STUDIES ON THE GROWTH OF FUNGUS SCYTALIDIUM ACIDOPHILUM IN HYDROLYSATES FROM SPHAGNUM PEAT MOSS

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BY

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ABSTRACT

Scytalidium acidophilum was cultured in varibus peat based substrates to determine which one would produce the highest concentration of biomass. Peat was hydrolyzed individually with sulfuric, hydrochloric, nitric and phosphoric acids and the hydrolysates used as substrates to prepare the media for culturing the fungus. The proximate composition of the hydrolysates were determined. The H_2SO_4- hydrolysate of peat was the richest in carbohydrate concentration. The constituent monosaccharides in the peat hydrolysates were determined by High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). Hexoses constituted more than 50 % of the total reducing sugars present in the peat hydrolysates.

Preliminary experiments with synthetic media showed that a 5 % (v/v) inoculum ratio produces the highest growth of the fungus. Growth of the fungus on the media containing different carbon sources indicated that hexoses were better utilized by the fungus than pentoses.

Undiluted and water-diluted peat hydrolysates were used for culturing the fungus. Peat hydrolyzed with sulfuric acid and diluted 1:1 with water produced the highest growth parameters of the fungus. Supplementation of the hydrolysates with 3 g/L yeast extract and 0.4 g/L magnesium sulfate improved the growth of the fungus compared to the other nutritional additives tested.

The effects of components of peat on the growth of the fungus were investigated. A study involving the removal of one peat component (modified peats) to avoid the potential inhibitory effect of peat is not a viable method of increasing the biomass production. A comparison of non-modified and modified peat hydrolysates showed that modification of peat results in a loss of nutrients.

The nutrient supplementation of modified peat hydrolysates caused no statistically significant increases ($P < 0.05$) in the growth parameters as compared with those obtained when non-supplemented modified peat hydrolysates were used.

Fractionation of humic substances of peat was completed to identify the growth inhibiting components. The fulvic acid fraction was found to show inhibitory effect on the growth of the fungus while the humic acids fraction was stimulatory.

Hydrolysates obtained from peat-fish offal compost were also used as substrates for the growth of the fungus. Growth curves have been constructed by growing *S. acidophilum* in peat and compost hydrolysates. The composition of biomass produced on peat hydrolysate was determined.

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ABBREVIATIONS

AOAC = Association of Official Analytical Chemists

DP = debituminized peat hydrolysate

DP-H = debituminized peat hydrolysate with humic acids removed

e = efficiency

HCl - P = peat hydrolysate obtained from hydrochloric acid hydrolysis of peat

HNO_3 - P = peat hydrolysate obtained from nitric acid hydrolysis of peat

H_3PO_4 - P = peat hydrolysate obtained from phosphoric acid hydrolysis of peat

HPLC = High Performance Liquid Chromatography

H_2SO_4 - P = peat hydrolysate obtained from sulfuric acid hydrolysis of peat

P = peat hydrolysate

P-H = peat hydrolysate with humic acids removed

x = biomass concentration

y = yield

CHAPTER 1

INTRODUCTION

As reserves of petroleum and coal diminish and their costs rise, alternative chemical raw materials are becoming more important. Peat is one of these alternatives. Therefore, nations that have significant peat resources, including Canada, have proceeded to re-examine the commercial utilization of peat (Cooper *et al.*, 1984).

There is growing interest in peat and its utilization in Canada, where peat is found throughout the wetlands. In Newfoundland, the peatlands cover an estimated land area of 2,000,000 ha. Here, the peatlands are commonly referred to as 'marsh', and sometimes as 'mish', 'bog' or 'swamp'. Ecological studies of Newfoundland peatlands have revealed many varieties of bogs and fens. Deposits of peat in Newfoundland bogs differ from those in southern Canada (Auer, 1930) and some parts of the United States (Waksman, 1942, 1943; Rigg, 1925, 1940a, b, 1958). The most important factor determining the formation of a specific peat variety on a site is the source of nutrients. It has been shown that the raised bogs of interior Newfoundland have more acidic peats than those of coastal bogs. This difference may be attributed to the higher precipitation and greater sea-salt concentrations experienced by the coastal bogs (Pollet and Wells, 1977).

The developments in the manufacture of agricultural and industrial products

using peat as an alternative organic raw material has resulted in intensive monitoring of peatlands and the development of technology for the utilization of peat (Taylor and Smith, 1980).

1.1 Peat

1.1.1 Definition

Peat is an organic soil accumulated from partly decomposed plants and decaying microorganisms under wet conditions where oxygen is limited or excluded (Chang, 1985). The formation of peat is influenced by biological, geological and climatic factors. Peat contains a complex mixture of organic materials in which the more chemically stable residues of plant tissues are predominant (Fuchsman, 1980). *Sphagnum* peat consists mainly of various *Sphagnum* species, slightly humified with leaves and stems partially identifiable and mostly intact. The color of peat varies from light brown to darker shades of brown. The highly humified (decayed) peats are amorphous and black while the less-decomposed peats are fibrous and brown. Characteristically, peat develops as individual mires (the accepted international term for bogs, swamps, fens etc.) in basins, hollows or valleys. Under climatic inducement and in the absence of tree-cover, peat may also form a thin blanket over the land surface (Taylor and Smith, 1980). The water-holding capacity is the most important feature of commercial *Sphagnum* peat moss, and ranges from 18 to 27 times the dry weight of the peat (Swinnerton, 1958).

1.1.2 World peat reserves

Peat reserves are distributed all over the world. Table 1.1 lists the countries in which the major peat resources are found. The large peat deposits of temperate Europe, Asia, and North America date from the Ice Age. The peat at the bottom of these bogs is mostly less than 10,000 years old (Naucke, 1966). In Northern Canada peat occurs in shallow deposits covering large areas, but in Southern Canada, it is mostly scattered in isolated deeper deposits. 'Reserve' is the term used to refer an area containing exploitable peat while the 'resource' refers to the total amount of peat present in the area of study. Therefore, the reserves may constitute only a small portion of resources. Canada has the second largest peat resource in the world. The reserves of peat in Canada consists of the 348 exploitable mires that exceed 40 ha in area, and the area encomposed by these mires totals 278, 000 ha (Fuchsman, 1980).

1.1.3 Deposition

The rate at which the peat accumulates varies from place to place depending on the kind of vegetation on the ground. The average rate of deposition is 1 meter of peat per 3000 or 4000 years (Fuchsman, 1980). This accumulation continues as long as the bog plants can live and die on the surface, and decomposition of peat by microbes is less than phytoproductivity. The accumulation of peat ceases when evapo-transpiration is more than precipitation or input of water from other sources. The peatlands of the world contain approximately 150,000

Table 1.1 The countries with the highest peat resources[1]

Country	Area of peat resource (Million ha)
U.S.S.R.	150
Canada	100
U.S.A.	38
Finland	20
Sweden	7

[1] Kevinin, 1980.

million tons of carbon. They represent, by extent and weight, one of the world's major untapped organic resources (Taylor and Smith, 1980).

1.1.4 Classification

Peat can be classified according to geological, botanical and physico-chemical characteristics. Geologically, a peatland is said to be "low-moor" if the bog water system is continuous with the mineral ground water system. These peatlands are transitional in character and are often covered with water. The second type is "high-moor" peatland, whose water system is significantly above the ground water system. The relationship between the peat and the ground water system controls the availability of dissolved inorganic material to the bog plants (Fuchsman, 1980).

The botanical classification of peat is based on the identification of plants that have grown most profusely in the bog. High-moor peats are mostly characterized by mosses (*Sphagnum*) and low-moor peats by woody plants or sedges. The botanical observations are used to supplement and confirm the geological classifications, and are applicable mainly to the peats of northern and temperate climatic regions.

The physico-chemical classification of peat is based on the degree of decomposition or humification. In general, low-moor peats are more decomposed than high-moor peats (Fuchsman, 1980). The main varieties of peat can also be dis-

tinguished by their acidity, and the amount of ash produced by burning the peat. The ash content of high-moor peat is less than that of low-moor peat (Fuchsman, 1980).

1.1.5 Composition of peat

Chemically, peats are primarily composed of organic material, therefore, peat which has been dried and burned leaves little ash. The composition of peat varies with the location and the depth of the mire from which it originates. Subsites within a given mire may also differ in composition. The variances in the chemical composition of peats result from differences in the extent of decomposition of the mire plants. Peat principally consists of carbohydrates, proteins, lipids, polyphenols, small amounts of nucleic acids, pigments, alkaloids, vitamins and other organic substances, along with inorganic materials (Fuchsman, 1980). The chemical composition of *Sphagnum* peat is listed in Table 1.2. Natural peat contains about 80-90 % of water on wet weight basis.

Various components of peat can be classified as "bitumens" (those substances that can be dissolved by suitable organic solvents), "humic acids" (characterized by their solubility in aqueous alkali), "carbohydrates" (the components which are removable by hydrolysis with acid), and "lignins" (the cementing material of living plants). In addition, small quantities of simple sugars, free-amino acids and other water-soluble components are present. The cellulose and hemicellulose content of peat carbohydrates decreases with increased decom-

Table 1.2 Chemical composition of *Sphagnum* peat[1]

Components	Range of content (%)[2]
Bitumen	3-9
Hemicellulose	9-21
Cellulose	10-24
Lignin and humic acids	26-64
Protein	6-7
Total reducing sugars	20-42
Total ash	2-3

[1] Fuchsman, 1980

[2] Dry weight basis

position (Puustjärvi and Robertson, 1977). Generally the content of monosaccharides in peat indicate that peat containing smaller particles are more decomposed than peat containing coarser materials (Morita and Levesque, 1980). Ion-exchange chromatographic results indicate that the amount of hexose containing carbohydrates decreases as the degree of decomposition of peat increases (Morita and Montgomery, 1980; Morita, 1981). It has been reported that the nitrogen content of peat increases with decomposition (Puustjärvi and Robertson, 1977).

A correlation appears between the depth from which the sample is obtained and the concentration of crude fats, nitrogen, total reducing substances and cellulose in the peat. The content of crude fat increases with depth, while the concentration of other constituents in the peat decreases (Black *et al.*, 1955). Cold-water soluble matter in peat ranges from 1% at top to 3 % at the bottom.

1.1.6 Carbohydrates

Carbohydrates comprise a large fraction of the organic matter of peat. Carbohydrates are grouped as: (a) water-soluble, easily hydrolyzable, *i.e.*, hemicelluloses, glycosides, pectins, and (b) water-insoluble and difficult to hydrolyze, *i.e.*, cellulose. Acid hydrolysis may be employed to convert much of the cellulose into fermentable sugars (Fuchsman, 1980).

A qualitative chromatographic examination of peat hydrolysates revealed the presence of galactose, glucose, mannose, arabinose, xylose, and trace amounts of

fucose (Black *et al.*, 1955). In acidic hydrolysates of peat, hexoses represented more than 50 % of the total content of reducing sugars, of which, glucose was the major hydrolysis product in sulfuric acid hydrolysate of peat (Le Duy, 1981; Morita and Montgomery, 1980; Morita and Levesque, 1980).

Various processes have been tested for the conversion of carbohydrates to easily fermentable sugars. Several operating variables such as type of acid, concentration of acid, temperature, holding time, ratio of peat : acid in the acid hydrolyzation process of peat were analyzed to obtain a maximum yield of carbohydrate (mg of total carbohydrate per gram of dry peat) (Martin and Bailey, 1984). A steam explosion process has been used for the production of fermentable carbohydrates. In this process the lignin fraction is also recovered (Forsberg *et al.*, 1986).

1.1.7 Bitumens

Bitumen, characterized by high concentration of methylene units in the hydrocarbon structure, is the component of peat which dissolves in hot organic solvents (Fuchsman, 1980). The extraction of this component, termed as debituminization, is achieved by using a non-polar solvent, thus removing the hydrophobic materials from the surface of the peat particles. This procedure renders the peat more amendable to treatment with aqueous reagents.

The solvents chosen for bitumen extraction range from less powerful solvents

to powerful solvents. Mixture of solvents have been used to obtain high yields of bitumens (Passer *et al.*,1968; Kander *et al.*, 1963). The yield of bitumen extracted is also dependent on the type of peat sample, and the type of process used for pre-drying of the samples (Kander *et al.*, 1963).

The bitumen obtained contains mixture of waxes and resins. Peat wax, a water-repellent solid at room temperature, is a mixture of esters, acids, alcohols and hydrocarbons. These components are soluble in aliphatic hydrocarbons. Resins are soluble in low-boiling alcohols. Another component of bitumen, the asphalts, are characterized by their solubility in light petroleum ether or in hot methanol (Cooper *et al.*, 1984). When the peat hydrolysates of debituminized peat are used to grow *Candida utilis*, high concentrations of biomass are produced at all levels of pH tested (Chang, 1985).

1.1.8 Humic acids

It has been reported that when decayed plant materials were extracted with alkali, they produced a black liquid, which on acidification gave a brown colored powder. It was identified as "humic acids" (Arnold *et al.*, 1935). Later, humic acids have been defined as the brown colored fraction of peat which is soluble in alkaline solutions but insoluble in acids (Fuchsman, 1980, 1983; Haworth, 1971; Smith and Lorimer, 1964). Humic acids give peat its water retentive character and its high cation-exchange capacity. Such properties justify treating peat as a distinctive class of naturally-occurring organic matter (Fuchsman, 1983).

The origin of humic acids in peat is not clear. Although, humic acids show a phenolic structure similar to lignin (Fuchsman, 1980) the carboxylic acid content of humic acids constitute a major distinction from lignins, since the lignins do not contain carboxy groups. The solubility of humic acids in mild alkaline solution reflects this difference in chemical structure between the humic acids and lignin (Fuchsman, 1980).

Humic acids are not a constituent of living organisms (Puustjärvi and Robertson, 1977). Instead, they contain a variety of compounds that were originally in the undecomposed plant material together with substances formed as a result of microbial decomposition. Fuchs, (1930) has tentatively proposed a formula for humic acids as $C_{60}H_{39}O_{19}(COOH)_4(OH)_4(-O-)(CH_2CO)(OCH_3)$.

Extraction of humic acids from peats generally requires a preliminary removal of the bitumens (Lishtvan and Korol', 1975; Smith and Lorimer, 1964; Haworth, 1971; Passer *et al.*, 1968; Dragunov, 1968). However, some other researchers have worked on humic acids derived from peat in which the bitumens were not removed (Souci, 1938a; White, 1957a).

Alkaline extraction of humic acids is still in question since humic acids oxidize in the presence of air (Kondratiev *et al.*, 1940; Arnold *et al.*, 1935; Passer *et al.*, 1968). To avoid this problem, Passer *et al.*, (1968) conducted the alkaline extraction under an atmosphere of nitrogen. A fraction of the lignin in peat will dissolve during the alkaline extraction and reprecipitate with mineral acids

(Arnold *et al.*, 1935). The type of alkali used and the severity of the treatment affects the solubility and oxidation during the extraction (Fuchsman, 1980).

Yields of 29-37 % humic acids can be obtained from peats without the prior removal of bitumens and other components (White, 1957a). The yield of a process depended upon the volume of NaOH used, the degree of agitation and the duration of the extraction process. Haworth (1971), using peat from which bitumens had been removed, reported that the humic acid content seldom exceeded 20 %.

Humic acids contain 4-6 mg nitrogen per milliequivalent of humic acids. This level is 1.5-2.0 times higher than the rest of the peat (Rakovskii and Pal'min, 1965). Humic acids have been reported to stimulate the growth of crops (Dragunov *et al.*, 1973; Reutov and Kravchenko, 1973; Sakurai, 1977). It has been reported that some microorganisms can utilize humic acids as a source of carbon and nitrogen (Mathur and Paul, 1966).

1.1.9 Lignins

Lignin is one of the components of living plants, and like humic acids, lignins contain phenolic structures. In peat, lignins are the organic residues remaining after the bitumens, hemicelluloses, humic acids and cellulose have been removed (Lishtvan and Korol', 1975). Woody plants are richer in lignin than herbs and bryophytes, and therefore lignin content of peats vary with their

botanical origin.

1.2 Uses of peat

Peat can be used for agricultural, metallurgical, energy and medicinal purposes (Lishtvan, 1981). Germany, the Soviet Union, Finland, and Ireland have been the major contributors to the understanding of the complexities and possibilities of utilizing peat as a raw material for chemical production. The rapid burning properties, the low ash and sulfur contents of dehydrated peat make it a potential substitute for oil in many utility and industrial applications (Rohrer, 1981).

The organic solvent extraction of peat produces waxes that are useful in pharmaceutical chemistry and industrial applications (Fuchsman, 1981). Acid hydrolysis of peat produces sugars for the production of high-protein livestock feed and alcohol by fermentation. It has been suggested that the residues from the acid hydrolysis of peat can be used as agricultural fertilizers (Sorokina and Yanchevskaya, 1977), or as horticultural fertilizers (Martin and Scott, 1987). Pyrolysis of peat leads to high purity metallurgical coke, and activated carbon. Alkaline extraction produces humic acids which are useful in a variety of surface-active agents, such as viscosity modifiers for the plastic and adhesive industries (Fuchsman, 1980). Peat humates have been reported to improve the flow properties of Portland cement mixtures (Rohrer, 1981).

Peat is also a potential source of organic materials such as phenols, nitrogen based products, and some aromatics, which are useful for the production of plastics, plant protectives and pharmaceuticals. Peat tar is used as an organic intermediate. Furfurals and polyhydric alcohols have also been produced from peat carbohydrates. Peat contains a high proportion of nitrogenous compounds of both organic and inorganic nature (McLoughlin and Küster, 1972a). The complex mixture of N-containing organic substances present in peat offers the potential for its use in a variety of medicinal products. Torfot, a preparation from peat, is used in the treatment of ophthalmic diseases, such as myopia (Nikitskii, 1964). Wrobel, (1974) stated that a preparation derived from peat, which he identified as TK_2, had favorable effects in asthma patients. In addition to the above mentioned products, some bactericidal preparations have also been prepared from peat (Fuchsman, 1980).

In Britain, experiments have shown that peat is a potential source of gaseous fuel (Morita, 1980). Studies have been conducted in Canada on the possible use of peat as a source of energy (Gallo and Shepperd, 1981; Chornet *et al.*, 1981). Peat is also used for horticultural and agricultural purposes in North America and Europe (Fuchsman, 1980; Sinclair, 1981). In Newfoundland, about 2 % of the total peat reserves have been used for afforestation trials and agricultural purposes (Pollet, 1972). Peat soils in Europe are extensively used for forestry and pasturage, while in Canada they are used mainly for vegetable production (Jasmin and Hamilton, 1977).

Peat also shows preservative properties. Recently, well preserved human skeleton and brains of 8,000 years old were recovered from the peatlands of Florida. The brains preserved in peat environment have yielded the oldest human DNA. It has been reported that peat baths are used in reducing body pains. North America's first peat fueled power plant is under construction in coastal Maine and is expected to feed Boston Edison Grid by 1989 (Levathes, 1987).

1.2.1 Peat as a fermentation substrate

The carbohydrate fractions are the focus of interest when peat is viewed as a fermentation substrate. Cellulose is the carbohydrate present in greatest quantity in most peats. They can be dissolved and then hydrolyzed by dilute mineral acids (Waksman and Hutchings, 1935). Of the polysaccharides present in peat, the cellulose is the most difficult fraction to hydrolyze. Cellulose is a homopolymer consisting of glucose units. Its conversion to glucose is important because it is the primary assimilable sugar for industrial fermentation processes (Fuchsman, 1980).

Hydrolysis is the principal technique employed in the conversion of peat polysaccharides to fermentable sugars. Hydrolysis of polysaccharides requires the addition of a molecule of water to effect the cleavage of monomeric glycosidic bonds with the aid of acids as promoters of the hydrolysis of oligomers. The primary step in hydrolysis is the initial breakdown of the cellulose to a water-soluble

form, followed by a second step in which these oligomers are converted to monosaccharides.

The sugars extracted from peat can serve as a substrate in such fermentation processes as microbial biomass production, and in other processes (Quierzy *et al.*, 1979; Le Duy, 1981; Boa and Le Duy, 1982; Mulligan and Cooper, 1985; Martin and White, 1985, 1986; Forsberg *et al.*, 1986). The second largest application of peat in the Soviet Union is as a raw material for the production of high-protein yeast (Carter, 1981). Recently, Martin (1986) has reviewed the fundamental process aspects for the production of mushroom mycelium in submerged culture with peat extract as the main nutrient source.

1.3 Peat hydrolysates

The liquid fraction produced from peat hydrolysis is called peat extract (Fuchsman, 1980; Martin and White, 1985, 1986) or peat hydrolysate (Boa and Le Duy, 1982). The peat hydrolysate produced by dilute acid hydrolysis in an autoclave constitutes the most appropriate culture medium (McLoughlin and Küster, 1972a). According to Quierzy *et al.*, (1979), acid-catalyzed peat hydrolysis produces a fermentable total carbohydrate concentration due to the efficient hydrolysis of cellulose. The steam explosion method has also been reported for the production of solvents (Forsberg *et al.*, 1986). Water extracts obtained during the drying of fuel grade peat extracts have been used as a substrate to produce extracellular polysaccharides (Mulligan and Cooper, 1985).

1.3.1 Production

Various extraction procedures have been used to obtain the nutrients from cellulose for further utilization as a substrate to produce microbial protein. The major methods of conversion of cellulose to glucose are by steam (Buchholz *et al.*, 1981); acid (Eklund *et al.*, 1976; Le Duy, 1979); alkali (Datta, 1981; Han, 1975) and the enzyme cellulase (Goldstein, 1980; Reese *et al.*, 1972).

Concerning peat hydrolysis, the processes have been reported to be influenced by factors such as reaction temperature, retention time, type of catalyst used, ratio of peat : catalyst (McLoughlin and Küster, 1972a, c; Chang, 1985; Quierzy *et al.*, 1979; Boa and Le Duy, 1982) and particle size of peat (Martin and Bailey, 1984; Morita and Levesque, 1980). It has also been reported that waxy substances, such as bitumens and water-insoluble components of peat affect the successful effectiveness of acid hydrolysis (Chang, 1985).

1.3.2 Composition

1.3.2.1 Carbohydrate-derived components

The carbohydrate components obtained by the hydrolysis of peat are of primary consideration in the production of microbial protein. Water-soluble and easily hydrolyzable substances constitute 42-54 % of peat, the cellulose fraction being about 24-27 %. One-half of the water-soluble substances are the reducing sugars. About 70-90 % of the reducing sugars are monosaccharides. As a result

of hydrolysis, 90-95 % of easily hydrolyzable components and 40-60 % of the cellulose are removed in the liquid phase. Under optimal conditions, about one-half of the organic content of peat goes into solution (Fuchsman, 1980). This fraction constitutes 70-77 % of the initial carbohydrate content of peat.

Peat hydrolysate contains mostly monosaccharides (Fuchsman, 1980). The lesser fraction consists of non-volatile organic acids such as sugar acids, hydroxy carboxylic acids, and C_2 - C_5 dicarboxylic acids. Sugar acids comprise 2-8 % of the organic material in the hydrolysate. Volatile acids comprise 0.7 to 0.9 %, and non-volatile organic acids account for 5-6 % of the hydrolysate. A group of non-acidic, unclearly characterized "humic acids" are also found in the hydrolysate (Fuchsman, 1980). Le Duy (1981) has reported that the major sugar in H_2SO_4 - hydrolyzed peat hydrolysate was glucose while xylose was the major sugar in HCl- P hydrolysate. Chang (1985) reported that HCl- P hydrolysate contained slightly higher concentrations of reducing sugars and total nitrogen than H_2SO_4 hydrolysate of peat.

