7.22	12.17	65.12	60.96	6.39
SIGMA	COWDUNG+UREA		.06		.05	.03		2.94	2.9	
C.V.			3.62		.61	.42		4.52	4.75	
XBAR	C.S.F.E+5%	6	7.58	20	8.22	7.64	7.06	64.8	61.12	5.68
SIGMA	COWDUNG WITHOUT UREA		.03		.05	.07		2.6	2.41	
C.V.			4.57		.61	.92		4.02	3.95	
XBAR	C.S.F.E+10%	20	30	58	8.22	5.02	38.93	64	57.92	9.5
SIGMA	COWDUNG+UREA		.09		.05	.02		2.2	1.49	
C.V.			3.66		.61	.39		3.43	2.58	
XBAR	C.S.F.E+10%	21	31.46	55	8.22	4.98	39.42	63.2	56.16	11.14
SIGMA	COWDUNG WITHOUT UREA		.06		.05	.03		3.322	1.94	
C.V.			2.42		.61	.4		5.26	3.45	
XBAR	C.S.F.E+20%	34	66.18	62	8.22	4.83	41.24	65.6	51.2	21.95
SIGMA	COWDUNG+UREA		.6		.05	.03		5.72	3.42	
C.V.			11.07		.61	.62		8.72	6.67	
XBAR	C.S.F.E+20%	68	130.2	59	8.22	4.72	42.58	64	24	62.5
SIGMA	COWDUNG		.39		.05	.04		1.62	.52	
C.V.			3.62		.61	.85		2.53	2.15	