1.3.2.2 Nitrogen compounds

Nitrogen constitutes 1-3 % of the dry weight of the peat, but only a part of it (which is in the form of amino acids) appears in the peat hydrolysate. Much of the nitrogen in peat is present in the form of polypeptides which are loosely bound to humic acids. The polypeptides, under appropriate conditions of hydrolysis, yield amino acids. Other nitrogen-containing compounds, such as glu-

cosamine, are also detectable in peat hydrolysate (Fuchsman, 1980).

Peats vary in the quantity and in their nature of nitrogen-containing compounds (Fuchsman, 1980). The amount of nitrogen released by 6 N HCl hydrolysis is greater than the nitrogen released by concentrated H_2SO_4 hydrolysis. The amount of amino acid in the hydrolysate increases with concentration of H_2SO_4 in the solution (Fuchsman, 1980).

1.3.2.3 Other components

Vitamins B_1 and B_2 are present in small quantities in peat. They are partially destroyed during hydrolysis. Peat hydrolysates are rich in minerals. Calcium, iron, magnesium and sodium are present in higher concentrations in peat hydrolysate than cobalt, copper, potassium, manganese, nickel and zinc. Small quantities of steroids are also present in peat hydrolysates (Fuchsman, 1980).

1.3.3 Inhibitory components

It has been reported that the most widely studied biologically active materials obtained from peat are those that inhibit the growth of microorganisms (Fuchsman, 1980). The hydrolysis of peat yields both nutrients and growth inhibitors. Therefore, peat hydrolysate might have an inhibiting, promoting or no effect on microbial growth and product synthesis (McLoughlin and Küster, 1972b, c). McLoughlin and Küster (1972a) have reported that peat hydrolysate produced from alkaline extraction and benzene : ethanol extraction inhibits the growth of *Candida utilis* .

The extraction of peat by HCl results in high concentrations of NaCl in hydrolysates which have an inhibitory effect on the growth of microorganisms (Dady and Chang, 1983). The effects of humic acids and bitumens on the growth of microorganisms are unclear. Chang (1985) observed that the yield of *Candida utilis* was increased when bitumen and humic acids were removed from peat hydrolysate. However, Dady and Chang (1983) reported a significant increase in the concentration of biomass of *Candida tropicalis* with the addition of synthetic humic acid up to a 0.5 % level. Martin (1983a, b) reported the need to dilute the peat hydrolysate with distilled water in order to reduce the concentration of the inhibitory substances and obtain higher yields of biomass of *Scytalidium acidophilum*.

1.4 Peat - fish offal compost

Peat has been used in the production of peat-fish offal compost. Studies have been conducted with different combinations of peat with fish, crab and seaweed (Mathur *et al.*,1986). The products met all the requirements for a high-quality compost. Lack of water-extractable aliphatic acids, and narrow C : N ratios proved the maturity of the compost. Subsequent studies showed that seaweed was not necessary for composting (Mathur *et al.*, 1986). The completion of composting was studied by using NMR (Preston *et al.*,1986). Tissue culture studies of mature composts showed an absence of toxins (Mathur and Johnson, 1987).

1.5 Compost hydrolysates

The liquid fraction obtained by treating peat-compost with H_2SO_4 is termed as compost hydrolysate. The proximate composition of the hydrolysate has been determined and further studies were conducted with compost hydrolysate as substrate for the growth of *S. acidophilum* fungus in this work.

1.6 Microbial biomass protein (MBP)

1.6.1 Definition

As the world's population increases rapidly, there is a growing need for the development of new unconventional sources of protein. The search for these sources of protein has resulted in the development of "microbial biomass protein" (MBP). The term MBP has recently been chosen to refer to single-cell protein, because the mass cultivation of multicellular fungi is also of commercial interest, in addition to the cultivation of single-celled bacteria and yeast (Moo-Young and Gregory, 1986). MBP compares very well with other high quality protein sources in terms of crude protein content and overall pattern of nutrients (Miller, 1968). Animal-feed experiments have shown that MBP is suitable as a supplement (Sibbald and Ivarson, unpubl.) and its refined form could be used to supplement human-diet (Dimmling and Seipenbusch, 1978; Litchfield, 1977). Microbial proteins are good dietary supplements because they are rich in lysine and low in sulfur-containing amino acids (Reed, 1982). The commercial production of mycoprotein for human consumption began in England in 1985. It has been sug-

gested that MBP produced from waste residues and surplus raw materials could help in the control of some forms of environmental pollution (Moo-Young and Gregory, 1986).

1.6.2 Microorganisms as MBP producers

For thousands of years people have used microorganisms in the production of foods such as alcoholic beverages, cheese, yogurt and soy sauce. Recently, the use of several types of microorganisms to produce protein has been studied. Certain types of algae, fungi, bacteria and yeast can produce valuable proteins which are used in aquaculture and also as mixed fodder constituent for intensive animal feeding.

Algae have been used to produce MBP because they have the photosynthetic ability, without the need of a carbohydrate substrate, to produce protein. Heterotrophic bacteria have also been studied as potential MBP sources. Several species of bacteria have been grown on hydrocarbons and carbohydrates. Indeed, their high rate of growth, metabolic versatility, and high content of protein have made bacteria an attractive candidate for MBP production.

Filamentous fungi have been used to produce protein condiments, and some drugs. Rapid growth rates, the ability to assimilate a variety of substrates, and high concentrations of protein have made yeasts an attractive choice as MBP producers. In choosing a microorganism as a dietary supplement, nutritional, toxicological, functional and cultural considerations are important (Miller, 1983-1984). Table 1.3 gives the proximate chemical composition of various classes of microbial biomass.

Table 1.3 Proximate composition of microbial biomass obtained from different microorganisms[1]

	Composition (%)			
Component	Filamentous Fungi	Algae	Yeast	Bacteria
Nitrogen	5-8	8-10	8-9	12-13
Protein	31-50	47-63	47-56	72-83
Nucleic acids	9-10	3-8	6-12	8-16
Ash	9-14	8-10	5-10	3-7
Lipids	2-8	7-20	2-6	2-3

[1] Reed, 1982.

1.7 Fungi

The filamentous structures which lack chlorophyll constitute the 'molds' of Kingdom Fungi. Filaments of mold are called hyphae, and a bundle of hyphae is called a mycelium. Molds reproduce by spores which are produced either sexually (as a result of mating between two different organisms or hyphae), or asexually (resulting from a simple internal division or external modification of individual hypha). The nutrient requirements of fungi are diverse. In general, fungal species need sources of energy, nitrogen and other micro-nutrients.

Fungi have been used as an indirect source of protein in diet of man for centuries. Many fungi can be grown in large quantities on inexpensive carbohydrate containing material such as molasses, vegetable waste, citrus wastes; or sulfite-waste liquor from industrial wood pulping processes (Dimmling and Seipenbusch, 1978; Lebaneiah *et al.*, 1979; Janardhanan *et al.*, 1970; Falanghe *et al.*, 1964). Because of this, fungal biomass has the potential to be used at the industrial level. High efficiencies in the conversion of carbohydrates to biomass protein have been reported for several fungal species (Robinson and Davidson, 1959).

1.7.1 Fungal protein

Many species of fungi produce mycelia with high concentrations of protein (Martin, 1983b). The usefulness of fungal protein depends upon its nutritional value, cost delivered to the consumer, and acceptability of the product. Different

substrates such as citrus wastes (Labaneiah *et al.*, 1979), peat hydrolysates (Martin, 1982; Martin and Bailey, 1983; Martin and White, 1985, 1986), and waste paper hydrolysates (Ivarson and Morita, 1982) have been used to produce the fungal protein.

Different values have been obtained for the content of crude protein in fungal biomass cultivated on various hydrolysates. *Morchella* species grown on peat hydrolysate have produced a protein content of 26 % (Martin, 1982) and on vegetable waste the protein content was about 26.7 % (Janardhanan *et al.*, 1970). On peat hydrolysate, *Agaricus campestris* contained 46.9 % protein (Martin, 1983b), whereas *S. acidophilum* contained 47 % protein (Martin and White, 1985).

1.8 *Scytalidium acidophilum* fungus

1.8.1 Classification

Scytalidium acidophilum belongs to the division Amastigomycota of the Kingdom Fungi. The division Amastigomycota is divided into four sub-divisions; Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. The sub-division Deuteromycotina contains class Deuteromycetes and this class is divided into three sub-classes, Blastomycetidae, Coetomycetidae and Hyphomycetidae. As Fungi imperfecti lack a perfect sexual stage, they are artificially classified into form-classes, form-orders, form-families, form-genera and form-species. The

fungus under investigation belongs to the form-genus *Scytalidium* of sub-class Hyphomycetidae. Figure 1.1 illustrates the classification of the fungus *Scytalidium acidophilum*.

1.8.2 Morphology

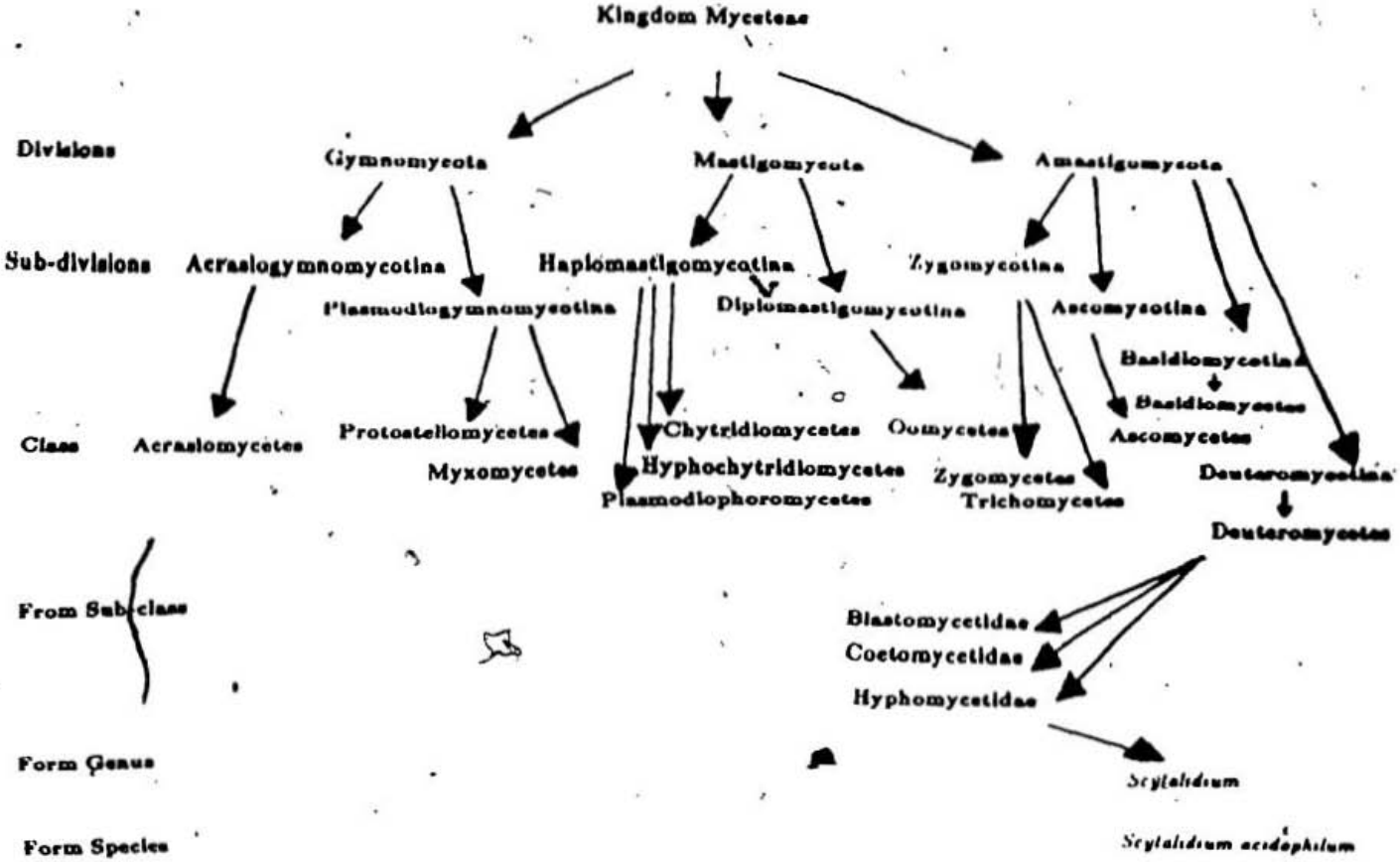
The new form-genus *Scytalidium* was established for six strains of fungi ; three isolated from acidic soil (pH 1.4-3.5) from a gas purification plant near Bowden, Alberta; another described by Starkey and Waksman (1943) and strain cultured by K.C. Ivarson in 1972 (Sigler and Carmichael, 1974; Ellis, 1971; Gould *et al.*, 1973; Starkey, 1973).

Colonies of *Scytalidium acidophilum* are moderately slow-growing. They reach a diameter of 21-26 mm in 21 days at 25 ° C. The colonies are flat and produce scant, velvety, aerial mycelium. An increase in addition of acid to the medium results in flatter colonies with more aerial mycelia (Sigler and Carmichael, 1974). The hyphae of this fungus are pale to medium brown in color and are septate. A yeast-like stage was reported by Miller *et al.*, (1984), when *S. acidophilum* was cultured on whey at pH 1.4. However, the yeast-like phase is favored by less acidic pH values (pH $\geq$ 1.4) (Sigler and Carmicheal, 1974).

1.8.3 Reproduction

S. acidophilum lacks a perfect sexual reproductive stage in its life cycle. Reproduction occurs by means of spores which are formed by the fragmentation

Figure 1.1 Classification of *Scytalidium acidophilum*


Kingdom Mycetese
Divisions
Gymnomycota
Mastigomycota
Amastigomycota
Sub-divisions
Acrasiogymnomycotina
Haplomastigomycotina
Zygomycotina
Plasmodiogymnomycotina
Diplomastigomycotina
Ascomycotina
Basidiomycotina
Basidiomycetes
Class
Acrasiomycetes
Protostelliomycetes
Myxomycetes
Chytridiomycetes
Hyphochytridiomycetes
Plasmodiophoromycetes
Oomycetes
Zygomycetes
Trichomycetes
Ascomycetes
Deuteromycotina
Deuteromycetes
From Sub-class
Blastomycetidae
Coelomycetidae
Hyphomycetidae
Form Genus
Scytalidium
Form Species
Scytalidium acidophilum

of hyphae. The spores, called arthroconidia, are formed in chains at terminal or intercalary positions. They are pale brown when young and later become darker and develop a thick wall. They are ellipsoidal, cylindrical or irregular shaped and show a constriction at the septum. Sporulation of *S. acidophilum* is enhanced in acid medium (Sigler and Carmichael, 1974). Other species of form-genera *Scytalidium* have been found to have a pycnidial stage (Ellis, 1976; Punithalingam and Waterson, 1970), but none have been observed in *S. acidophilum* (Campbell, 1974; Sigler and Carmichael, 1974).

1.8.4 Cultivation

S. acidophilum culture has been maintained on various media by several workers. The media includes (a) synthetic medium containing glucose, ammonium sulfate, magnesium sulfate, calcium chloride, and ferrous sulfate (Starkey and Waksman, 1943); (b) a medium of glucose with ammonium sulfate, potassium phosphate monobasic, potassium phosphate dibasic, magnesium sulfate, calcium chloride, and ferrous sulfate (Starkey, 1973); (c) nutrient broth (Gould *et al.*, 1974); (d) fries medium (Booth, 1971); (e) pablum cereal agar (Sigler and Carmichael, 1974); (f) modified synthetic medium (Ivarson and Morita, 1982); and (g) peat extract media (Martin and White, 1985, 1986). Larger scale experiments were conducted by transferring the culture to the modified synthetic medium of Ivarson and Morita (Miller *et al.*,1984), waste paper hydrolysate (Ivarson and Morita, 1982) or supplemented peat hydrolysates (Martin and White, 1985, 1986).

1.8.5 Reasons for using *S. acidophilum* as a MBP producer

The use of *S. acidophilum* has many inherent advantages. They include,

(a) the low pH values at which the fungus grows facilitates aseptic operation of the fermentation process

(b) a wide range of sugars can be fermented using the fungus,

(c) the fungus grows well in acidic environment; therefore only minimal neutralization procedures are necessary if acidic media are used as substrates

(d) the filamentous growth of the fungus permits low-cost filtration methods for mycelial recovery,

(e) the fungus is tolerant to high salt concentrations (Gould *et al.*, 1973; Starkey, 1973). Therefore, the pH of the hydrolysates can be adjusted by adding strong bases (Ivarson and Morita, 1982).

1.8.6 Submerged fermentation

Studies conducted with *S. acidophilum* on hydrolysates of waste paper (Ivarson and Morita, 1982) and peat (Martin and White, 1985, 1986) indicated that no contamination was observed during the fermentations. Freshly harvested, dried, ground mycelia gave yeasty odor which was reported to disappear after a few days (Ivarson and Morita, 1982). On hydrolysates of waste paper, the average biomass yields were about 38 %, and the protein content was 47.3 % (Ivarson and Morita, 1982). A content of 42.1 % protein and biomass of about 41 % were

observed on supplemented peat hydrolysates by studies of Martin and White, (1985) and Martin and White, (1986) respectively. Previous studies showed that the content amino acids in the mycelia obtained from different processes were relatively constant (Ivarson and Morita, 1982; Martin and White, 1985). Table 1.4 presents the content of protein in *S. acidophilum* cultivated on different hydrolysates. The composition of essential amino acids in *S. acidophilum* is shown in Table 1.5.

1.9 Present investigation

The growth of the acidophilic fungus *S. acidophilum* has been investigated in acidic peat hydrolysates. In order to enhance the biomass production of the fungus, different methods were employed to extract peat carbohydrates. The hydrolysates produced are employed as substrates to grow the fungus. In addition, the supplementation of peat hydrolysates with nutrients was also investigated. The objective of this study was to provide the information required for producing fungal protein utilizing peat as a raw material. Peat-fish offal compost has been used in some studies.

Table 1.1 Content of protein in the biomass of *S. acidophilum* and unidentified fungus cultured in various substrates[1]

Organism	substrate	% Protein[2]
S. acidophilum[3]	Waste paper hydrolysate	47.3±1.1
S. acidophilum[1]	Non-suppl. peat extract	29.2±1.9
S. acidophilum[1]	Suppl. peat hydrolysate	42.1±2.2
Unidentified fungus[4]	Glucose	35.1
Unidentified fungus[4]	Peat hydrolysate	24.3

[1] Martin and White, 1985

[2] % protein = % nitrogen x 6.25

[3] Ivarson and Morita, 1982

[4] Boa and Le Duy, 1982

Table 1.5 Content of essential amino acids (g/100 g protein) in the biomass of *S. acidophilum* cultured on waste paper hydrolysate[1]

Amino acid	Waste paper hydrolysate[2]	Peat hydrolysate[1]
Isoleucine	3.9±0.4	2.7±0.3
Leucine	6.1±0.4	4.9±0.5
Lysine	5.4±0.4	4.9±0.4
Methionine	1.4±0.4	1.5±0.2
Phenylalanine	3.4±0.2	2.9±0.2
Threonine	5.2±0.4	4.9±0.3
Tryptophan	nd	nd
Valine	4.9±0.4	3.0±0.3

[1] Martin and White, 1985

[2] Ivarson and Morita, 1982

nd = not determined.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 *Sphagnum* peat moss

A high-moor peat moss was obtained from the Sundew peat mire, St. John's, Newfoundland, Canada. Samples were collected from the upper layers of the peatland at a depth of about 20 cm. The peat was of a low degree of decomposition and had a humification value corresponding to H_2 of von Post scale (Fuchsman, 1980). The initial moisture content of the peat was approximately 80 %.

2.1.2 Culture

The culture of *Scytalidium acidophilum* ATCC 26774 was obtained from the American Type Culture Collection (Rockville, MD., U.S.A.). The culture was maintained on potato dextrose agar slopes, incubated for 2 weeks at room temperature, and then stored at 4°C. Fresh transfers were made every two months.

2.1.3 Chemicals

Unless specified, all the chemicals used in this work were of reagent or laboratory grade. The chemicals were obtained from Fisher Scientific Company, 18 Morris Drive, Darmouth, NS., and are listed below :

Acetic acid, acetic anhydride, ammonium carbonate, ammonium chloride, ammonium citrate, ammonium hydroxide, ammonium nitrate, ammonium phosphate (monobasic, dibasic), ammonium sulfate, barium hydroxide, buffer solutions (pH 1.0, 4.0, 7.0 and 10.0), calcium acetate, calcium hydroxide, calcium nitrate, calcium sulfate, chloroform (HPLC grade), ethanol, ether, ferric chloride, Kjeltabs-S 3,5, magnesium carbonate, magnesium chloride, potassium citrate, potassium dichromate, potassium hydroxide, potassium hydrogen phthalate, pyridine, and sodium molybdate.

The following chemicals were obtained from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO :

Ammonium ferric sulfate, anthrone, arabinose, boric acid, calcium chloride, glucose, galactose, mannose, manganous chloride, rhamnose, sodium carbonate, sodium bicarbonate, sodium hydroxide, urea and xylose.

The following chemicals were obtained from BDH Chemicals, 105 Akerley Blvd, Dartmouth, NS :

Acetone (HPLC grade), amberlite-XAD 2, benzene, diethyl ether, hydrochloric acid (concentrated, 1, 0.1N), hydrogen peroxide, hydroxylamine hydrochloride, magnesium phosphate, methylene blue, oxalic acid, potassium phosphate, sulfuric acid (concentrated, 10N, 1N, 0.1N) and toluene.

Cupric sulfate, ferrous sulfate, magnesium sulfate, potassium chromate and triethanol amine were obtained from J.T. Baker Chemical Co. Phillipsburg, NJ. Potato dextrose agar and yeast extract were obtained from Difco Company Methyl red was obtained from Aldrich Chemical Company, 940 West Saint Paul Avenue, Milwaukee, Wisconsin 53233.

2.2 Methods

2.2.1 Preparation of the synthetic medium

The synthetic medium was prepared according to the method described by Ivarson and Morita (1982). The composition of the synthetic medium is given in Table 2.1. *Scytalidium acidophilum* fungus was activated by repeated transfers in this medium until the response was similar. The culture of *S. acidophilum* grown on synthetic medium was used as the inoculum for experiments with peat hydrolysates.

2.2.2 Preparation of peat hydrolysates

The peat was air-dried for 2 days (moisture content of about 60 %) and used to produce peat hydrolysates according to the method of Martin and Bailey (1984). The procedure involved mixing peat with 1.5 % H_2SO_4 to give a ratio of 25 g of dry peat to 100 mL of acid solution. The resulting mixture was autoclaved at 121±1 °C for 2 hours. The liquid fraction which constituted the hydrolysate was separated using a Carver Laboratory Press (Model C, F.S. Carver Inc.,

Table 2.1 Composition of synthetic medium[1]

Component	Concentration
KOH	2.5 g
NaOH	2.0 g
$FeCl_3$	0.1 g
$MgCO_3$	25.0 g
Glucose	12.0 g
H_2SO_4 (10 N)	100.0 mL
NH_4OH (29 %)	5.0 mL
H_3PO_4 (pH 0.5)	6.3 mL
Trace element solution	2.0 ml
Distilled water to bring the volume to 1 L.	
Trace element solution	
H_3BO_4	120 mg
$NaMoO_4.2H_2O$	500 mg
$CuSO_4.5H_2O$	800 mg
$MnCl_2.4H_2O$	150 mg
Distilled water to bring the volume to 1 L	

[1] Ivarson and Morita, 1982.

WS., USA). The hydrolysate was then filtered using Whatman No. 1 filter paper under vacuum to remove any particles of peat present. This peat hydrolysate (P) was either used directly or diluted with distilled water at a 1:1 ratio when used for further experiments.

Other hydrolysates of peat were produced from peat by treating the air-dried peat with HCl, HNO_3 or H_3PO_4 according to the procedure described above. In this case, the hydrolysates were used, directly, without dilution in fermentations.

2.2.3 Modified-peat hydrolysates

The hydrolysates produced by treating the peats with H_2SO_4 after removing either the bitumen or humic acids or both fractions were termed 'modified-peat hydrolysates'. The procedures involved in the preparation of the modified peat hydrolysates are discussed in the following sections.

2.2.3.1 Debituminized-peat hydrolysate

Sphagnum peat moss was air-dried for 2 days and ground in a Waring blender. After being sieved through a wire mesh, the peat was extracted for 7 hours with toluene:ethanol (1:1) using a Soxhlet extraction apparatus. This solvent mixture resulted in the removal of the bitumens from peat. The debituminized peat was allowed to dry for 24 hours and then was treated with H_2SO_4 as described in Section 2.2.2 to produce the debituminized peat hydrolysate (DP).

2.2.3.2 Peat hydrolysate with humic acids removed

A modified Souci method (Fuchsman, 1986) was used to remove the humic substances from peat. The procedure included treating the finely ground air-dried peat with 1 % NaOH solution at 60 °C under a nitrogen atmosphere for 2 hours. The nitrogen atmosphere prevented the oxidation of the humic compounds during the alkaline extraction. The alkali extracts humic substances which contain both the humic and fulvic acids. The resulting mixture was centrifuged using a Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge to separate the solid residue from the dissolved humic acids. The solid residue was washed with distilled water 4-5 times to ensure the complete removal of dissolved humic substances. The remaining peat was treated with 1 % HCl solution until it was slightly acidic to the pH paper. Excess acid was removed by suspending the peat in distilled water and centrifuging. The solid obtained after centrifugation was dried in a Blue M Single Wall gravity convection laboratory oven at 60 °C for 12 hours. After drying, the sample was ground and the hydrolysate (P-H) was produced as described in Section 2.2.2.

2.2.3.3 Debituminized peat hydrolysate with humic acids removed

Air-dried, ground peat was treated first by toluene:ethanol (1:1) as in Section 2.2.3.1 to remove bitumens followed by the procedure to remove the humic substances as explained in Section 2.2.3.2. The hydrolysate (DP-H) from this modified-peat was produced according to the method in Section 2.2.2.

The modified-peat hydrolysates were used in further experiments without being diluted. The nomenclature used to identify growth media is presented in Table 2.2. All the media (peat hydrolysates from different acid hydrolyses of peat and the modified-peats) were adjusted to pH 2.0 using 15 N NaOH. Prior to inoculation, the media were sterilized in an autoclave at 121±1°C for 20 minutes.

2.2.3.4 Extraction of humic and fulvic acids

The humic acids were separated from the peat according to the method of Schnitzer (1978). Ten grams of air-dried peat were weighed into a 200 mL flask and 100 mL of 0.1 N NaOH was added. The air present in the flask was displaced by nitrogen. The flask was mechanically agitated at room temperature for 24 hours. The supernatant was then separated from the residual peat by centrifugation at 10,000 rpm for 10 minutes. The peat was washed with distilled water and the washings were added to the supernatant. The pH of the supernatant and the washings was adjusted to 2.0 with 2 N HCl and the solution was allowed to stand at room temperature for 24 hours. The soluble fraction, fulvic acid, was separated from the coagulated humic acid fraction by centrifugation. Both fractions were freeze-dried.

2.2.4 Preparation of inoculum

The mycelial growth from one fresh potato dextrose agar slope was blended

Table 2.2 Symbols to identify the nutrient supplemented hydrolysates of peat

Nutrient supplement	Symbols for the growth media[1]			
5 g/L yeast extract	P^2	DP^2	$P\text{-}H^2$	$DP\text{-}H^2$
5 g/L K_2HPO_4				
–	P^3	DP^3	$P\text{-}H^3$	$DP\text{-}H^3$
5 g/L $(NH_4)_2SO_4$				
5 g/L K_2HPO_4				
+				
5 g/L $(NH_4)_2SO_4$	P^4	DP^4	$P\text{-}H^4$	$DP\text{-}H^4$
+				
0.4 g/L $MgSO_4$				
3 g/L yeast extract				
+	P^5	DP^5	$P\text{-}H^5$	$DP\text{-}H^5$
0.4 g/L $MgSO_4$				
3 g/L yeast extract	P^6	DP^6	$P\text{-}H^6$	$DP\text{-}H^6$

[1] P, DP, P-H, and DP-H symbolizes the peat hydrolysate, debituminized peat hydrolysate, peat hydrolysate with humic acids removed, and debituminized peat hydrolysate with humic acids removed respectively.

with 50 mL of sterile water for 30 seconds in a previously sterilized Waring blender. The blended mycelial suspension was aseptically inoculated into 50 mL of sterile synthetic medium in 250 mL Erlenmeyer flasks. An inoculum ratio of 5 % (v/v) was employed in all the experiments. It has been reported that propagation of the *S. acidophilum* in the synthetic medium contributes to the adaptation of the fungus to grow in submerged fermentation with peat hydrolysate as the main nutrient source (Martin and White, 1984).

The cultured medium activated after incubating for 8 days at 25±1 °C in the synthetic medium flasks was used to inoculate the peat hydrolysate media. Inoculated media were incubated in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co., Inc., Edison, NJ, USA), at 25±1 °C for 8 days, with an agitation of 150 rpm. The pH of all the growth media were adjusted to 2.00 (unless otherwise specified) before inoculation.

2.2.5 Growth of *S. acidophilum* in the synthetic medium

Preliminary studies were conducted with synthetic medium in order to determine the optimal inoculum ratio for adding cultured synthetic media to peat hydrolysate media fermentations and to determine the the necessity of pH adjustment. Two ratios of 5 % and 10 % (v/v) inoculum additions were studied. The results were expressed as yield and efficiency. Yield was defined as the grams of dry biomass produced per gram of total carbohydrate consumed. Efficiency is the grams of dry biomass produced per gram of carbohydrate initially supplied. Both

these parameters are expressed as percent in fermentation processes.

2.2.5.1 Effect of humic acids and fulvic acids on the growth of *S. acidophilum*

In order to study the influence of humic acids on the growth of *S. acidophilum*, studies were conducted where humic acids were added to synthetic medium. The humic acid levels studied were 0.00, 0.10, 0.15, 0.20 and 0.23 % at pH 2.00 and 0.12, 0.25, 0.37, and 0.50 % (w/v) at pH 8.00. The growth parameters, such as biomass concentration, yield and efficiency were recorded for each level and the results were compared. Studies were also conducted by adding the fulvic acid fraction of the peat in 0.25, 0.50, and 0.75 (w/v) levels to synthetic medium at pH 2.00 and pH 8.00.

2.2.5.2 Various carbon sources

Experiments were conducted using different sugars as carbon sources. The glucose of the synthetic medium was replaced with arabinose, galactose, mannose, rhamnose or xylose in separate experiments. The results obtained with the substitute sugars were compared with those obtained by using glucose as the reference in order to determine the most favorable carbon source. The concentration of the carbon source in each case was 12 g/L.

2.2.5.3 Glucose and yeast extract

Experiments were conducted with yeast extract (1,3, or 5 g/L) which were individually supplemented to 15 g/L level of glucose in order to enrich the nitrogen content of growth media.

2.2.6 Growth of *S. acidophilum* in non-supplemented peat hydrolysates

The peat hydrolysates obtained from H_2SO_4, HCl, HNO_3, and H_3PO_4 acid hydrolyses of peat were used as substrates for culturing of the fungus. The total carbohydrate content of these hydrolysates were calculated to determine the composition of appropriate growth media for the growth of the fungus.

2.2.6.1 Effect of nutrients on the growth of the *S. acidophilum*

To enhance the growth of the fungus, the peat hydrolysates were supplemented with nutrient mixtures. They include: 5 g/L yeast extract; 5 g/L K_2HPO_4 + 5 g/L $(NH_4)_2SO_4$; 5 g/L K_2HPO_4 + 5 g/L $(NH_4)_2SO_4$ + 0.4 g/L $MgSO_4$; 3 g/L yeast extract + 0.4 g/L $MgSO_4$, and 3 g/L yeast extract.

A growth curve for *S. acidophilum* was obtained by culturing the fungus in peat hydrolysate.

2.2.7 Growth of *S. acidophilum* in modified-peat hydrolysates

The peat hydrolysates obtained from modified-peats as described in Section 2.2.3 were utilized as substrates for the growth of *S. acidophilum* . The effects of bitumens were observed by comparing peat with and without removal of bitumens. The mineral nutrients which are described in Section 2.2.6.1, were added in the media of modified-peat hydrolysate experiments.

2.2.8 Growth of *S. acidophilum* in peat-fish offal compost hydrolysate

Experiments were conducted with supplemented, as well as non-supplemented fish-offal compost hydrolysates. The symbols identifying the growth media in this study are presented in Table 2.3. A growth curve for *S. acidophilum* was obtained by culturing the fungus in peat-fish offal compost hydrolysates. The determinations of the concentration of biomass and total carbohydrate were carried out at 24 hour intervals.

2.3 Analytical methods

2.3.1 pH of the peat

The pH of the peat was determined according to the method of the A.O.A.C. (2.172a, 1980). The method involved weighing about 3.0 g of air-dried peat into a 100 mL beaker and adding 50 mL of H_2O. The peat was allowed to soak for 30 minutes with occasional stirring and the pH was read with a pre-standardized pH meter (Model 5652-00, Cole-Parmer Instrument Co.)

Table 2.3 Symbols used to identify nutrient supplemented peat-fish offal compost hydrolysates

Nutrient supplement	Symbols for the growth media [1]
5 g/L yeast extract	C^2
5 g/L K_2HPO_4 + 5 g/L $(NH_4)_2SO_4$	C^3
5 g/L K_2HPO_4 + 5 g/L $(NH_4)_2SO_4$ + 0.4 g/L $MgSO_4$	C^4
3 g/L yeast extract + 0.4 g/L $MgSO_4$	C^5

[1] C is the symbol for the peat-fish offal compost hydrolysate.

2.3.2 Total solids

The total solid content of the peat hydrolysates and compost hydrolysates were determined according to the method of Reusser *et al.*, (1958). The pH of the samples were adjusted to 7.0 with 10 N NaOH solution and the neutralized samples were transferred to a dry pre-weighed glass dish. The samples were evaporated to dryness in a vacuum oven at 70 ° C to constant weight.

2.3.3 Dissolved solids

About 50 mL of peat hydrolysate was filtered through a dry and pre-weighed Whatman No. 541 filter paper. The filter paper was dried at 70 ° C to a constant weight. The dissolved solid content of the peat hydrolysate was calculated as the difference between the total solids and the total weight of the dry residue on the filter paper per unit volume of the peat hydrolysate.

2.3.4 Total reducing sugars

Before the determination of the total reducing sugars, the peat hydrolysates were purified according to the method of Morita and Montgomery (1980). The process involved neutralizing 50 mL of the sample using a saturated aqueous solution of barium hydroxide. The mixture was centrifuged at 2000 x g for 30 minutes, and the supernatant was concentrated to 15 mL. The supernatant was purified by ion-exchange chromatography using 3 columns in series, 5 mL each of Rexyn 101 cation exchanger, Rexyn 201 anion exchanger and Rexyn 101 cation

exchanger, respectively. The sample was eluted from the columns with deionized water.

The content of total reducing sugar in the solution was estimated using the colorimetric method of Nelson and Somogyi (Hodge and Hofretier, 1962). The method involved the addition of an equal volume of low-alkalinity copper reagent to 1 mL of sample. A standard curve was constructed using at glucose concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/mL. The standards, as well as the sample solutions, were heated for 10 minutes in a vigorously boiling water bath and then cooled to room temperature. Two mL of arsenomolybdate reagent was added and the solution was mixed throughly to ensure the dissolution. The solutions were diluted to 25 mL with deionized water and allowed to stand for 20 minutes. The absorbances of the samples were read at 500 nm with a Beckman spectrophotometer (Model Du-8).

2.3.5 Total carbohydrate (TCH)

The content of total carbohydrate in the peat hydrolysates before and after fermentation were determined by the anthrone reagent method (Morris, 1948; Neish, 1952; Le Duy *et al.*, 1975). The method involved the dilution of the samples to the appropriate range and addition of 4 mL of anthrone reagent to 2 mL of the samples. The anthrone reagent was prepared by dissolving 0.2 g of anthrone in 100 mL of concentrated H_2SO_4. The absorbance was measured at 540 nm using a Beckman spectrophotometer (Model DU-8). The TCH was calculated

from the standard curve of glucose and the results were expressed as equivalent concentrations of glucose.

2.3.6 Monosaccharide analysis by G.C

The concentrations of arabinose, galactose, glucose, mannose, rhamnose and xylose in the peat hydrolysate were determined according to the modified method of Morita and Montgomery (1980). The peat hydrolysates were purified according to the procedure described for the determination of total reducing sugars (Section 2.3.4). The monosaccharide containing effluent from the columns were freeze-dried. Five mg of dry sample, was treated with 2-3 drops of sodium borohydride solution for 1 hour at room temperature in order to convert the monosaccharides to alditols. Excess sodium borohydride was neutralized with glacial acetic acid until the solution was neutral to pH paper. The solution was evaporated to dryness. The residue was washed with 5 % acetic acid in order to remove borate.

The sugars were then acetylated with a mixture of pyridine:acetic anhydride (1:1) for 15 minutes at 100 °C. The samples were evaporated to dryness and washed with methanol until the odour of pyridine is removed. The samples were dissolved in chloroform and then filtered through sintered glass funnel. The filtered fraction was evaporated to dryness and redissolved in chloroform. A 10μl aliquot was injected into a gas chromatograph for the analysis of monosaccharides.

Samples, and standard acetylated alditols were run on a Perkin Elmer gas chromatograph (Model 8310) fitted with dual columns (2 mm id x 180 cm) packed with 3 % Silar 10CP on 100-200 mesh Chromosorb WHP. Dual flame ionization detectors were used. An oven temperature of 200 ° C, and a nitrogen carrier gas flow rate of 56 mL/min were used.

2.3.7 Monosaccharide analysis by HPLC

The standard sugar solution for the analysis of monosaccharides of peat hydrolysate was prepared by dissolving 0.030 g of arabinose, 0.0334 g of galactose, 0.0454 g of glucose, 0.0086 g of mannose, and 0.083 g rhamnose, and 0.0316 g of xylose in 4 mL of HPLC grade water and the volume was increased to 10 mL by adding 6 mL of acetonitrile. The concentration of these sugar standards were made to match the expected values to be found in peat hydrolysate by H.P.L.C.

The sample was prepared by diluting the peat hydrolysate (H_2SO_4- P) in 1:1 ratio with distilled water, followed by neutralization to pH 7.0 with saturated $Ba(OH)_2$ solution. The resulting solution was filtered through Whatman No. 1 filter paper to separate the residue. The supernatant was evaporated to dryness using a Rotavap. Then, the sample was dissolved in 4 mL of water and 6 mL of acetonitrile was added to the solution. The mixture was thoroughly mixed and passed through an amino-cartridge. The clear sample collected was filtered through 0.45 μL filter.

A Waters Associate Liquid Chromatograph (pump Model 6000 A, Waters Assoc. Milford, MS., USA) was used in this study. Acetonitrile : water (80:20) (v/v) was degassed before using as the solvent. The flow rate was set at 1.5 mL/min. Two Supelcosil LC-NH_2 columns attached in series, {2 x (25 cm x 4.6 mm); Supelco Canada Ltd., Oakville, ON, Cat. No. 6-9068). These analytical columns were protected by using a guard column (2 cm x 4.6 mm, Supelco, cat no. 6-9068).

Injections of 25μL of the peat sample as well as standard solution were made using a 25μL syringe (Supelco. Cat. No. 5-8656) into a chromatograph injector system (Waters Assoc., Model U6K). Differential refractometer (Model R 401, Waters Assoc. Milford, MS. USA) were used to monitor the effluent. The reference cell of the refractometer was filled with the HPLC solvent. The chromatograms were recorded on a Barber Colman recorder (Model PR 25, E.H. Sargent & Co.,). The attenuation of detector was set at 2X and integrator was 4X during the recording of the chromatograms. The chart speed was 0.5 in/min and pressure during the experiment was 1000 psi.

2.3.8 Moisture

The contents of moisture in the peats were determined according to the method of the AOAC (7.003; 1980). The determination involved drying a quantity of sample containing approximately 1 g of dry matter to constant weight at 90 - 100 °C under vacuum.

2.3.9 Biomass

The biomass determination was carried out by filtering the fermented media through pre-dried Whatman No.1 filter papers. The filter papers containing the mycelial biomass were washed with distilled water to remove the fermentation broth and oven dried at 60 °C to constant weight. The dry weight of initial inoculum was subtracted from the dry weight of the total total biomass to obtain the dry weight of the biomass produced in each fermentation.

2.3.10 Total nitrogen and crude protein

The contents of total nitrogen in raw peats, peat hydrolysates and the biomass were determined by a modified micro-Kjeldahl method (AOAC 47.021; 1980). The method involved digestion, distillation, and titration. In the digestion step, 1 g of ground dry sample was placed in a Kjeldahl digestion tube. Two Kjeltabs (S 3,5) were added to each tube containing the sample and the blank. Twenty five mL of concentrated H_2SO_4 was carefully added to the digestion tubes and digested on Kjeltec Digestion System 6 (1007 digestor, Tecator Inc.,

Boulder, Colorado, USA). The digestion was continued until the sample turned colorless or clear. About 100 mL of water was carefully added as soon as the liquid reached room temperature.

To each digestion tube was added 50 mL of 40 % NaOH solution before connecting on to a Ammonia Distillation Unit (Kjeltec system 1002). The distillate was collected in 50 mL of 4 % boric acid solution with indicator. The distillation was continued until a total of 150 mL solution was collected. The flasks containing the distillate were capped tightly until further use in titration.

The third step involved the titration of the distillate against a standard solution of 0.1 M H_2SO_4. The values obtained for the titration were used to calculate the % nitrogen values. The content of crude protein was calculated from the % nitrogen content using the conversion factor (N x 6.25).

2.3.11 Total lipids

The content of total lipid in the peats, peat hydrolysates and the biomass were determined according to a modified form of method reported by Folch *et al.*, (1957). The method involved homogenizing 1 g of the powdered sample with 19 mL of chloroform:methanol (2:1 v/v) mixing with a polytron setting of 20 for 2 minutes. The homogenate was allowed to equilibrate and the final volume was adjusted to 20 mL with chloroform:methanol mixture (2:1 mixture). It was then filtered through glass wool washed with 7.5 mL of the chloroform:methanol mix-

ture. To the crude lipid extract was added an amount of 0.9 % NaCl solution equivalent to 20 % of its volume. The mixture was shaken vigorously and the phases were allowed to separate upon standing. The volume of the chloroform layer was recorded, and the water/alcohol layer was removed by siphoning. The inner surface of the cylinder was washed with methanol and the final volume was made up to 20 mL by the addition of the chloroform:methanol mixture. The mixture was allowed to separate into two phases by standing and the upper layer was removed as described before. The chloroform layer, which contained the lipids, was transferred to a weighed flask and evaporated to dryness in a oven at 50 ° C to remove the solvent. The lipid residue was redissolved in chloroform and the non-soluble portion was separated by filtration. The filtrate was finally evaporated to dryness and the flask was weighed to determine the total lipids.

2.3.12 Ash

The content of ash in peats, peat hydrolysates and the biomass was determined according to the method of the AOAC (14.006; 1980). The method involved weighing 1 g of sample into a previously dried, cooled porcelain crucible. The sample was ignited in a muffle furnace at 600.° C for 16 hours (until only a gray ash remained). The crucible and the contents were allowed to cool in a desicator to room temperature. The ash was moistened with distilled water to dissolve the soluble salts, dried slowly on a hot plate, and heated again at 600 ° C.

2.3.13 Amino acids

The previously dried samples were hydrolyzed with 6 N HCl under vacuum for 24 hours at 110° C. The samples were reconstituted with 0.6 M lithium citrate buffer and analyzed with a Beckman 121 MB amino acid analyzer using a single column method.

2.3.14 Total acidity

The total acidity of the humic acids obtained from the peat was determined according to the method of Schnitzer (1978). About 0.05 to 0.1 g of the humic material was weighed in a 125 mL ground-glass stoppered Erlenmeyer flask and 20 mL of 0.2 N $Ba(OH)_2$ solution was added. Simultaneously, a blank was made with only the 20 mL of 0.2 N $Ba(OH)_2$. The air in the flasks was displaced by N_2, and the flasks were stoppered carefully and kept on a shaker for 24 hours at room temperature. Afterwards, the suspension was filtered and the residue washed with CO_2-free water. The filtrate was titrated against 0.5 N HCl to pH 8.4. The total acidity was calculated as follows :

$$\frac{\text{(Volume of blank - volume of sample) X normality of acid X 1000}}{\text{weight of sample}}$$

= meq. of total acidity / g of humic material

2.3.15 Total carboxylic groups

About 50 mg of the humic acid material was weighed into a 125 mL ground-glass stoppered flask and 10 mL of 1 N $(CH_3COO)_2Ca$ solution and 40

mL of CO_2-free water were added. A blank was set up simultaneously. After shaking for 24 hours at room temperature, the suspension was filtered and washed with CO_2-free water. The filtrates were combined and titrated against a standard 0.1 N NaOH solution to pH 9.8. The calculation used to determine the total carboxylic groups of humic acids is as follows.

$$\frac{\text{(Volume of sample – volume of blank) X normality of base X 1000}}{\text{weight of sample in mg}}$$

$$= \text{meq. carboxy groups / g of humic material}$$

2.3.16 Phenolic hydroxy groups

The concentration of phenolic hydroxy groups was obtained by calculating the difference between the total acidity and the total carboxy groups (Schnitzer, 1978).

2.4 Statistical analysis

The data obtained from the shaker flask experiments represent the mean value of three experiments. The data for the proximate analysis of peats, peat hydrolysates, compost, compost hydrolysate, represent the mean values of three determinations. Comparison between the means were made using the Duncan's multiple range method-one way program in SPSSx subroutine of VAX at Memorial University of Newfoundland Computing Services.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Chemical composition of peats

The contents of carbohydrate, nitrogen, lipid, amino acid, and ash in the *Sphagnum* peat and the modified peats produced by removing either bitumen, humic acids or both were determined. The results of the chemical analyses of the peat and modified peats are presented in Sections 3.1.1 and 3.1.2.

3.1.1 Proximate composition of peat and modified peats

The proximate analysis of the peat and modified peats are presented in Table 3.1. The peat used in this work was acidic, with its pH ranging from 4.2 to 4.8, which agrees with the range reported by Smith *et al.*, (1958) and by Fuchsman (1980). However, this range is higher than the one reported by Pollett and Wells (1977) for some Newfoundland peats.

It has been reported that the content of moisture in peat is approximately 85 % by weight (Puustjarvi and Robertson, 1977; Fuchsman, 1980). In this work, the moisture content of the peat was reduced to approximately 67 % during storage. The modified peats showed low moisture contents because their preparation involved either air-drying or oven-drying. The contents of moisture in the modified peats was found to be in the range of 6 to 10 %.

Table 3.1 Proximate composition of the peat and modified peats[1]

Components	Content (%)[2]			
	Peat	Debituminized peat	Peat with humic acids removed	Debituminized peat with humic acids removed
Moisture[3]	66.6 ± 1.2^{a}	10.3 ± 1.0^{b}	6.1 ± 0.6^{d}	8.4 ± 0.9^{c}
Total solids[4]	33.4 ± 0.7^{b}	89.7 ± 1.0^{a}	93.9 ± 0.6^{a}	91.6 ± 0.8^{a}
Total lipids	4.8 ± 1.8^{b}	1.15 ± 0.01^{c}	10.7 ± 2.1^{a}	0.85 ± 0.03^{d}
Total nitrogen	0.66 ± 0.05^{a}	0.46 ± 0.05^{b}	0.38 ± 0.04^{b}	0.24 ± 0.05^{c}
Ash	4.3 ± 0.2^{c}	$4.8 \pm 0.9^{b,c}$	6.6 ± 1.4^{b}	10.6 ± 1.2^{a}

[1] Mean values of three experiments ± standard deviations.

[2] Dry weight basis.

[3] Percentage of wet weight.

[4] Determined by the difference between total dry weight and dry solids.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).

The amount of total solids was lower in the peat than in the modified peats. The content of total solids was higher in the peat from which humic acids had been removed than in the other modified peats. No statistically significant differences ($P > 0.05$) were observed between the content of total solids in modified peats.

In general, peat has a content of lipid ranging from 2 to 6 % on a dry weight basis (Fuchsman, 1980). The lipid content of the peat used in this work is comparable with the values reported by Black *et al.*, (1955). As expected, the content of lipid was higher in the peat from which the humic acids were removed than in debituminized peats.

The content of total nitrogen in the peat was within the range reported for Newfoundland peats (Pollet, 1972). The values of the modified peats were below the values observed for the peat. It has been reported that the nitrogen content increases with degree of decomposition (Puustjärvi and Robertson, 1977), and the depth from which the samples were obtained (Black *et al.*,1955). The content of nitrogen was lower in peats from which the humic acids were removed than in the other peats. Humic acids contain amino acids in their structure (Fuchsman, 1980). So, the removal of the humic acids could account for the lower nitrogen content (Fuchsman, 1980). The content of ash in the peat was within the range reported by Black *et al.*, (1955) and was slightly higher than the value reported by Fuchsman (1980).

3.1.2 Amino acid composition of the peat and modified peats

The content of amino acids in the peat and modified peats are shown in Table 3.2. In the peat the predominant amino acids were glycine, alanine, aspartic acid, serine, glutamic acid, threonine, and valine, in decreasing order of concentration. Generally, significant differences ($P > 0.05$) were not observed in the amino acids between the peat and the debituminized peat. As expected, when humic acids were removed, there were decreases in the concentrations of most of the amino acids of peat. It was observed that some amino acids, such as cysteic acid and hydroxyproline were decreased in concentration during the removal of humic acids. However, significant increases were observed in the concentrations of arginine and glycine in the same samples. This could be due to the location of these amino acids in peat. They show decreased concentration if they are a part of the humic acid complex. If amino acids are found in protein form, when the humic acids are removed from peat, their concentrations may show an increase in relation to the total peat weight.

3.2 Composition of peat and modified peat hydrolysates obtained from hydrolysis of peats with H_2SO_4

3.2.1 Proximate analysis

The proximate composition of peat and modified peat hydrolysates are given in Table 3.3. The main carbohydrate constituent of peat is cellulose. When

Table 3.2 Content of amino acids (%) in peat and modified peats[1]

Amino acid	Peat	Debituminized peat	Peat with humic acids removed	Debituminized peat with humic acids removed
Alanine	11.70 ± 0.67^{a}	10.99 ± 2.47^{a}	12.09 ± 2.35^{a}	11.59 ± 0.64^{a}
Arginine	3.06 ± 0.49^{b}	2.49 ± 0.25^{b}	11.11 ± 0.21^{a}	2.04 ± 0.04^{c}
Aspartic acid	9.88 ± 0.86^{a}	$7.76 \pm 0.94^{b,c}$	$9.07 \pm 0.42^{a,b}$	$9.03 \pm 0.30^{a,c}$
Cysteic acid	0.29 ± 0.00^{b}	0.54 ± 0.01^{a}	-----	-----
Glutamic acid	$8.47 \pm 0.94^{b,c}$	7.18 ± 0.59^{b}	9.9 ± 0.74^{a}	$8.70 \pm 0.20^{c,d}$
Glycine	$13.44 \pm 0.66^{b,c}$	11.98 ± 1.07^{b}	17.48 ± 0.86^{a}	14.69 ± 1.36^{c}
Histidine	$3.33 \pm 0.56^{a,b}$	2.43 ± 0.53^{a}	$5.95 \pm 2.20^{b,c}$	4.83 ± 0.24^{c}
Hydroxylysine	0.59 ± 0.13^{a}	0.19 ± 0.04^{b}	0.42 ± 0.28^{a}	0.46 ± 0.08^{a}
Hydroxyproline	1.24 ± 0.34^{a}	0.93 ± 0.02^{b}	-----	-----
Isoleucine	3.4 ± 0.02^{a}	3.99 ± 0.75^{a}	3.52 ± 0.33^{a}	3.88 ± 0.38^{a}
Leucine	5.61 ± 0.69^{a}	6.46 ± 0.74^{a}	4.38 ± 0.01^{b}	6.38 ± 0.51^{a}
Lysine	3.86 ± 0.28^{a}	$3.6 \pm 0.42^{a,b}$	4.07 ± 0.45^{a}	2.96 ± 0.21^{b}
Methionine	0.73 ± 0.00^{a}	0.64 ± 0.03^{b}	0.22 ± 0.00^{c}	0.66 ± 0.19^{b}
Phenylalanine	3.23 ± 0.26^{a}	3.54 ± 0.81^{a}	2.02 ± 1.00^{a}	3.16 ± 0.07^{a}
Proline	4.42 ± 0.99^{b}	7.61 ± 0.02^{a}	2.58 ± 1.00^{b}	6.51 ± 0.69^{a}
Serine	9.08 ± 0.08^{a}	7.64 ± 0.54^{b}	$8.53 \pm 1.20^{a,b}$	7.13 ± 1.28^{b}
Threonine	8.42 ± 0.52^{a}	7.47 ± 0.68^{a}	6.49 ± 1.43^{a}	7.06 ± 0.81^{a}
Tyrosine	1.24 ± 0.32^{a}	1.92 ± 0.09^{b}	0.48 ± 0.21^{b}	1.05 ± 0.35^{a}
Valine	7.53 ± 1.08^{a}	5.35 ± 1.73^{a}	5.90 ± 1.37^{a}	0.71 ± 0.40^{b}

[1] Mean values of three determinations ± standard deviations.
----- Trace amounts.
Values in the same row with the same superscript are not statistically different ($P > 0.05$).

Table 3.3 Proximate composition of hydrolysate of peat and modified peatss[1]

Component	Concentration (g/L)			
	P	DP	P-H	DP-H
TCH	49.0±0.4^{a}	24.1±0.6^{b}	26.4±2.4^{b}	18.2±0.9^{b}
Total reducing sugars	11.56±1.28^{b,c}	14.98±0.36^{a}	11.14±1.08^{b}	13.10±0.50^{c}
Total lipids	11.4±0.4^{b}	2.1±0.7^{c}	20.6±3.7^{a}	1.40±0.04^{c}
Total nitrogen	1.2±0.1^{c}	1.6±0.1^{a}	0.75±0.03^{d}	1.40±0.02^{b}
Ash	8.6±0.6^{d}	10.3±0.4^{c}	12.5±0.8^{b}	17.4±1.2^{a}
Total solids	105.3±3.2^{a,b}	101.45±0.14^{a}	115.23±2.16^{c}	109.33±4.35^{b,c}
Dissolved solids	74.88±3.18^{a}	42.24±0.41^{c}	56.07±1.92^{b}	54.2±0.16^{b}

[1] Mean values of three determinations ± standard deviations.
Values in the same row with the same superscript are not statistically different ($P > 0.05$).

hydrolyzed, this component produces simple sugars suitable for the use as carbon and energy sources in fermentation processes (Martin and Bailey, 1984). The hydrolysis process is designed to produce a maximal content of fermentable carbohydrates in the hydrolysate.

The total carbohydrate (TCH) concentration of the modified peat hydrolysates are lower than that of the undiluted peat hydrolysate, indicating that some carbohydrate must have been lost during the removal of bitumens and humic acids. Therefore, as might be expected, DP-H had a lower TCH value than any other hydrolysate.

The total reducing sugar content was the highest for DP hydrolysate when compared to other hydrolysates. There was no statistically significant difference ($P > 0.05$) for the total reducing content in P and P-H as well as P and DP-H hydrolysates. The reason for the high value for total reducing sugar in DP could be due to better digestion of these substances in absence of bitumens. Chang (1985) reported that debituminized peat produces higher values of total reducing sugar in the solution compared to the peat.

As expected, the content of lipid in the DP hydrolysates were the lowest and the P-H hydrolysate showed the highest lipid content. The lipid content of DP and DP-H were not statistically different ($P > 0.05$).

The content of total nitrogen in the peat hydrolysate increased with the

removal of bitumens. The contents of ash in P-H and DP-H hydrolysates were higher compared to those of the hydrolysates from which the humic acids were not removed.

The type of peat used, degree of decomposition of peat, temperature, and retention time for hydrolysis, and the method of separation of the hydrolysate will influence the concentration of total solids in peat hydrolysates (Manu-Tawiah, 1987). The content of total solid in the P and DP hydrolysates were not statistically different ($P \geq 0.05$). The dissolved solids were found to be higher for P than for the modified hydrolysates. This may be due to the removal of some of the dissolved solids during the modification protocols. No statistically significant differences ($P > 0.05$) were obtained for the dissolved solids in P-H and DP-H hydrolysates.

3.2.2 Content of amino acids

The content of amino acids in the peat and modified peat hydrolysates are presented in Table 3.4. Usually, significantly higher concentrations of amino acids were obtained in the DP compared to the P hydrolysate. The explanation for this could be the removal of bitumens facilitates better digestion of proteins into the solution. As it was observed in solid peat samples (Table 3.2), P hydrolysate contained higher concentration of alanine, aspartic acid, glycine, threonine and valine than the other amino acids. In general, lower concentrations of amino acids were observed in DP-H compared to those in P, DP, and P-H hydrolysates.

Table 3.4 Content of amino acids (%) in peat and modified peat hydrolysates[1]

Amino acid	P	DP	P-H	DP-H
Alanine	12.60±0.55^{b}	15.4±0.04^{a}	12.73±0.04^{b}	12.22±0.58^{b}
Arginine	1.56±0.00^{b}	1.76±0.01^{a}	0.57±0.00^{c}	0.57±0.08^{c}
Aspartic acid	14.75±0.76	16.24±0.04	15.91±0.73^{a}	15.14±0.02^{a}
Cysteic acid	1.60±0.51^{a}	-----	-----	-----
Cystine	0.14±0.01^{b}	0.33±0.01^{a}	0.10±0.01^{c}	0.05±0.04^{d}
Glutamic acid	6.15±0.47^{c}	7.36±0.06^{a}	7.50±0.20^{a,b}	7.88±0.20^{b}
Glycine	15.51±0.57^{b}	18.63±0.06^{a}	14.64±0.11^{b,c}	14.53±0.66^{c}
Histidine	1.49±0.03^{b}	1.99±0.04^{a}	0.92±0.01^{c}	0.98±0.05^{c}
Hydroxylysine	0.80±0.25^{c}	1.63±0.01^{a}	1.00±0.00^{b}	0.97±0.04^{c}
Hydroxyproline	1.51±0.00^{a}	-----	-----	-----
Isoleucine	2.82±0.06^{b}	3.86±0.00^{a}	1.81±0.03^{c}	1.9±0.1^{c}
Leucine	4.68±0.01^{b}	5.71±0.08^{a}	2.89±0.16^{c}	2.48±0.68^{c}
Lysine	2.73±0.06^{b}	3.42±0.06^{a}	1.17±0.10^{d}	1.39±0.06^{c}
Methionine	2.75±0.91^{a}	0.34±0.03^{c}	0.35±0.01^{c}	0.53±0.08^{b}
Phenylalanine	1.62±0.04^{b}	2.03±0.01^{a}	0.86±0.00^{c}	0.61±0.11^{d}
Proline	3.75±0.01^{b}	4.71±0.01^{a}	3.65±0.00^{c}	2.89±0.18^{d}
Serine	7.54±1.85^{a}	3.23±0.01^{b}	7.69±0.33^{a}	8.01±0.34^{a}
Threonine	9.53±0.01^{a}	6.07±0.28^{c}	7.57±0.41^{b}	6.76±0.42^{b,c}
Tyrosine	2.27±0.26^{a}	0.32±0.00^{c}	1.47±0.52^{a}	1.03±0.00^{b}
Valine	7.77±1.34^{a}	7.10±0.11^{a}	7.25±0.87^{a}	8.48±1.56^{a}

[1] Mean values of three determinations ± standard deviations.
----- Trace amounts.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).

3.2.3 Chromatographic analysis of monosaccharides

Carbohydrates are widely distributed in nature. Since they are present in nature in various forms and there are many isomers and analogues, separation of carbohydrates involves more difficult problems than those of proteins and nucleic acids (Kakehi and Honda, 1986). However, analysis of sugar and sugar mixtures is of considerable and growing importance in food industry (Aitzetmüller, 1978; Conrad and Palmer, 1976; Yang *et al.*, 1981).

Carbohydrates have been analyzed by classical chemical and enzymatic techniques (e.g., gas, paper, thin-layer, liquid-partition, ion-exchange, and gel-filtration chromatography). Though ion-exchange, liquid-partition, and gel-filtration methods have excellent resolving power, however, they are slow and time consuming. Rapid separations can be achieved by gas chromatography (GC), but only after formation of volatile derivatives. Recent developments in high performance liquid chromatography (HPLC) equipment and the microparticulate column packings allow direct and rapid determination of sugars, including oligosaccharides in food and beverage industry (Conrad and Palmer, 1976). It has been reported that HPLC offers a rapid analysis of a large spectrum of saccharides and requires a minimum sample preparation (Linder and Lawhed, 1975). Recent developments in HPLC combined with fluorescence spectroscopy (Kato and Kinoshita, 1980) as detector has been investigated to determine the component sugars in hydrolysates of soil polysaccharides (Hamada and Ono, 1984).

Several researchers worked on analysis of sugars from soil samples (Morita and Montgomery, 1980; Hamada and Ono, 1984). Their work included the technique of ion-exchange chromatography, gas chromatography, and HPLC combined with fluorescence spectroscopy. In the present work, the analysis of peat monosaccharides in the hydrolysates was carried out using the GC technique. In addition, preliminary studies on the usefulness of HPLC for carbohydrate analysis was carried out.

3.2.3.1 Analysis by GC technique

The temperature and duration of the hydrolysis procedure influences the amount of free sugar produced during the hydrolysis of peat. Longer reaction times at high temperature will result in a net decrease in free sugar concentration because of the destruction of free sugars (Le Duy and Laroche, 1983; Forsberg *et al.*, 1986). It has been reported that the amount of hexoses decreases with an increase in the decomposition of the peat sample (Morita and Montgomery, 1980). In general, 121±1 °C is considered as a optimal temperature to hydrolyze peat in order to use the hydrolysate as fermentation substrate (Martin and Bailey, 1984).

The content of major monosaccharides present in the hydrolysates of peat are summarized in Table 3.5. The values of monosaccharide composition in DP and P-H hydrolysates of this study are compared with those values in P hydrolysate taken from the study of Manu-Tawiah, 1987. The content of each of the monosaccharides in the peat hydrolysates are presented as percent of the total

Table 3.5 Content of monosaccharides in hydrolysates of peat[1]

	Percent of total reducing sugars		
Monosaccharides	P[!]	DP	P-H
Arabinose	2.48±[illegible][a,b]	2.40±0.02[a]	3.68±0.06[b]
Galactose	19.07±1.67[a]	15.83±0.11[b]	21.95±1.48[a]
Glucose	38.20±1.31[a]	23.16±1.23[b]	23.61±1.09[b]
Mannose	16.46±1.83[a]	17.96±0.05[a]	9.20±0.07[b]
Rhamnose	6.96±1.62[b]	13.68±0.90[a]	12.3±1.42[a]
Xylose	12.03±1.15[b]	23.63±1.10[a]	23.56±1.25[a]

[1] Mean values of three determinations ± standard deviations.

[!] Taken from Manu-Tawiah, 1987.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).

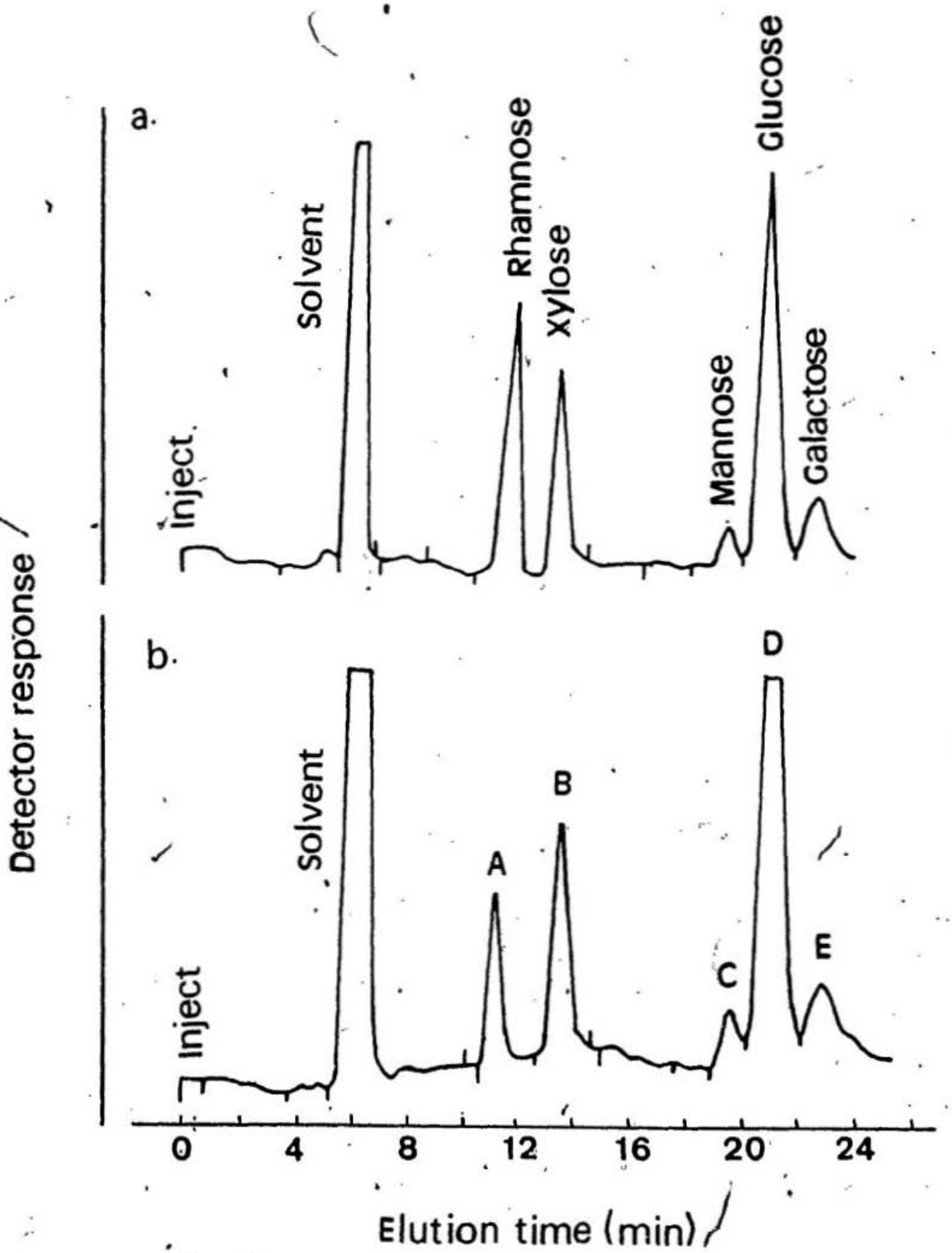
reducing sugars. Hexoses represent more than 50 % of total sugars in which glucose was the most abundant hexose sugar followed by galactose and mannose, except in DP hydrolysate where the glucose was followed by mannose and galactose. Similar observations were reported by Fuchsman, (1980), Morita and Levesque, (1980), Morita and Montgomery (1980), and Black *et al.*, (1955). The concentration of hexoses was lower in DP and P-H compared to P. Interestingly the concentrations of rhamnose and xylose increased almost 2 fold compared to the P hydrolysate.

3.2.3.2 Analysis by HPLC technique

The qualitative analysis of monosaccharides in hydrolysates of peat by HPLC are presented in Figure 3.1. Figure 3.1.a. shows the chromatogram of a mixture of monosaccharide standards comprised of five major monosaccharides found in peat hydrolysates; rhamnose, xylose, mannose, glucose and galactose. Figure 3.1.b shows the chromatogram of a peat hydrolysate sample, which was prepared following the procedure described in Chapter 2 in Section 2.3.7. The sample was concentrated 3 fold from the original hydrolysate solution. By comparing the elution times of the peat sample with those of the standard it can be seen that all five monosaccharides are present in the peat hydrolysate. Arabinose was not detected although this pentose was observed to be present in low concentration by GC technique.

Quantitatively, the results of HPLC analysis of the monosaccharides in peat

Figure 3.1 a. Reconstructive HPLC chromatogram of mixture of sugar standards. b. Reconstructive HPLC chromatogram of processed peat hydrolysate (H_2SO_4- P). Experimental conditions: flow rate = 1.5 ml/min, detector attn. 2X, integrator attn. 4X, and chart speed 1 in/min.


a.
Inject
Solvent
Rhamnose
Xylose
Mannose
Glucose
Galactose
b.
Inject
Solvent
A
B
C
D
E
Detector response
0
4
8
12
16
20
24
Elution time (min)

hydrolysates are unsatisfactory at the present time. The total amount of monosaccharides detected by HPLC is substantially lower than the that observed by GC analysis. In addition, there is a discrepancy between the ratios of the monosaccharides measured by HPLC and GC.

Sample preparation involved in HPLC is more important and complex than that involved in GC analysis. Desalting and concentrating sample are frequent prerequisites for the quantitative HPLC analysis of sugars in extracts of a biological material. Also application of these techniques is severely compromised by protein, lipid, and cationic contaminants (Honda, 1984). Sample concentration procedures involving freeze-drying results in removal of low and intermediate molecular weight components by the vacuum which may make the quantitative HPLC analysis of neutral sugar extract of plant material almost impossible (Keeling and James, 1986). Any or all of these problems could have accounted for the unsatisfactory analysis by HPLC. More work needs to be done in this area if HPLC is the analysis technique of choice, particularly the use of a more sensitive detector.

3.3 Chemical composition of undiluted peat hydrolysates obtained from hydrolysis of peat with HCl, HNO_3, and H_3PO_4

The acids HCl, HNO_3, and H_3PO_4 were used besides H_2SO_4 in order to obtain the hydrolysates from peat. The comparison of these hydrolysates are discussed in subsequent Sections.

3.3.1 Proximate analysis

The proximate analyses of undiluted peat hydrolysates obtained with HCl, HNO_3, and H_3PO_4 are presented in Table 3.6. These values are compared with the values of hydrolysate obtained from the H_2SO_4 hydrolysis of peat. These hydrolysates showed lower concentration of TCH compared to the H_2SO_4 hydrolyzed peat (H_2SO_4- P). The lowest value of TCH was observed in HCl- P.

The concentrations of total reducing sugars in the HCl- P, HNO_3- P, and H_3PO_4- P were also lower compared to that of H_2SO_4- P. Statistically significant differences were not observed between the HCl- P and H_3PO_4- P hydrolysates for the concentrations of total reducing sugars.

The contents of total lipid in the H_2SO_4- P and HNO_3- P were not statistically different ($P > 0.05$), and neither were the lipid contents of HCl- P, HNO_3 and H_3PO_4- P.

The highest value of total nitrogen content was obtained for the HNO_3- P hydrolysate, mainly due to the presence of nitrogen in nitric acid.

The content of ash in all the hydrolysates were not statistically different ($P > 0.05$). Therefore, it can be inferred that the hydrolysis process did not effect the percent ash content.

Lower values of total solids were observed in HCl- P, HNO_3- P, and H_3PO_4-

Table 3.6 Proximate composition of undiluted hydrolysates of peat obtained with different acids[1]

	Concentration (g/L)			
Components	H_2SO_4- P	HCl- P	HNO_3- P	H_3PO_4- P
TCH	49.4[a]	24.1±0.6[b]	26 4±2.4[b]	18.2±0.9[b]
Total reducing sugars	11.56±1.28[a]	7.28±0.62[b]	4.34±0.20[c]	7.25±0.21[b]
Total lipids	11.4±0.4[a]	9.47±0.54[b]	10.13±1.07[a,b]	8.42±0.89[b]
Total nitrogen	1.2±0.1[a,b,d]	1.1±0.1[a,c]	1.5±0.2[b]	0.7±0.3[c,d]
Ash	8.6±0.6[a]	7.5±4.1[a]	9.12±2.2[a]	7.1±3.0[a]
Total solids	105.3±3.2[a]	-78.78±3.03[b]	37.60±1.23[c]	72.23±5.73[b]
Dissolved solids	74.88±3.18ua	18.35±1.56[c]	13.85±5.62[c]	29.63±0.55[b]

[1] Mean values of three determinations ± standard deviations.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).

P compared to H_2SO_4- P. The amount of total solids was the lowest in HNO_3- P compared to the other hydrolysates. Similar observation was also made with dissolved solids. Only 23 % of the total solids in HCl- P, 37 % in HNO_3- P, and 41 % in H_3PO_4- P were recovered as dissolved solids. However, the HCl- P and HNO_3- P did not show statistical difference ($P > 0.05$) in their dissolved solid values.

3.3.2. Content of amino acids

The content of amino acids in the undiluted hydrolysates of peat obtained with H_2SO_4, HCl, HNO_3, and H_3PO_4 hydrolysis of peat are listed in Table 3.7. Alanine, aspartic acid, glycine, serine, threonine, and valine were the predominant amino acids in most of the hydrolysates. The concentrations of methionine and proline were the highest in HNO_3 -P hydrolysate. In general, hydrolysis of peat with HNO_3 resulted in the lowest concentrations of amino acids. The concentrations of many of the amino acids did not show statistically significant differences ($P > 0.05$) between HCl- P and H_3PO_4- P hydrolysates.

Table 3.7 Content of amino acids (%) in undiluted peat hydrolysates obtained by hydrolysis of peat with different acids[1]

Amino acid	H_2SO_4- P	HCl- P	HNO_3- P	H_3PO_4- P
Alanine	14.17 ± 0.10^{a}	13.32 ± 0.45^{b}	12.12 ± 0.71^{b}	12.43 ± 0.61^{b}
Arginine	1.45 ± 0.01^{a}	0.75 ± 0.11^{c}	0.94 ± 0.01^{b}	0.79 ± 0.02^{c}
Aspartic acid	16.06 ± 0.03^{d}	19.74 ± 0.36^{a}	8.98 ± 0.09^{c}	17.69 ± 0.18^{b}
Cystine	0.19 ± 0.02^{b}	0.07 ± 0.03^{c}	0.34 ± 0.01^{a}	0.36 ± 0.06^{a}
Glutamic acid	6.50 ± 0.03^{b}	5.45 ± 0.11^{c}	7.02 ± 0.08^{a}	5.59 ± 0.16^{a}
Glycine	17.25 ± 0.10^{b}	14.65 ± 0.29^{c}	21.77 ± 0.52^{a}	14.33 ± 0.29^{c}
Histidine	1.60 ± 0.02^{a}	0.85 ± 0.09^{c}	1.28 ± 0.08^{b}	0.96 ± 0.06^{c}
Hydroxylysine	1.31 ± 0.01^{c}	$1.58\pm0.17^{b,c}$	5.61 ± 0.36^{a}	1.74 ± 0.07^{b}
Isoleucine	2.93 ± 0.02^{b}	2.02 ± 0.07^{c}	4.34 ± 0.04^{a}	2.13 ± 0.28^{c}
Leucine	4.56 ± 0.04^{b}	3.26 ± 0.04^{c}	5.40 ± 0.00^{a}	3.08 ± 0.25^{c}
Lysine	2.90 ± 0.04^{a}	1.85 ± 0.05^{b}	$1.62\pm0.21^{b,c}$	1.39 ± 0.01^{c}
Methionine	0.38 ± 0.01^{b}	0.33 ± 0.00^{c}	13.32 ± 0.68^{a}	3.44 ± 0.16^{a}
Phenylalanine	1.60 ± 0.03^{a}	1.06 ± 0.06^{c}	0.41 ± 0.11^{a}	1.31 ± 0.09^{b}
Proline	4.11 ± 0.04^{a}	3.08 ± 0.00^{d}	13.32 ± 0.68^{c}	3.44 ± 0.16^{b}
Serine	4.91 ± 0.05^{c}	9.12 ± 0.11^{a}	2.20 ± 0.23^{d}	7.98 ± 0.09^{b}
Threonine	8.44 ± 0.03^{b}	9.16 ± 0.24^{a}	1.24 ± 0.14^{c}	9.15 ± 0.04^{a}
Tyrosine	1.54 ± 0.03^{a}	0.90 ± 0.02^{c}	0.12 ± 0.04^{d}	1.40 ± 0.09^{b}
Valine	6.05 ± 0.04^{c}	11.56 ± 1.33^{a}	7.60 ± 0.20^{b}	9.94 ± 0.70^{b}

[1] Mean values of three determinations ± standard deviations.
Values in the same row with the same superscript are not statistically different ($P > 0.05$).

3.4 Chemical composition of peat-fish offal compost and compost hydrolysates

3.4.1 Proximate analysis

The proximate analysis of peat-fish offal compost is presented in Table 3.8, and the composition of compost was compared with that of peat. The moisture content of the compost as received was lower than that of the peat. The total solids in compost were higher, whereas the total lipid content of compost was much lower than that of peat. However, the contents of total nitrogen and ash of the compost were about four times higher than that in the peat. The total nitrogen content in the compost is within the range reported by Mathur *et al.*, (1986).

The proximate analysis of the compost hydrolysate (C) is listed in Table 3.9. and the comparison between P and C hydrolysates is also presented. The TCH of C hydrolysate was significantly ($P > 0.05$) lower than that of the P hydrolysate. As the compost was prepared by microbial decomposition of a mixture of peat and fish waste (Mathur *et al*), it should be less organic and poorer in carbohydrates than in peat. Nevertheless, the contents of total reducing sugar in P and C hydrolysates did not show statistically significant differences ($P > 0.05$).

It is surprising that the total lipid content value of the P hydrolysate was higher than that of the compost hydrolysate.

Table 3.8 Proximate composition of peat and compost[1]

	Concentration (% dry weight)	
Components	Peat	Compost
Moisture[2]	66.6±1.2[a]	27.07±0.77[b]
Total solids[3]	33.4±0.7[b]	72.93±0.77[a]
Total lipids	4.8±1.8[a]	0.90±0.04[b]
Total nitrogen	0.66±0.05[b]	2.5±0.1[a]
Ash	4.3±0.2[b]	19.5±2.8[a]

[1] Mean values of three determinations ± standard deviations.

[2] Percentage of wet weight.

[3] Determined by difference between the total weight and percent moisture.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).

Table 3.9 Proximate composition of peat hydrolysate and compost hydrolysate [1]

	Concentration (g/L)	
Components	P	C
TCH	49.0 ± 0.4^{a}	14.45 ± 0.89^{b}
Total lipids	11.4 ± 0.1^{a}	1.98 ± 0.02^{b}
Total nitrogen	1.2 ± 0.1^{b}	2.7 ± 0.5^{a}
Ash	8.6 ± 0.6^{b}	13.85 ± 0.56^{a}
Total solids	105.3 ± 3.2^{a}	43.57 ± 3.33^{b}
Dissolved solids	74.88 ± 3.18^{a}	40.37 ± 3.51^{b}
Total reducing sugars	11.56 ± 1.28^{a}	11.64 ± 0.22^{a}

[1] Mean values of three determinations ± standard deviations.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).

The total nitrogen content in C hydrolysate was about 2.3 times higher than that in P hydrolysate. The proteinaceous animal tissues degraded to produce higher values of nitrogen content in compost and compost hydrolysates.

The content of ash in C hydrolysate was higher than that of the P hydrolysate. The inorganic content of compost contributes to the formation of higher ash contents in compost and compost hydrolysates.

The total solids and dissolved solids of C hydrolysates were statistically lower ($P > 0.05$) than those of P hydrolysate.

3.4.2 Content of amino acids

The content of amino acids in compost and compost hydrolysate are presented in Table 3.10. The concentrations of alanine, aspartic acid, glutamic acid, glycine, proline, serine, threonine and valine were high compared to the other amino acids in compost as well as in compost hydrolysate. Leucine was also high in compost. The concentrations of alanine, cystine, serine, and threonine showed no statistically significant differences ($P > 0.05$) for compost and compost hydrolysate.

3.5 Growth of *S. acidophilum* in synthetic medium

The results obtained from the preliminary studies with the synthetic medium are presented in Table 3.11. The highest values of growth parameters

Table 3.10 Content of amino acids (%) in compost and compost hydrolysate[1]

Amino acid	Compost	Compost hydrolysate
Alanine	10.13±0.49[a]	10.77±0.03[a]
Arginine	3.34±0.27[a]	0.83±0.05[b]
Aspartic acid	9.98±0.20[b]	16.63±0.24[a]
Cysteic acid	0.39±0.01[b]	3.79±0.04[a]
Cystine	0.34±0.01[a]	0.45±0.24[a]
Glutamic acid	9.63±0.06[b]	10.10±0.06[a]
Glycine	12.30±2.43[b]	16.90±0.06[a]
Histidine	2.76±0.21[a]	0.64±0.03[b]
Hydroxylysine	0.62±0.02[b]	2.66±0.05[a]
Hydroxyproline	1.36±0.94[b]	3.92±0.04[a]
Isoleucine	3.91±0.16[a]	1.53±0.02[b]
Leucine	6.55±0.19[a]	2.34±0.08[b]
Lysine	2.96±1.22[a]	1.38±0.01[b]
Methionine	1.03±0.16[a]	-----
Phenylalanine	3.57±0.43[a]	1.46±0.01[b]
Proline	6.58±0.15[a]	4.32±0.04[b]
Serine	7.47±0.87[a]	7.79±0.01[a]
Threonine	6.40±0.67[a]	5.40±0.01[a]
Tyrosine	1.96±0.08[a]	0.66±0.00[b]
Valine	8.07±0.05[a]	6.66±0.12[b]

[1] Mean values of three determinations ± standard deviations.
----- Trace amount.
Values in the same row with the same superscript are not statistically different ($P > 0.05$).

Table 3.11 Effect of initial pH and inoculum ratios on the growth of *S. acidophilum* in synthetic medium[1]

	Inoculum ratio (v/v)			
	5 %		10 %	
	pH adjusted to 2.00	pH not[2] adjusted	pH adjusted to 2.00	pH not[2] adjusted
Biomass conc. (g/L)	8.02±1.0[a]	7.71±0.74[a]	7.66±1.3[a]	7.44±0.02[a]
Yield (%)	80.52±1.0[a]	75.09±2.4[b]	77.1±2.9[a,b]	72.2±1.0[b]
Efficiency (%)	66.83±1.6[a]	64.25±3.9[a,b]	63.83±1.4[a,c]	61.5±2.4[b,c]

[1] Mean values of three experiments ± standard deviations.
[2] pH = 1.3 to 1.5.
Values in the same row with the same superscript are not statistically different ($P > 0.05$).

were obtained with 5 % inoculum at pH 2.00. However, no statistically different ($P > 0.05$) values were observed for biomass concentration with 5 and 10 % (v/v) inoculum ratios in both pH adjusted and non-adjusted media. The values of yield and efficiency were in the same range in all cases. The highest value of efficiency was observed in media with pH 2.00 and 5 % inoculum.

From these results it can be concluded that 5 % (v/v) inoculum is better compared to 10 % inoculum. This result was attributed to the production and accumulation of metabolites from propagation of *S. acidophilum*. Therefore, with higher inoculum ratio, a higher concentration of growth retarding products are transferred to the growth media and decrease the growth parameters of the fungus (Martin and White, 1985). In this work, at pH 2.00 and 5 % inoculum (v/v) a significantly higher value of yield was observed.

3.5.1 Effect of humic and fulvic acids on the growth of *S. acidophilum*

The chemical composition of humic acids is presented in Table 3.12. The major functional groups of humic acid containing oxygen are COOH, phenolic-OH, and ketone groups. The occurrence of dissociable hydrogen in aromatic, aliphatic COOH groups and phenolic-OH groups contributes to the acidic nature of the humic acids (Schnitzer, 1978). The concentrations of total acidity, total carboxy groups and phenolic hydroxy groups of peat humic acids are higher than the values reported for the soil humic acids. The humification level of peat humus is

Table 3.12 Chemical characterization of peat humic acids[1]

Component	Concentration (m eq/g of humic material) Peat	Soil[2]
Total acidity	9.50±0.30	6.6
Total carboxy groups	6.33±0.11	4.5
Phenolic hydroxy groups	3.17±0.32	2.1

[1] Mean values of three determinations ± standard deviations.

[2] Taken from Schnitzer and Khan, 1972.

lower than that of soil humus in aerated soils.

To study the influence of humic and fulvic acids on the growth of *S. acidophilum*, various concentrations of these substances were added to the synthetic medium. The growth parameters obtained at various levels of humic acids at pH 2.00 is shown in Figure 3.2 (Appendix 1). As the concentrations of humic acids were increased from 0.10 % to 0.20 %, a continuous increase in the biomass concentration was observed. However the values obtained for 0.10 % were not statistically different than that of 0.00 % humic acids level. When the humic acid concentration was increased beyond 0.20 %, a sudden decrease in the concentration of biomass was observed. The optimal concentration of humic acids was considered to be 0.20 % with pH of medium at 2.00. The yield and efficiency values of the experiments showed similar pattern.

As the humic acids are not completely soluble at low pH values, the experiment was also conducted at pH 8.00. The results obtained with these two experiments were compared. The growth parameters of fungus obtained in humic acid supplemented synthetic medium experiment at pH 8.00 are shown in Figure 3.3 (Appendix 2). At pH 8.00, higher concentration of humic acids were dissolved in synthetic medium compared to pH 2.00. With the increase in the concentration of humic acids from 0.12 % to 0.50 %, a gradual increase in growth parameters obtained. However, the biomass concentration values were not statistically different ($P > 0.05$). The highest values of growth parameters were observed with 0.37 % humic acid level.

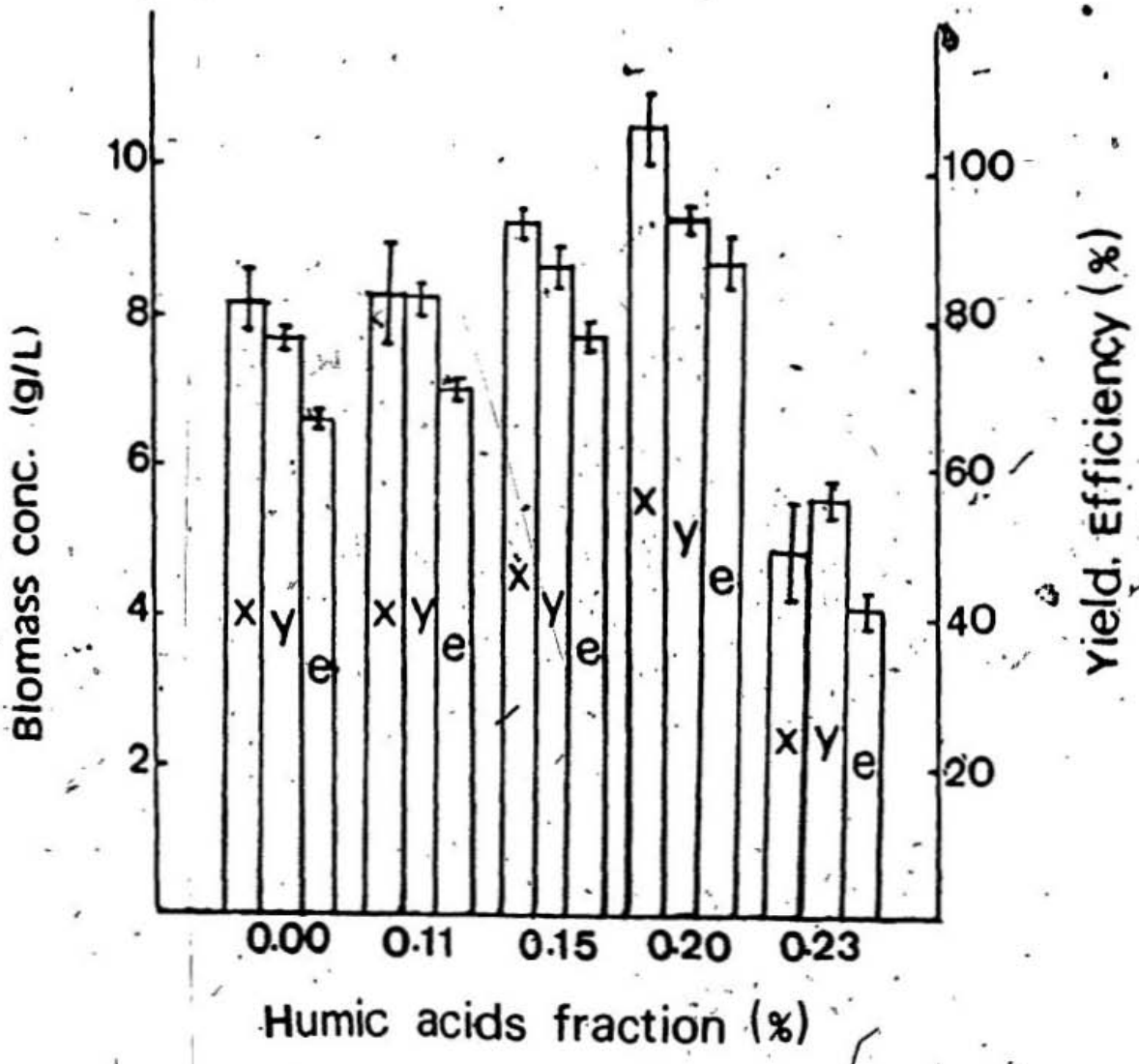


Figure 3.2 Effect of increasing concentration of the humic acids fraction on the growth of *S. acidophilum* in synthetic medium at pH 2.00. x = biomass concentration, y = yield, e = efficiency.

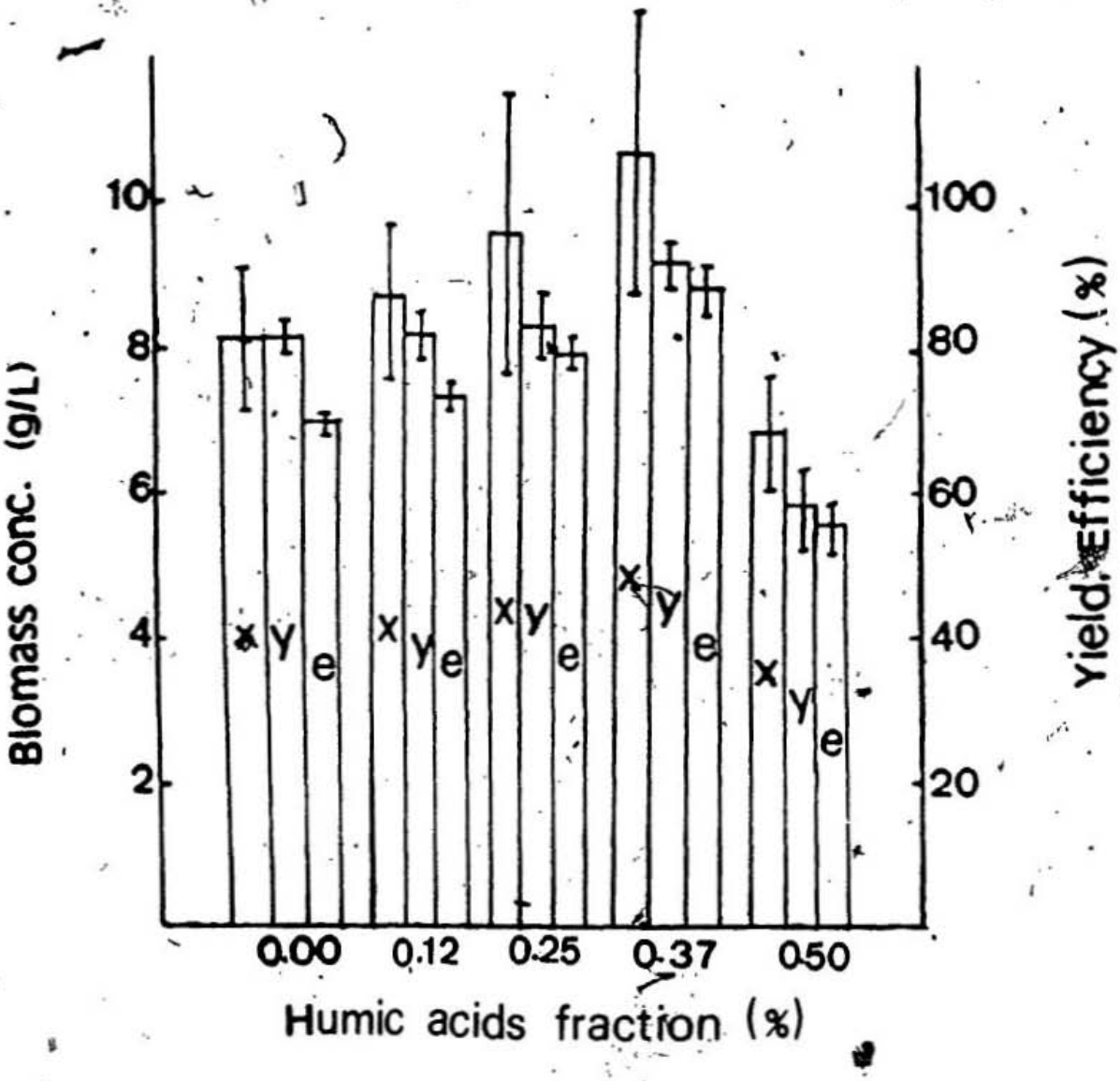


Figure 3.3 Effect of increasing concentration of humic acids fraction on the growth of *S. acidophilum* in synthetic medium at pH 8.00. x = biomass concentration, y = yield, e = efficiency.

The growth parameters were higher when the experiment was done at pH 8.00 due to the higher concentration of humic acids. But, these values were not statistically different from the values obtained at pH 2.00. A concentration of 0.20 % of humic acids in pH 2.00 medium produced higher growth parameters (Figure 3.2) compared to that of 0.25 % humic acids at pH 8.00 (Figure 3.3). It was reported that the optimum pH for the growth of the fungus is 2.00 (Martin and White, 1985). However, the fungus was reported to grow well at a wide range of pH values (Miller *et al.*,1984). Low values of biomass at high humic acid levels could be due to the formation of micellar aggregates, which will inhibit the growth of the fungus (Fuchsman, 1980).

These results indicate that addition of humic acids up to certain level is growth promoting and after that level they become growth inhibitory. These observations agree with the results obtained by Dady and Chang (1983). Humic acids are reported to stimulate growth of crops (Dragunov *et al.*,1973), sugar beet plants (Dragunov, 1968), and barley plants (Reutov and Kravchenko, 1973). According to Sakurai (1977), the crops treated with humic acids are more resistant to pests. Christewa (1958) noted that humic acid complexes were growth-stimulating to crop plants only if present at low concentrations and at higher concentrations they become toxic.

The fulvic acid fraction which was extracted from peat was also supplemented to the synthetic medium. The results obtained for these experiment at pH 2.00 are shown in Figure 3.4 (Appendix 3). Addition of fulvic acids resulted

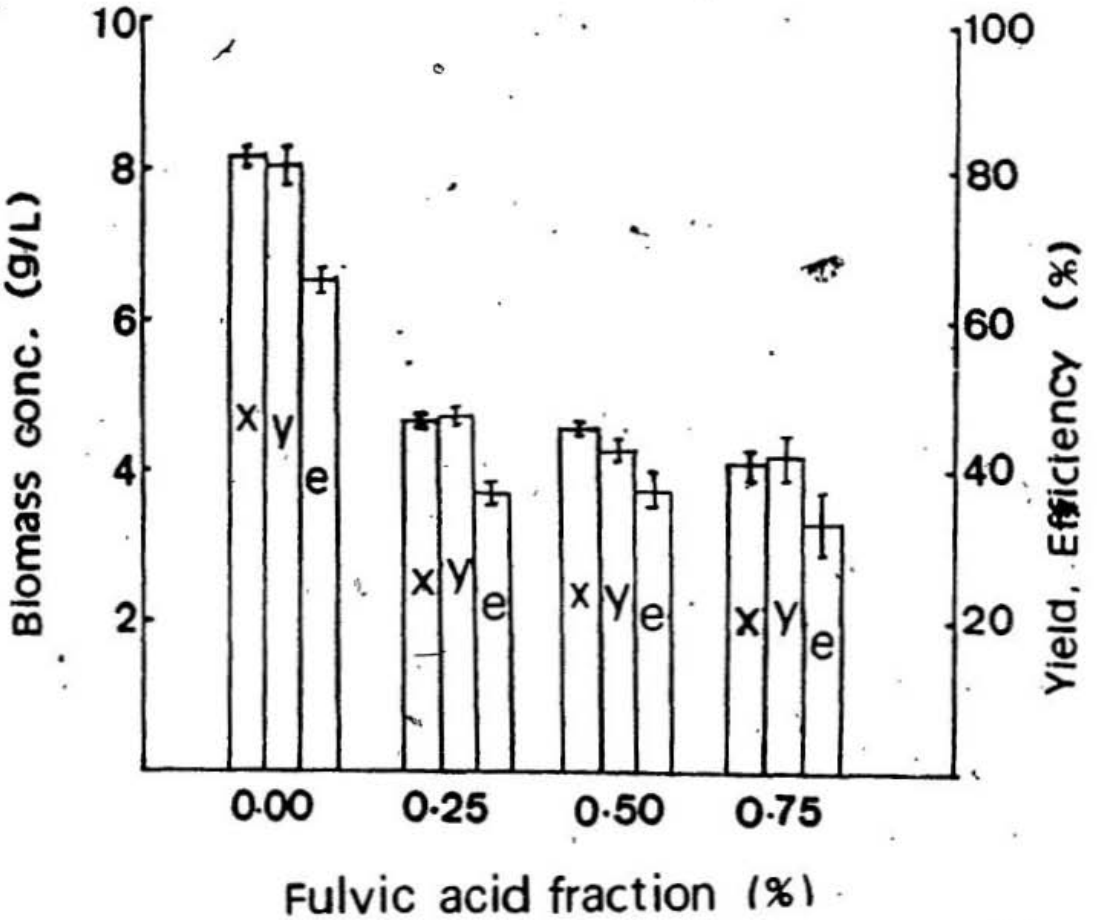


Figure 3.4 Effect of increasing concentration of fulvic acid fraction on the growth parameters of *S. acidophilum* in synthetic medium at pH 2.00. x = biomass concentration, y = yield, e = efficiency.

in the decrease of growth of the fungus. This suggests that the fulvic acids show an inhibitory effect on *S. acidophilum*. However, increasing the concentration of fulvic acid fraction did not cause significant suppression ($P > 0.05$) in the growth parameters of the fungus. The values observed for the concentration of biomass and efficiency at 0.25 %, 0.50 %, and 0.75 % levels of fulvic acid were not statistically different ($P > 0.05$). Similarly, no significant differences were observed in growth parameters of the fungus for 0.50 % and 0.75 % levels of fulvic acids. Similar observations were made in concentration of biomass when the experiment was conducted at pH 8.00. The results of this experiment are shown in Figure 3.5 (Appendix 4). Though there were reports about the stimulatory effect of fulvic acids (Dragunov, 1968) on the growth of plants, this work infers *S. acidophilum* can not grow well in presence of fulvic acids.

3.5.2 Different carbon sources

Almost all fungi use glucose as a source of energy for their growth. Many fungi can grow equally well on mannose and fructose. The utilization of any sugar depends upon how easily it can be converted to a phosphorylated derivative of glucose which can enter the respiratory pathways. In general pentoses appear to be poor sources of carbon (Moore-Landecker, 1982) for the growth of the fungus.

The major monosaccharides detected in peat were arabinose, galactose, glucose, mannose, rhamnose and xylose (Table 3.5). These sugars were tested on the

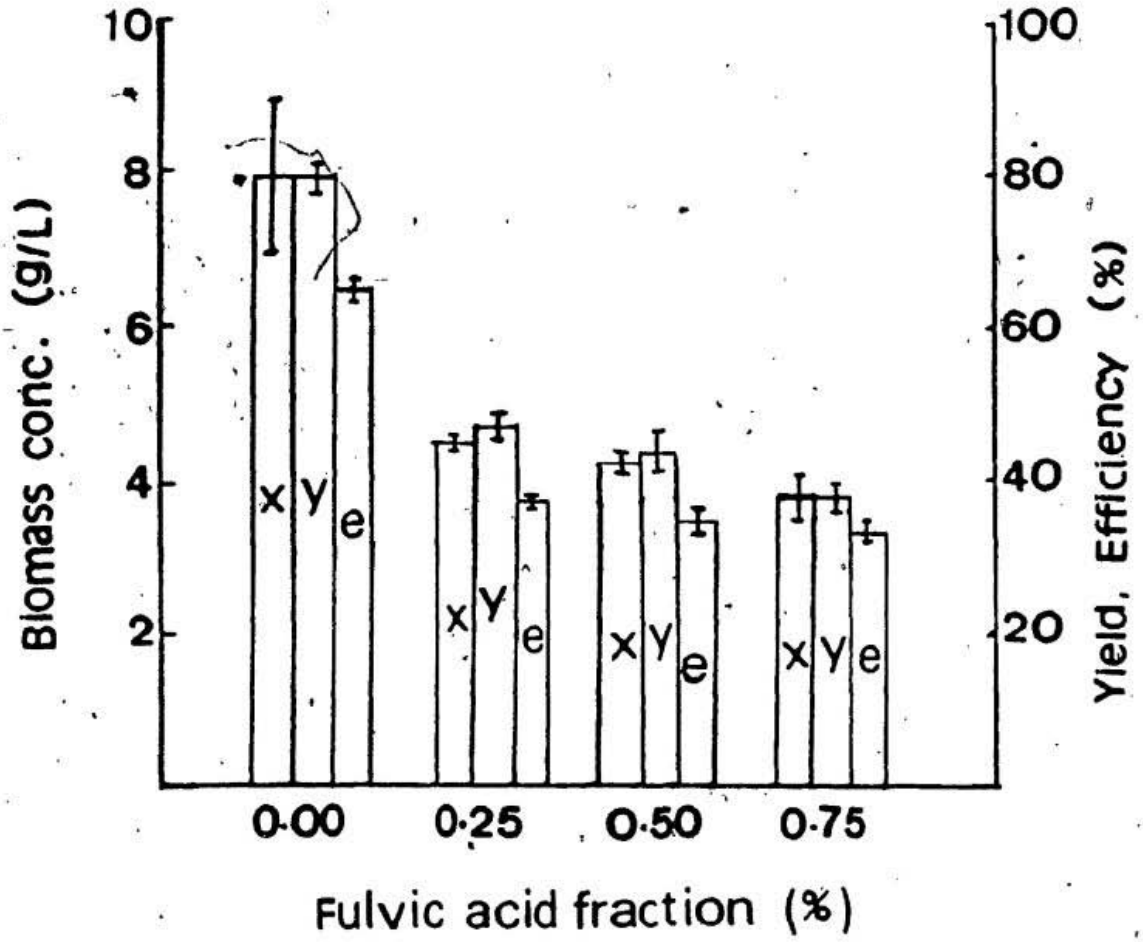


Figure 3.5 Effect of increasing concentrations of fulvic acid fraction on the growth of *S. acidophilum* in synthetic medium at pH 8.00. x = biomass concentration, y = yield, e = efficiency.

growth of *S. acidophilum* by adding them individually to the synthetic medium. Table 3.13 presents the growth parameters in synthetic medium with various carbon sources. The optimal growth parameters were observed when glucose was used as carbon source, followed by galactose. Growth was poor when xylose and arabinose were used. This could mean that hexoses were more efficiently metabolized than the pentoses by the fungus.

The growth parameters obtained when *S. acidophilum* was grown on arabinose and xylose as carbon sources did not show statistically significant differences ($P > 0.05$). Similarly, galactose and mannose did not show significant differences ($P > 0.05$) in biomass concentration. The corresponding yield and efficiency values followed the same pattern. Although the glucose supplemented media produced lower yield values compared to the values of galactose and mannose, their values did not differ statistically ($P > 0.05$) with the others.

Studies with the supplementation of different monosaccharides as carbon sources in separate experiments showed that glucose was the most easily fermentable sugar. Therefore, the subsequent experiments were conducted with media in which glucose was the carbon source and the nitrogen was supplemented in the form of yeast extract as it was reported to be a good source of nitrogen for the growth of the fungus (Martin and White, 1986).

Table 3.13 Effect of different sources of carbon on the growth of *S. acidophilum* in synthetic medium[1]

Carbon source (12 g/L)	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
Arabinose	0.63 ± 0.08^{c}	39.26 ± 2.7^{c}	5.38 ± 0.09^{d}
Galactose	2.45 ± 0.29^{b}	88.79 ± 6.6^{a}	20.29 ± 2.1^{b}
Glucose	8.02 ± 1.0^{a}	80.52 ± 2.2^{a}	66.83 ± 1.62^{a}
Mannose	2.28 ± 0.36^{b}	$85.95\pm9.2^{a,b}$	18.33 ± 0.7^{b}
Rhamnose	1.02 ± 0.05^{c}	72.66 ± 6.4^{b}	8.56 ± 0.4^{c}
Xylose	0.71 ± 0.12^{c}	50.28 ± 8.8^{c}	5.98 ± 1.0^{d}

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

3.5.3 Glucose and yeast extract as the nutrient media

Yeast extract (1, 3, or 5 g/L) was supplemented individually to medium of glucose (15 g/L) in order to determine the optimum concentration of yeast extract for growth of the fungus. The concentration of glucose chosen was 15 g/L because this value is in the range total carbohydrate value of (1:1) diluted peat hydrolysates.

The growth parameters obtained by increasing the concentration of yeast extract are presented in Table 3.14. For the glucose concentration of 15 g/L, the addition of yeast extract from 1 g/L to 3 g/L showed an increase in biomass concentration. The highest yield and efficiency values were obtained with 3 g/L yeast extract and these values decreased significantly at concentration of 5 g/L. This type of inhibitory effect of yeast extract at high concentration was also observed by Martin and White (1984). Hence, 3 g/L yeast extract was considered as the optimal addition of nitrogen source for the growth of the fungus.

3.6 Growth of *S. acidophilum* in peat hydrolysates

3.6.1 Non-supplemented undiluted H_2SO_4, HCl, HNO_3, and H_3PO_4 peat hydrolysates

Different peat hydrolysates obtained from hydrolysis of peat with H_2SO_4, HCl, HNO_3, and H_3PO_4 were used to grow *S. acidophilum*. The growth parameters of *S. acidophilum* in different undiluted peat hydrolysates are shown in Figure 3.6 (Appendix 5). The highest values of biomass concentration and yield were

Table 3.14 Effect of increasing concentration of yeast extract on the growth of *S. acidophilum* in glucose medium[1]

	Glucose (15 g/L)		
Yeast extract (g/L)	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
1	3.82 ± 0.14^{b}	48.27 ± 1.30^{b}	24.38 ± 0.15^{b}
3	7.14 ± 0.20^{a}	53.55 ± 2.50^{a}	44.61 ± 1.35^{a}
5	1.90 ± 0.56^{c}	38.70 ± 2.89^{c}	12.68 ± 3.73^{c}

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

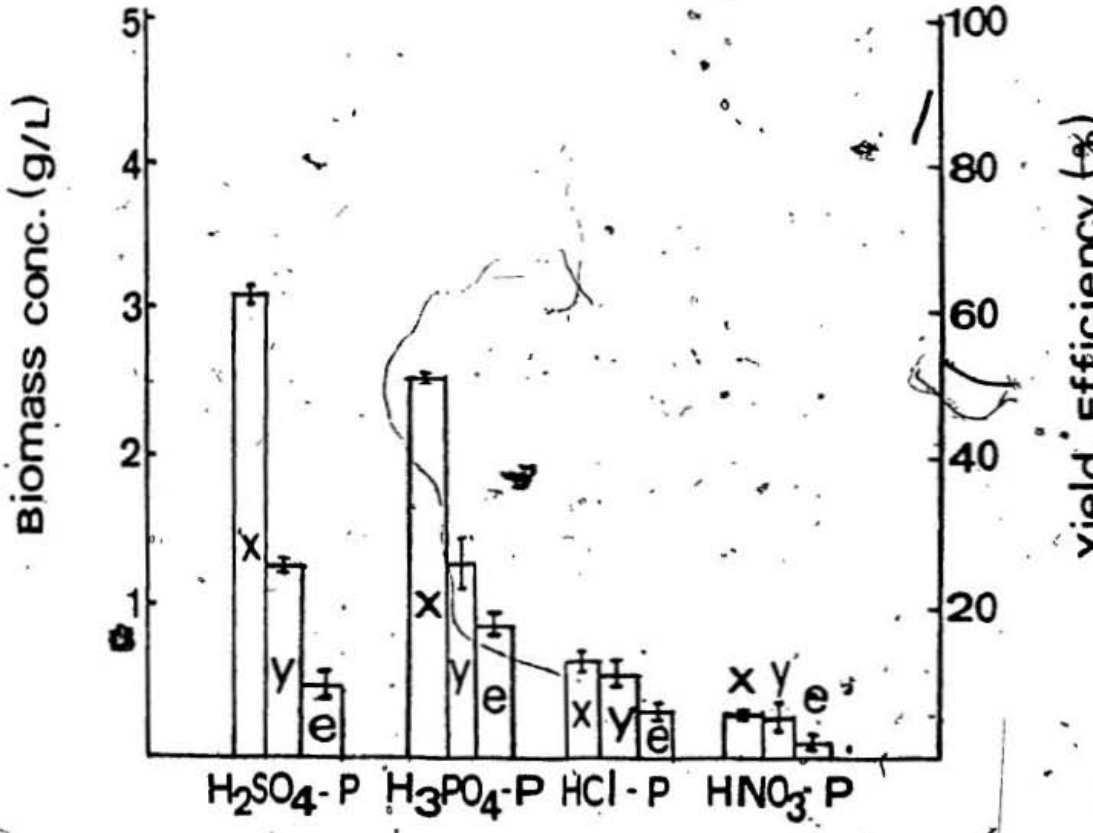


Figure 3.6 Comparison of growth parameters of *S. acidophilum* in undiluted H_2SO_4- P, HCl- P, HNO_3- P, and H_3PO_4- P growth media. x = biomass concentration, y = yield, e = efficiency.

observed when H_2SO_4- P hydrolysate was used as a substrate. The highest efficiency value was observed in H_3PO_4- P hydrolysate. No statistically significant differences ($P > 0.05$) were observed between the yield values of H_2SO_4- P and H_3PO_4- P hydrolysates. The lowest values of growth parameters were obtained with HNO_3- P hydrolysate as a substrate. It appears that there were differences in the abilities of the acids to break the glycosidic bonds associated with the cellulose microstructure during hydrolysis and there by releasing the fermentable sugars.

In an attempt to improve the growth parameters, these hydrolysates were diluted (1:1) with water to reduce the the concentration of potential inhibitory components. It has been reported that the dilution of peat hydrolysate from H_2SO_4 hydrolysis of peat resulted in increased growth parameters of the fungus (Martin and White, 1984). The results obtained by growing the fungus in these diluted hydrolysates are shown in Figure 3.7 (Appendix 6). The growth parameters increased with the dilution of the hydrolysates. The growth parameters followed simila pattern as in undiluted peat hydrolysates as in the following order, H_2SO_4- P > H_3PO_4- P > HCl- P > HNO_3- P.

In order to further enhance the growth of the fungus, different nutrient supplementations were studied. The results are discussed in subsequent Sections.

It has been reported that HCl is not a preferred acid for hydrolysis of polysaccharides. However, in wood saccharification, HCl is used (Bergius *et al.*,

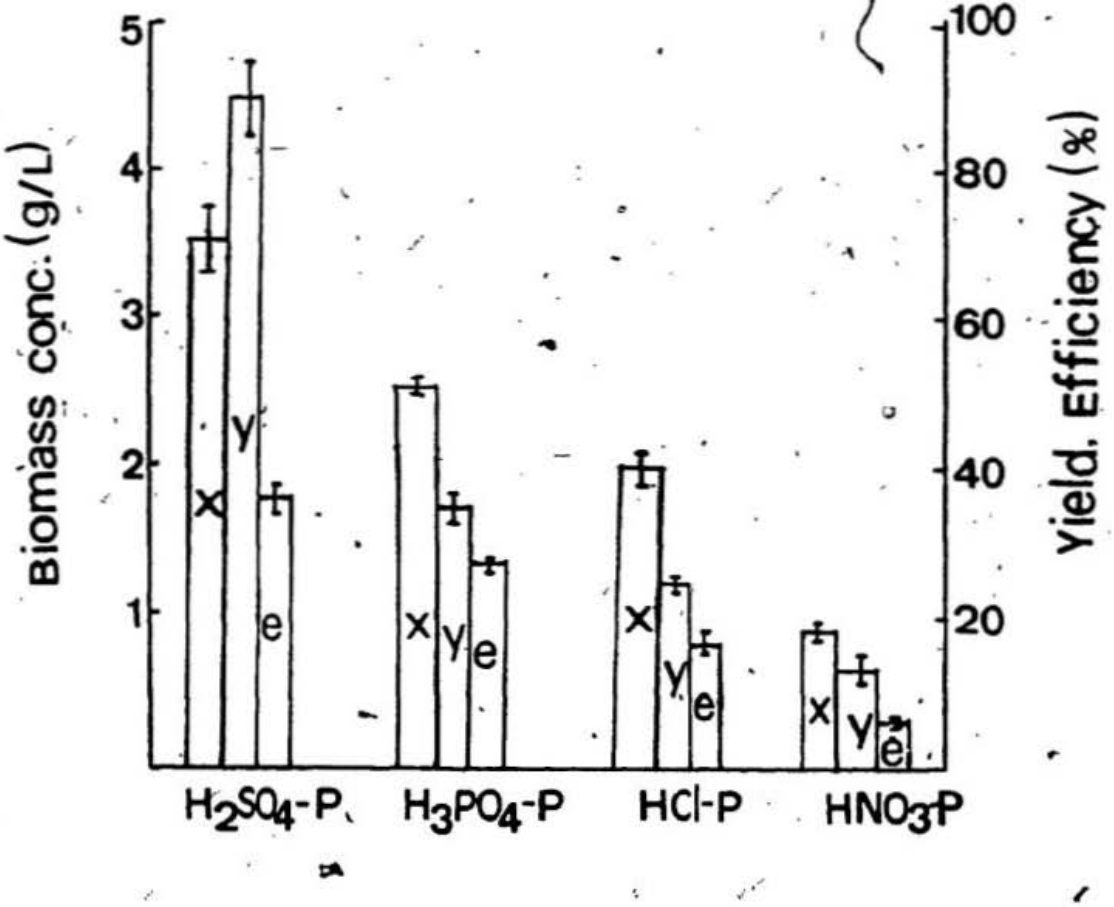


Figure 3.7 Comparison of growth parameters of *S. acidophilum* in (1:1) diluted H_2SO_4- P, HCl- P, HNO_3- P, and H_3PO_4- P growth media. x = biomass concentration, y = yield, e = efficiency.

1931). In 1956, Leibnitz and Hrapia considered the Bergius process as an appropriate model for hydrolysis of peat. It was reported that with 41 % HCl, a yield of 49-50 % reducing sugars (dry basis) were obtained from peat in five hours at room temperature. Higher concentrations and longer reaction time did not increase the yield. However, lower concentration and shorter times decreased the yield (Fuchsman, 1980). Higher concentration of acid resulted in overall loss of sugars (Leibnitz and Hrapia, 1956). Under milder conditions, the hydrolysis was reported to be incomplete. The saccharification of peat by HCl does not seem to be appropriate because of the high concentrations of acid required and added cost of drying peat (Fuchsman, 1980).

Sulfuric acid has been the preferred acid catalyst for the hydrolysis of polysaccharides in peat. One of the principal drawbacks to the various processes have been the high consumption of acid. The acid must be present in sufficient quantity to assure adequate homogenity. Low concentrations of acid were tested at different temperatures. At 160 °C, 1 % H_2SO_4 effectively hydrolyses all the peat hemicelluloses, but very little of the cellulose. Prolonged reactions hydrolyzed some of the cellulose, but caused degradation of the previously hydrolyzed hemicelluloses (Tolchel'nikova and Lisenkova, 1976). A process of using low concentration of H_2SO_4 and mechanical stress is described in a Soviet patent (Chalov *et al.*, 1971).

3.6.2 Supplemented H_2SO_4, HCl, HNO_3, and H_3PO_4 peat hydrolysates

H_2SO_4- P hydrolysates

The H_2SO_4- P hydrolysate was supplemented with different nutrients as described in Section 2.2.6.1. The results are tabulated in Table 3.15. No significant differences were observed for, H_2SO_4- P, H_2SO_4- P^2, H_2SO_4- P^3, H_2SO_4- P^4 and H_2SO_4- P^6 hydrolysates in the concentration of biomass. The highest biomass concentration was observed in the H_2SO_4- P^5 hydrolysate. The yield values obtained for H_2SO_4- P, and H_2SO_4- P^5 hydrolysates were not statistically different ($P > 0.05$). Increased values of efficiency were observed in substrates where the yeast extract was supplemented to the peat hydrolysates (H_2SO_4- P^2 and H_2SO_4- P^5 hydrolysates and H_2SO_4- P^6 hydrolysates). The values of yield and efficiency were lower in the H_2SO_4- P^3 and H_2SO_4- P^4 hydrolysates than in H_2SO_4- P, H_2SO_4- P^2, H_2SO_4- P^5 and H_2SO_4- P^6 hydrolysates. Although the concentration of yeast extract was lower in H_2SO_4 - P^5 and H_2SO_4- P^6 hydrolysates than that in the H_2SO_4- P^2 hydrolysate, the values for the concentration of biomass, yield and efficiencies were higher compared to the H_2SO_4- P^2 hydrolysate. This could be due to the inhibitory action of yeast extract at higher concentrations (Martin and White, 1984). Addition of magnesium sulfate to yeast extract in peat hydrolysate enhanced the growth of the fungus (H_2SO_4- P^5).

Table 3.15 Effect of nutrient-supplementation of the peat hydrolysates (1:1 diluted) obtained from H_2SO_4 hydrolysis of peat on the growth of *S. acidophilum*[1]

Nutrient symbol[2]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P	3.5±0.4	90.0±5.4^{a}	37.25±1.2^{d}
P^2	4.7±0.8^{a,c}	67.92±1.9^{c}	45.17±1.77^{c}
P^3	4.0±2.2^{a}	52.6±1.86^{d}	35.36±1.92^{d}
P^4	4.1±0.2^{a,b}	66.16±2.11^{b,c}	36.54±2.23^{d}
P^5	5.9±0.1^{b,c,d}	88.16±3.27^{a}	57.11±0.58^{a}
P^6	5.2±0.3^{a,d}	75.69±1.39^{b}	50.39±0.68^{b}

[1] Mean values of three exeriments ± standard deviations.

[2] See Table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

HCl- P hydrolysates

The results obtained from the study of nutrient supplementation to the HCl- P hydrolysate are listed in Table 3.16. The values obtained for the biomass concentration of HCl- P^5 and HCl- P^6 hydrolysates were not statistically different ($P > 0.05$), and neither were the values of HCl- P^2 and HCl- P^6 hydrolysates. The highest values of yield and efficiencies were obtained in HCl- P^5 hydrolysates. The yield values obtained for HCl- P and HCl- P^4 hydrolysates were not statistically different ($P > 0.05$). Although the concentration of biomass obtained for the HCl- P^3 hydrolysate was higher than that obtained for HCl- P, the corresponding yield value was lower. Comparison of the growth parameters obtained for the HCl- P, HCl- P^3 indicated that, addition of the K_2HPO_4, and $(NH_4)_2SO_4$ did not improve the growth parameters of the fungus. However, the addition of $MgSO_4$ increased the growth parameters in the case of the HCl- P^4 hydrolysate, as compared to the HCl- P^3 hydrolysate in which there was no $MgSO_4$. Hence, the combination of 3 g/L yeast extract and $MgSO_4$ was found to be the best nutrient for the fungal growth.

HNO_3- P hydrolysates

Similar set of experiments were conducted with HNO_3- P hydrolysates and the results are presented in Table 3.17. The highest biomass concentration was obtained with HNO_3- P^5 hydrolysate, however, the value was not statistically different ($P > 0.05$) than that of HNO_3- P^2, and HNO_3- P^6 hydrolysates. No significant differences ($P > 0.05$) in biomass concentration were observed for the growth parameters when HNO_3- P^3 and HNO_3- P^4 hydrolysates were used as

Table 3.16 Effect of nutrient-supplementation of undiluted peat hydrolysates obtained from HCl hydrolysis of peat on the growth of *S. acidophilum*[1]

Nutrient symbol[!]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P	0.59 ± 0.04^{e}	10.17 ± 1.63^{d}	6.09 ± 0.22^{f}
P^2	1.01 ± 0.07^{b}	17.08 ± 0.20^{c}	8.63 ± 0.04^{c}
P^3	0.68 ± 0.01^{d}	6.06 ± 0.03^{e}	6.78 ± 0.03^{e}
P^4	0.79 ± 0.02^{c}	9.34 ± 0.12^{d}	7.78 ± 0.02^{d}
P^5	1.09 ± 0.01^{a}	23.20 ± 0.28^{a}	10.68 ± 0.02^{a}
P^6	$1.04\pm0.02^{b,a}$	20.76 ± 0.32^{b}	10.22 ± 0.01^{b}

[1] Mean values of three determinations ± standard deviations.

[!] See Table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table 3.17 Effect of nutrient-supplementation of undiluted peat hydrolysates obtained from HNO_3 hydrolysis of peat on the growth of *S. acidophilum*[1]

Nutrient symbol[2]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P	0.30 ± 0.01^{c}	5.11 ± 0.30^{e}	1.48 ± 0.40^{c}
P^2	0.66 ± 0.04^{a}	16.13 ± 1.27^{c}	2.82 ± 0.07^{a}
P^3	0.47 ± 0.03^{b}	6.65 ± 0.09^{d}	$1.98\pm0.05^{b,c}$
P^4	0.51 ± 0.01^{b}	21.4 ± 1.3^{a}	$2.13\pm0.88^{a,b,c}$
P^5	0.70 ± 0.13^{a}	17.5 ± 0.13^{b}	2.90 ± 0.45^{a}
P^6	0.67 ± 0.03^{a}	$16.50\pm0.07^{b,c}$	$2.71\pm0.03^{a,b}$

[1] Mean values of three experiments ± standard deviations.

[2] See Table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

substrates. The highest value of yield was observed in the HNO_3- P^4 hydrolysate, whereas the efficiency was found to be the highest in the HNO_3- P^5 hydrolysate. The efficiency value of HNO_3- P^5 hydrolysate was not statistically significant ($P > 0.05$) than that of HNO_3- P^4, and HNO_3- P^6 hydrolysates. Though the values produced by the media supplemented with yeast extract were higher than those of the unsupplemented media, the highest values were produced when the supplement was a mixture of K_2HPO_4, $(NH_4)_2SO_4$, and $MgSO_4$.

H_3PO_4- P hydrolysates

The H_3PO_4- peat hydrolysates were also used as substrate to grow *S. acidophilum*. The growth parameters obtained with these hydrolysates are presented in Table 3.18. The highest values for growth parameters were obtained with H_3PO_4- P^2 hydrolysate and this is indicating that H_3PO_4- peat hydrolysates are deficient in nitrogen. Addition of K_2HPO_4, $(NH_4)_2SO_4$, and $MgSO_4$ did not show significant increase in biomass concentration of the fungus compared to the non-supplemented H_3PO_4- P hydrolysate. The values of yield and efficiency were significantly lower in the H_3PO_4- P^3 and H_3PO_4- P^4 hydrolysates than those in the H_3PO_4- peat hydrolysate supplemented with yeast extract. Addition of $MgSO_4$ to the 3 g/L yeast extract in H_3PO_4- P^5 hydrolysate did not improve the biomass concentration, yield or efficiency compared to the H_3PO_4- P^2 hydrolysate. It shows that high concentration of yeast extract is required in H_3PO_4 hydrolysates to obtain high growth parameters. The results obtained for the

Table 3.18 Effect of nutrient-supplementation of undiluted peat hydrolysates obtained from H_3PO_4 hydrolysis of peat on the growth of *S. acidophilum*[1]

Nutrient symbol[2]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P	2.15±0.01^{c}	76.56±3.38^{a}	17.92±0.16^{d}
P^2	4.37±0.09^{a}	79.36±5.2^{a}	32.42±1.92^{a}
P^3	2.89±0.01^{d}	58.27±3.4^{b}	20.61±0.10^{c}
P^4	2.2±0.19^{f}	61.30±1.01^{b}	14.75±1.9^{e}
P^5	3.6±0.02^{b}	60.30±3.29^{b}	25.12±0.2^{b}
P^6	3.35±0.07^{c}	55.65±1.28^{b}	23.24±1.8^{b}

1 Mean values of three experiments ± standard deviations.

2 See table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

H_3PO_4- P^3 and H_3PO_4- P^4 hydrolysates showed that apparently these hydrolysates have enough phosphorus and hence the addition of the K_2HPO_4 did not increase the growth parameters compared to the non-supplemented H_3PO_4- P hydrolysates.

Comparison of hydrolysates from different acid hydrolyses of peat

In general, the growth parameters obtained for the H_2SO_4- peat hydrolysates were relatively higher than those obtained for any other acid - peat hydrolysates, followed by those obtained for the H_3PO_4- peat hydrolysates. The growth parameters obtained for the HCl - peat and HNO_3- peat hydrolysates were very much lower than those obtained for the other two acid - peat hydrolysates. The values obtained for the HNO_3- peat hydrolysate were the lowest.

3.6.3 Growth curve for *S. acidophilum* on peat hydrolysate

In general, after the inoculation of a sterile medium with microorganisms, four different phases of growth are observed. They are (a) lag phase, (b) log phase, (c) stationary phase, and (d) death phase. Each phase is briefly explained here.

(a) Lag phase : This is the phase of adaptation of the microorganisms to the medium. Therefore, initially, there will be no increase in number of cells. However, several changes such as change in pH value, and decrease of growth inhibitors may occur. The length of lag phase is a function of physiological condition

of the inoculum. For example, the inoculum, taken from a culture in which growth has stopped due to several factors, may take more time to adopt to the new substrate. It is also reported that the concentration of inoculum has influence on the length of the lag phase.

(b) Log phase : Rapid growth of the microorganism can be seen in this phase. The term "log phase" is used for this because, the biomass concentration increases logarithmically with time. Changes in the substrate concentration are also observed. Growth rate is independent of substrate concentration as long as substrate is present in sufficient amounts *i.e.* it is not a limiting factor.

(c) Stationary phase : The biomass concentration remains constant. Breakage of cells may result in release of carbohydrate and proteins, which will serve as the energy sources for the survival of the remaining cells.

(d) Death phase : This phase occurs when the energy reserves of the cells are exhausted. Cells start to die exponentially with time. The fermentation process and organism are the factors determining the length of stationary and death phases (Crueger and Crueger, 1984).

The concentration of biomass and the TCH were determined for the growth of *S. acidophilum* at 24 hour intervals and the results were plotted against the number of days in Figure 3.8 (Appendix 7). The concentration of biomass was found to be decreasing in the first two days due to the death of some fungal cells

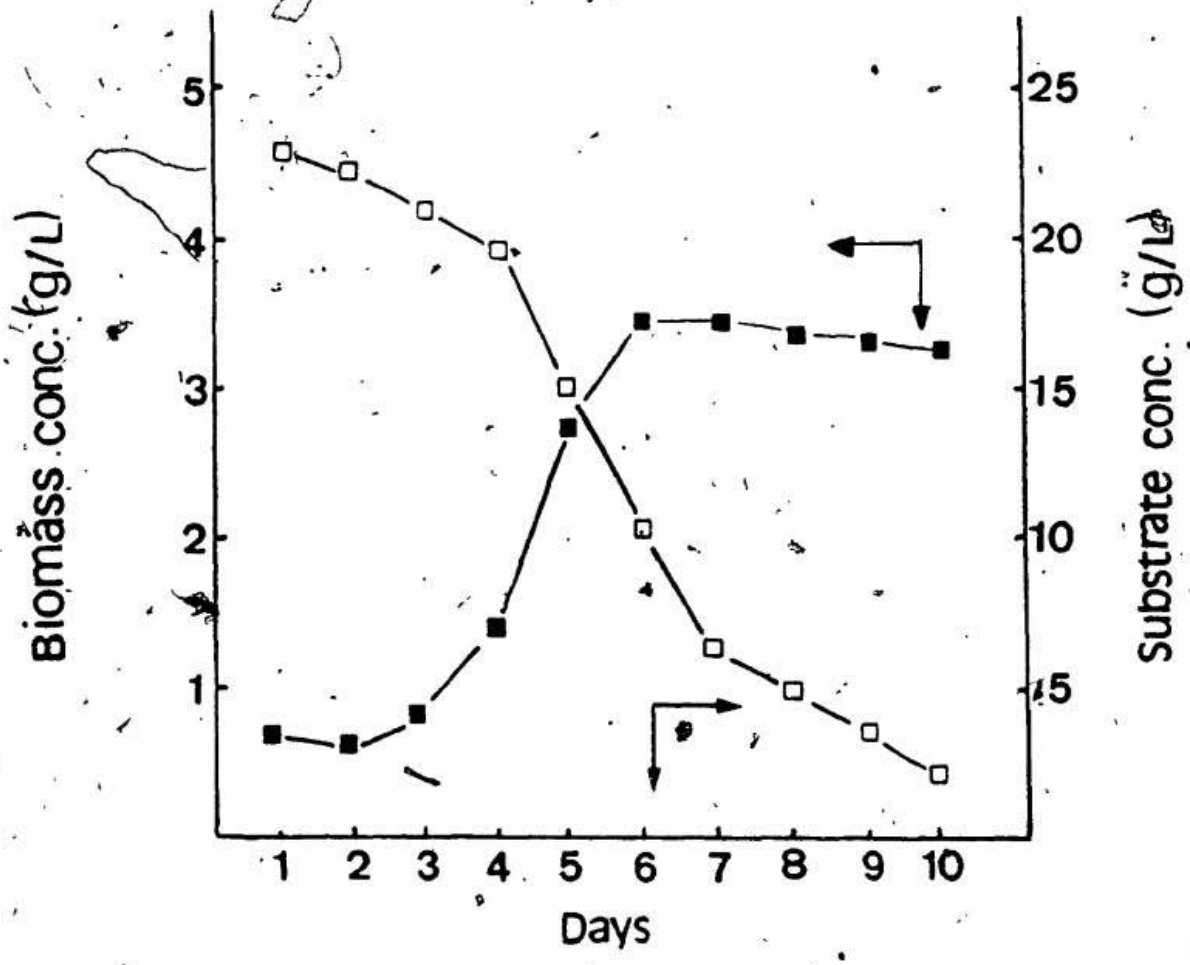


Figure 3.8 Growth curve of *S. acidophilum* in peat hydrolysate.

during the adaptation process. Significant increase in the biomass was observed from the third day, and the biomass concentration attained the maximum range at the sixth day. The substrate concentration decreased continuously from the first day until the tenth day. In this experiment the lag phase was observed between the first and the second days, the accelerated growth phase between the third day to the sixth day followed by the stationary phase between the sixth day to the tenth day. The experiment was stopped at this stage.

3.6.4 Growth of *S. acidophilum* in modified peat hydrolysates

The results of growth parameters of *S. acidophilum* in undiluted modified hydrolysates are shown in Figure 3.9 (Appendix 8). The highest values for the biomass concentration was obtained in cultures where P hydrolysate was used as substrate. The biomass concentration was comparatively low in the modified peat hydrolysates. This could be due to the alteration of carbohydrate pattern of peat during modification process which leads to the decrease in the concentration of easily metabolizable sugars in the modified peat hydrolysates. Biomass concentration values decreased in the order P > DP > P-H > DP-H. The values of biomass concentration and efficiency of P-H and DP-H substrates were not statistically different ($P > 0.05$). The highest value of yield and efficiency were obtained with the DP hydrolysates.

These preliminary results indicated that, removal of bitumen (DP) results in better utilization of the substrate by the fungus as shown by increases in the

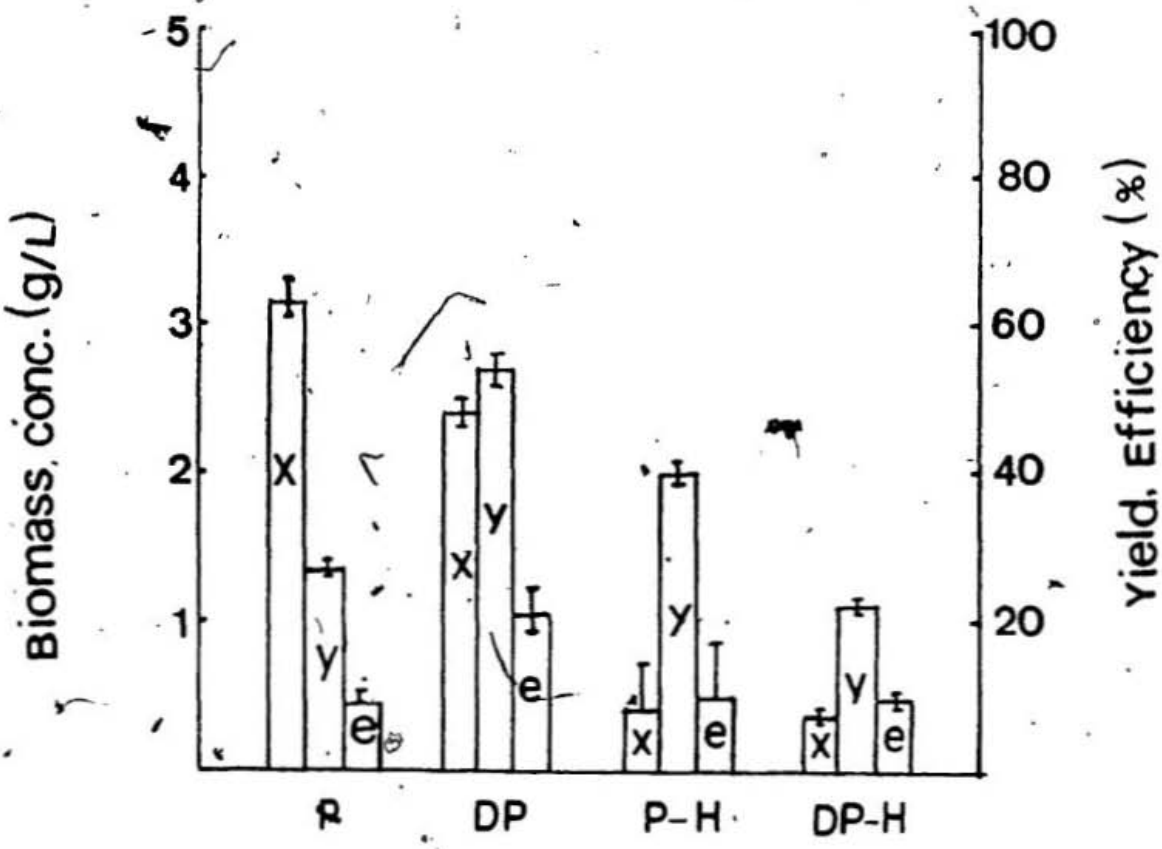


Figure 3.9 Comparison of the growth parameters of *S. acidophilum* in undiluted peat hydrolysate and modified peat hydrolysates. x = biomass concentration, y = yield, e = efficiency.

yield and efficiency values (Figure 3.9). However, this process also results in the removal of carbohydrates and other nutrients. Therefore, the experiments were conducted with the P- hydrolysate by diluting it with water (1:1) in order to decrease the concentration of inhibitory components. These results were compared with the undiluted modified peat hydrolysates as shown in Figure 3.10 (Appendix 9). As explained before, diluted P- hydrolysate produced higher growth parameters than the undiluted P hydrolysate and modified peat hydrolysates.

However, to study the effect and to improve the growth of the fungus, the modified peat hydrolysates were supplemented with different nutrients.

3.6.4.1 Nutrient supplementation

DP hydrolysates

The debituminized peat hydrolysate was supplemented with different nutrients as described in Section 2.2.6.1. The modified peat hydrolysates were not supplemented with 3 g/L yeast extract alone because the previous experiments have shown that 3 g/L yeast extract added with 0.4 g/L $MgSO_4$ is a better nutrient than 3 g/L yeast extract. The results obtained from this study are given in Table 3.19. The biomass concentrations and efficiency obtained for the DP, DP^2, DP^3, and DP^4 hydrolysates did not show statistically significant differences ($P > 0.05$). The concentration of the biomass and the efficiency were the lowest

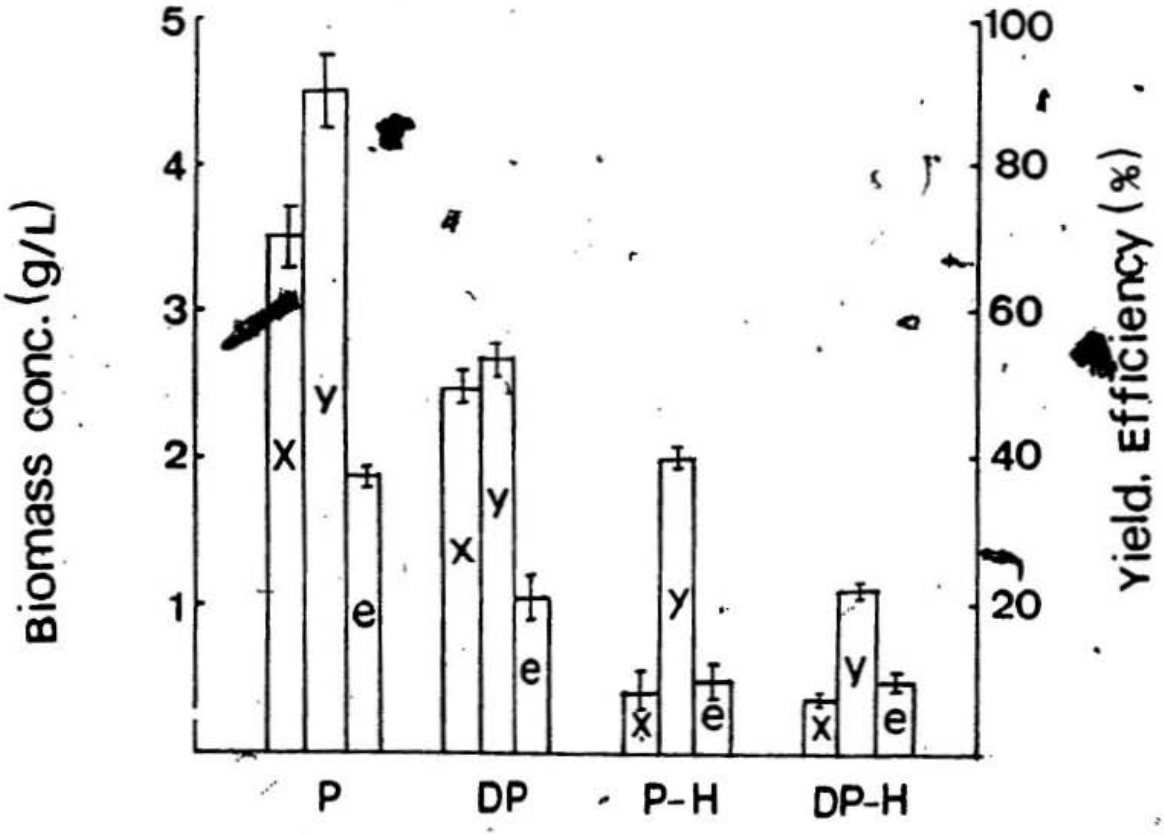


Figure 3.10. Comparison of growth parameters of *S. acidophilum* in peat hydrolysate (1:1 diluted) and modified peat hydrolysates.

Table 3.19 Effect of nutrient-supplementation of undiluted debituminized peat hydrolysates on the growth of *S. acidophilum*[1]

Nutrient symbol[2]	Biomass concentration (g/L)	Yield[1] (%)	Efficiency (%)
DP	2.42±0.06[a]	53.69±2.07[a]	26.36±2.77[a]
DP[2]	2.49±0.33[a]	45.65±2.21[b]	25.62±1.05[a]
DP[3]	2.25±0.39[a]	34.23±4.22[c]	23.14±1.32[a]
DP[4]	2.32±0.19[a]	40.55±0.84[d]	24.58±1.16[a]
DP[5]	1.69±0.15[b]	37.36±2.17[c,d]	10.81±0.08[b]

[1] Mean values of three experiments ± standard deviations.

[2] See Table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

for DP^5 hydrolysate. The yield was found to be highest for the DP hydrolysate. The yield values obtained for the DP^3, and DP^5 hydrolysates were statistically ($P > 0.05$) similar.

P-H hydrolysates

The results obtained with P-H hydrolysates are presented in Table 3.20. When the P-H hydrolysates were supplemented with nutrients, no significant differences ($P > 0.05$) were observed in the biomass concentration between the P-H, $P\text{-}H^2$ and similarly between the values of $P\text{-}H^4$, and $P\text{-}H^5$ substrates. Although higher value of biomass was observed in $P\text{-}H^5$, the hydrolysates. Statistically similar values ($P > 0.05$) were observed between the yield values of P-H, $P\text{-}H^3$, and $P\text{-}H^4$ hydrolysates. Highest yield value was observed in $P\text{-}H^5$ hydrolysate, whereas the highest efficiency was obtained with $P\text{-}H^3$ hydrolysate.

DP-H hydrolysates

DP-H hydrolysates were also supplemented with different nutrients and the results are presented in Table 3.21. Non-supplemented DP-H hydrolysates showed lower growth parameters compared to the supplemented DP-H hydrolysates. The highest biomass concentration was obtained for the $DP\text{-}H^4$ hydrolysate and this value is not statistically different ($P > 0.05$) from that obtained for the $DP\text{-}H^2$ and $DP\text{-}H^5$ hydrolysates. The yield was found to be the highest for the DP^5 hydrolysate which is statistically similar ($P > 0.05$) to the value

Table 3.20 Effect of nutrient-supplementation of undiluted peat hydrolysates with humic acids removed on the growth of *S. acidophilum*[1]

Nutrient symbol[2]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P-H	0.8±0.3[c]	39.9±1.4[c]	9.50±2.90[d]
P-H[2]	0.9±0.2[c]	46.34±2.07[b]	9.34±2.50[d]
P-H[3]	2.8±0.2[a]	35.58±7.29[c]	30.04±2.11[a]
P-H[4]	1.9±0.1[b]	39.54±2.66[c]	18.58±0.29[c]
P-H[5]	2.3±0.6[a,b]	59.41±3.07[a]	23.27±0.10[b]

[1] Mean values of three experiments ± standard deviations.

[2] See Table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table 3.21 Effect of nutrient-supplementation of the undiluted debituminized peat hydrolysates with humic acids removed on the growth of *S. acidophilum* [1]

Nutrient symbol[2]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
DP-H	0.69 ± 0.04^{c}	22.10 ± 0.90^{d}	8.18 ± 1.00^{c}
DP-H[2]	2.01 ± 0.01^{a}	$50.95 \pm 0.19^{b,c}$	22.61 ± 0.03^{a}
DP-H[3]	1.79 ± 0.08^{b}	46.78 ± 0.21^{b}	20.27 ± 0.07^{b}
DP-H[4]	2.12 ± 0.17^{a}	$58.49 \pm 6.53^{a,c}$	23.58 ± 0.91^{a}
DP-H[5]	2.04 ± 0.18^{a}	$59.11 \pm 7.06^{a,c}$	23.53 ± 0.99^{a}

[1] Mean values of three experiments ± standard deviations.

[2] See Table 2.2.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

obtained for DP^4 hydrolysate. The efficiency values obtained for the supplemented DP-H hydrolysates were very close to each other. The efficiency obtained for the $DP\text{-}H^4$ hydrolysate was the highest and was not statistically different ($P > 0.05$) from that obtained for the $DP\text{-}H^2$ and $DP\text{-}H^5$ hydrolysates.

Comparison between the non-modified and modified peat hydrolysates

Comparison of the results obtained for the non-modified and modified peat hydrolysates showed that the modification is not a good alternative to improve the growth of the fungus. When the modified peat hydrolysates were supplemented with different nutrients, the desired enhancement in the growth of the fungus was not observed. The growth parameters obtained for the non-modified peat hydrolysate were relatively higher compared to those obtained for the modified-supplemented hydrolysates. When the non-modified peat hydrolysate was supplemented with 3 g/L yeast extract and 0.4 g/L $MgSO_4$ the growth parameters were found to be the highest.

3.6.5 Growth of *S. acidophilum* in compost hydrolysate

S. acidophilum was grown in undiluted peat-fish offal compost hydrolysate and the results obtained with different nutrients are presented in Table 3.22. The biomass concentration obtained for C^5 hydrolysate was found to be the highest. However, the values obtained for C and C^3 hydrolysates were statisti-

Table 3.22 Effect of nutrient-supplementation on the growth of *S. acidophilum* in compost hydrolysate[a]

Substrate symbol[!]	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
C^1	2.51 ± 0.10^{c}	59.88 ± 3.89^{b}	$17.43 \pm 2.34^{b,c}$
C^2	3.08 ± 0.12^{b}	59.63 ± 1.57^{b}	14.80 ± 2.04^{c}
C^3	2.33 ± 0.08^{c}	48.56 ± 3.13^{c}	14.86 ± 1.98^{c}
C^4	3.36 ± 0.09^{b}	53.19 ± 2.59^{c}	21.14 ± 3.01^{b}
C^5	4.76 ± 0.38^{a}	66.49 ± 5.43^{a}	30.68 ± 3.80^{a}

[a] Mean values of three experiments ± standard deviations.

[!] See Table 2.3.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

cally not different ($P > 0.05$). Higher values of efficiency were observed when yeast extract was supplemented with $MgSO_4$ as in C^5 hydrolysates compared to the other compost hydrolysates.

The growth curve of *S. acidophilum* in compost hydrolysate is shown in Figure 3.11 (Appendix 10). The growth of *S. acidophilum* in compost hydrolysate can also be explained with four phases namely, lag phase, accelerated growth phase, stationary phase, and death phase. However, no clear stationary phase was observed in this experiment. Instead an accelerated death phase was observed. The biomass concentration increased until the seventh day and then decreased. The growth parameters of *S. acidophilum* in peat (P) and compost (C) hydrolysates are compared in Figure 3.12 (Appendix 11). Although the biomass concentration and the efficiency values of *S. acidophilum* in compost hydrolysate were lower than those in the peat hydrolysate, the yield values in both hydrolysates were not statistically different ($P > 0.05$). Therefore, peat hydrolysate (1:1 diluted) is a better substrate for the growth of *S. acidophilum* than compost hydrolysate.

3.7 Proximate composition of *S. acidophilum* biomass

Proximate analysis of the *S. acidophilum* biomass is presented in Table 3.23. Analysis of the biomass showed that *S. acidophilum* is a good source of protein. The protein content was about 38 % and this compares well with the value reported by Boa and Le Duy (1982), and lower than the value obtained by Martin

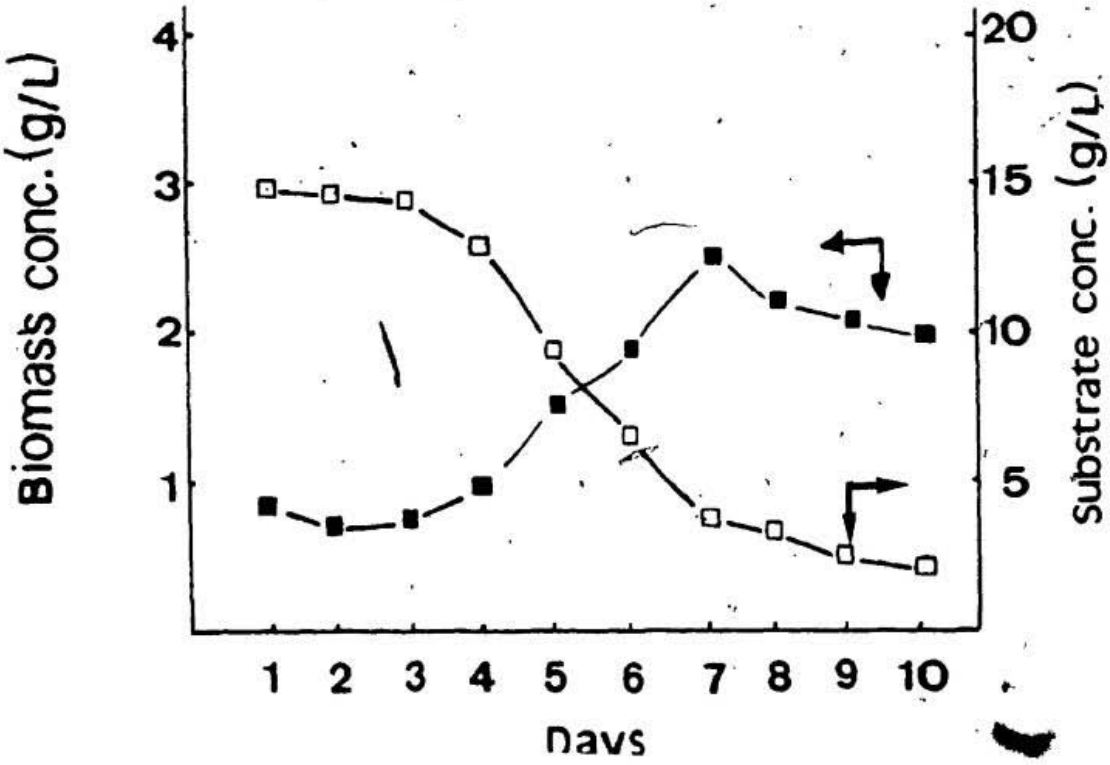


Figure 3.11 Growth curve of *S. acidophilum* in compost hydrolysate.

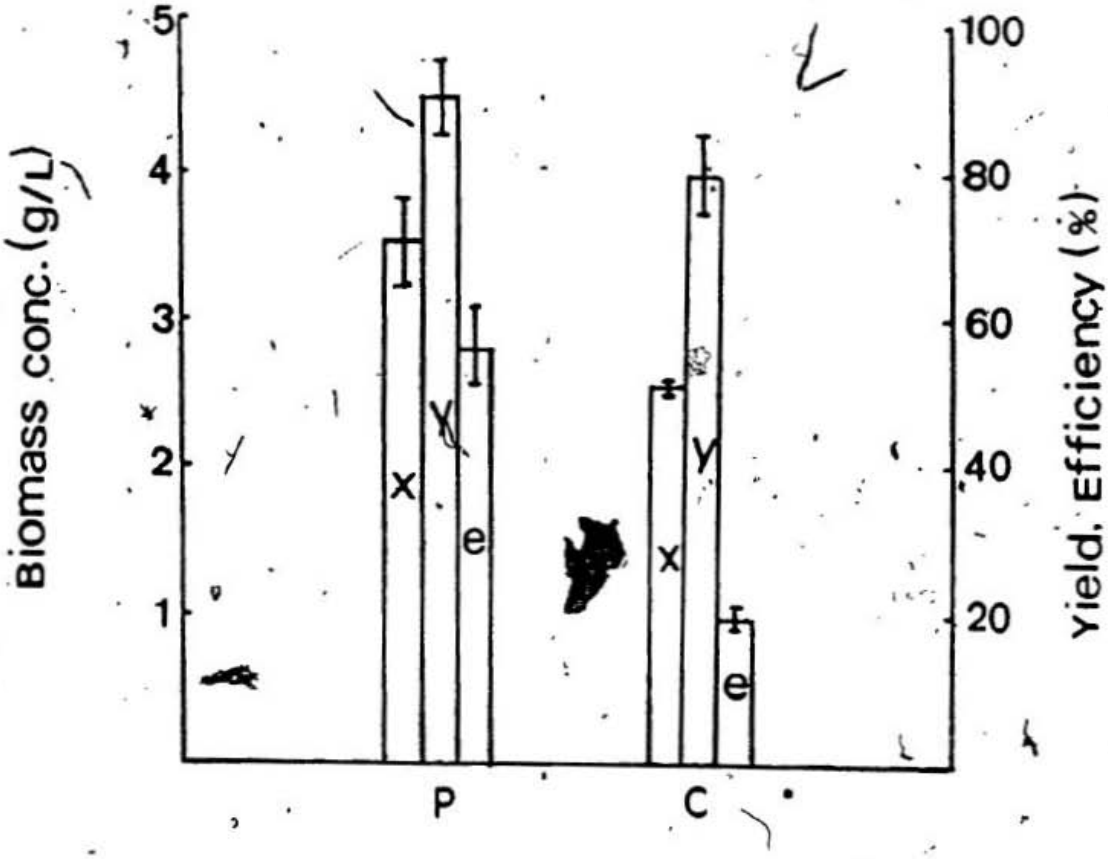


Figure 3.12 Comparison of growth parameters of *S. acidophilum* on peat hydrolysate (1:1 diluted) and compost hydrolysate. x = biomass concentration, y = yield, e = efficiency.

Table 3.23 Composition of *S. acidophilum* biomass produced on peat hydrolysate supplemented with 3 g/L yeast extract and 0.4 g/L $MgSO_4$[1]

Component	% dry weight
Crude protein	37.52±0.83
Crude lipid	1.91±0.07
Ash	10.88±0.61
Carbohydrate[2]	49.55±1.24

[1] Mean values of three determinations ± standard deviation

[2] Calculated by difference

and White (1985). The protein, lipid, and ash values obtained in this work are within the range of the values reported by Reed (1982) for the filamentous fungi. The high ash content of the biomass in peat hydrolysates was attributed to the large amount of minerals present in the medium (Boa and Le Duy, 1982).

The composition of amino acids of the fungal biomass are listed in Table 3.24. The values observed in this work compares with values reported by Boa and Le Duy (1982). The levels of essential amino acids, lysine, leucine, isoleucine, threonine were higher than the values reported by Boa and Le Duy (1982)..

Table 3.24 Content of amino acids (%) in biomass of *S. acidophilum*[1]

Amino acid	*S. acidophilum* biomass
Alanine	8.99±0.01
Arginine	4.72±0.01
Aspartic acid	9.69±0.03
Cysteic acid	0.10±0.03
Cystine	0.48±0.03
Glutamic acid	14.17±0.03
Glycine	9.82±0.01
Histidine	2.43±0.02
Hydroxylysine	0.06±0.00
Isoleucine	4.86±0.01
Leucine	6.84±0.02
Lysine	6.17±0.01
Methionine	0.10±0.02
Phenylalanine	3.69±0.01
Proline	5.05±0.02
Serine	5.83±0.01
Taurine	0.10±0.00
Threonine	6.31±0.03
Tyrosine	3.02±0.01
Valine	6.71±0.02

[1] Mean values of three determinations ± standard deviation

CONCLUSIONS

Included as parts of this thesis are the optimal growth media and the nutritional requirements of the fungus *Scytalidium acidophilum*. Of the various growth media investigated, non-modified H_2SO_4- peat hydrolysate diluted with 1:1 water proved to be the best in terms of fungal growth parameters produced. The undiluted non-modified peat hydrolysate produced a higher concentration of biomass than the modified hydrolysates, but these values were not as good as in the diluted non-modified hydrolysate. About half of the biomass was obtained using peat hydrolysate as a carbon source compared to glucose media.

It was observed that there was a decrease in carbohydrate content during the modification process. However, removal of bitumens enabled a better extraction of nitrogen and amino acid contents. By comparing the undiluted non-modified and modified hydrolysates, it was shown that the removal of bitumen or humic acids from peat does not improve the fungal growth parameters.

The supplementation of the growth media with various nutrients was studied. The addition of 3 g/L yeast extract and 0.4 g/L $MgSO_4$ produced higher growth parameter values than other nutrient supplements in all the growth media except H_3PO_4- peat hydrolysates. The addition of humic and fulvic acid fractions, obtained by fractionation of humic substances, to the synthetic medium, influenced the growth of the fungus. The preliminary studies have shown that humic acids fraction was stimulatory whereas the fulvic acid fraction

was inhibitory to the growth of the fungus.

Cultivation of *S. acidophilum* on compost hydrolysates showed that they can provide nutrient media for the production of MBP. Because the concentration of carbohydrates in compost hydrolysate is low compared to that of peat hydrolysates, the microbial biomass grown was low, although the yield values were similar. Since the content of nitrogenous nutrient in compost is high, growth of fungi on solid compost substrate could be a promising approach for the production of MBP.

S. acidophilum biomass produced in peat hydrolysates had a high protein content, which merits its consideration as a source of microbial protein for animal diet supplementation.

SUGGESTIONS

1. Since *S. acidophilum* fungus produces a high quality protein, it has a potential use in food and feeds. The use of H_2SO_4- peat hydrolysates as substrates is recommended in those areas where this resource is available and its price is competitive.

2. Because *S. acidophilum* grows at low pH values, at which most proteins lose their functional properties, future studies should concentrate on the functional properties of the protein and enzymes of this fungus.

3. Since *S. acidophilum* proteins are produced at low pH values, a low temperature pasteurization processes should be developed for the variety of products formulated using them.

4. Feeding trials should be conducted with animals on the utilization of this microbial biomass protein as a dietary-supplement.

5. It is suggested to supplement the compost hydrolysates with carbohydrates in order to enhance the growth of the fungus in that medium.

6. Further study is required to identify the precise effects of the humic and fulvic acid fractions of peat on microbial growth.

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Table A 1. Effect of increasing concentration of the humic acids fraction on the growth of *S. acidophilum* in synthetic medium at pH 2.00[1]

Humic acids (%)	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
0.00	8.04±0.40^{c}	78.51±1.03^{c}	67.00±1.23^{d}
0.11	8.58±0.78^{c}	84.78±2.10^{b}	71.5±1.09^{c}
0.15	9.54±0.21^{b}	87.84±2.58^{b}	79.5±2.46^{b}
0.20	10.68±0.53^{a}	95.18±1.14^{a}	89.00±3.09^{a}
0.23	5.07±0.67^{d}	57.22±1.36^{d}	42.25±2.02^{e}

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 2. Effect of increasing concentration of humic acids fraction on the growth of *S. acidophilum* in synthetic medium at pH 8.00[1]

Humic acids (%)	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
0.00	$8.16 \pm 1.00^{a,b}$	81.76 ± 2.20^{b}	68.00 ± 1.62^{d}
0.12	$8.90 \pm 1.27^{a,b}$	83.96 ± 3.11^{b}	74.17 ± 1.83^{c}
0.25	$9.72 \pm 2.15^{a,b}$	84.52 ± 3.30^{b}	81.01 ± 2.24^{b}
0.37	$10.71 \pm 2.06^{a,b}$	92.32 ± 2.90^{a}	89.25 ± 3.01^{a}
0.50	6.83 ± 0.70^{b}	59.49 ± 4.73^{c}	56.90 ± 3.05^{e}

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 3. Effect of increasing concentration of the fulvic acid fraction on the growth of *S. acidophilum* in synthetic medium at pH 2.00[1]

Fulvic acids (%)	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
0.00	8.02 ± 1.00^{a}	80.52 ± 2.20^{a}	66.83 ± 1.62^{a}
0.25	4.61 ± 0.03^{b}	47.11 ± 0.76^{b}	38.41 ± 0.45^{b}
0.50	4.59 ± 0.62^{b}	43.36 ± 0.45^{c}	38.25 ± 2.35^{b}
0.75	4.09 ± 1.08^{b}	42.86 ± 3.08^{c}	34.08 ± 4.34^{b}

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 4. Effect of increasing concentration of the fulvic acid fraction on the growth of *S. acidophilum* in synthetic medium at pH 8.00[1]

Fulvic acids (%)	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
0.00	8.02±1.00[a]	80.52±2.20[a]	66.83±1.62[a]
0.25	4.58±0.01[b]	47.86±1.74[b]	38.17±0.33[b]
0.50	4.30±0.12[b]	44.56±1.08[b]	35.83±0.06[c]
0.75	3.89±0.32[b]	38.44±3.21[c]	32.42±0.12[d]

[1] Mean values of three experiments ± standard deviations.

[2] Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 5. Comparison of the growth parameters of *S. acidophilum* on undiluted H_2SO_4, H_3PO_4, HCl, and HNO_3 hydrolysates of peat[1]

Hydrolysates	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
H_2SO_4- P	3.29±0.06[a]	26.15±0.50[a]	8.40±0.15[b]
H_3PO_4- P	2.15±0.01[b]	24.56±3.38[a]	17.92±0.16[a]
HCl- P	0.59±0.04[c]	10.17±1.63[b]	6.09±0.22[c]
HNO_3- P	0.30±0.01[d]	5.11±0.30[c]	1.48±0.14[d]

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 6. Comparison of growth parameters of *S. acidophilum* on (1.1 diluted) H_2SO_4, H_3PO_4, HCl, and HNO_3 hydrolysates of peat[1]

Hydrolysates	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
H_2SO_4- P	3.50 ± 0.10^a	90.01 ± 5.40^a	37.25 ± 1.20^a
H_3PO_4- P	2.56 ± 0.09^b	36.76 ± 0.42^b	28.47 ± 0.11^b
HCl- P	2.15 ± 0.23^b	25.44 ± 0.20^c	16.69 ± 1.32^c
HNO_3- P	0.99 ± 0.01^c	13.34 ± 0.17^d	6.39 ± 0.03^d

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 7. Growth curve of *S. acidophilum* in (1:1 diluted) peat hydrolysate

Days	Biomass concentration (g/L)	Substrate concentration (g/L)
1	0 67 ±0 03	22.75± 1.24
2	0 60±0 02	22.01± 2.01
3	0 82±0 04	20.52± 2.06
4	1.58±0.01	19.58± 1.86
5	2.76±0.12	15.00± 1.03
6	3.50±0.09	10.40± 1.01
7	3.50±0.16	5.63±0.59
8	3.48±0.20	5.03±0.50
9	3.47±0.23	4.34±0.53
10	3.46±0.22	2.25±0.18

Mean values of three experiments ± standard deviations.

Table A 8. Comparison of the growth parameters of *S. acidophilum* on undiluted peat hydrolysate and the modified peat hydrolysates[1]

Hydrolysates	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P	3.29±0.06[a]	26.15±0.50[c]	8.40±0.15[b]
DP	2.42±0.06[b]	53.69±2.07[a]	20.36±2.77[a]
P-H	0.80±0.30[c]	39.90±1.40[b]	9.50±2.90[b]
DP-H	0.69±0.04[c]	22.70±0.90[d]	8.18±1.00[b]

[1] Mean values of three experiments ± standard deviations.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 9. Comparison of the growth parameters of *S. acidophilum* on peat hydrolysate and the modified peat hydrolysates[1]

Hydrolysates	Biomass concentration (g/L)	Yield (%)	Efficiency (%)
P[!]	3.50 ± 0.40^{a}	90.01 ± 5.40^{a}	37.25 ± 1.20^{a}
DP	2.42 ± 0.06^{b}	53.69 ± 2.07^{b}	20.36 ± 2.77^{b}
P-H	0.80 ± 0.30^{c}	39.90 ± 1.40^{c}	9.50 ± 2.90^{c}
DP-H	0.69 ± 0.04^{c}	22.70 ± 0.90^{d}	8.18 ± 1.00^{c}

[1] Mean values of three experiments ± standard deviations.

[!] Diluted (1:1) with water.

Values in the same column with the same superscript are not statistically different ($P > 0.05$).

Table A 10. Growth curve of *S. acidophilum* in Compost hydrolysate

Days	Biomass concentration (g/L)	Substrate concentration (g/L)
1	0.87±0.01	14.88±0.24
2	0.70±0.02	14.80±0.32
3	0.76±0.01	14.63±0 29
4	0.78±0.01	14.25±0.35
5	1.60±0.04	9.50±0.30
6	1.89±0.03	7.00±0.48
7	2.51±0.06	4.25±0.22
8	2.25±0.03	4.02±0.20
9	2.20±0.04	2.50±0.19
10	2.01±0.02	2.46±0.26

Mean values of three experiments ± standard deviations.

Table A 11. Comparison of growth parameters of *S. acidophilum* in peat hydrolysate (1:1 diluted) and compost hydrolysate[1]

Growth parameters	P	C
Biomass concentration (g/L)	3.5±0.4	2.51±0.07
Yield (%)	90.0±5.4[a]	79.88±5.60[a]
Efficiency (%)	37.25±1.2	17.43±1.65

[1] Mean values of three experiments ± standard deviations.

Values in the same row with the same superscript are not statistically different ($P > 0.05$).