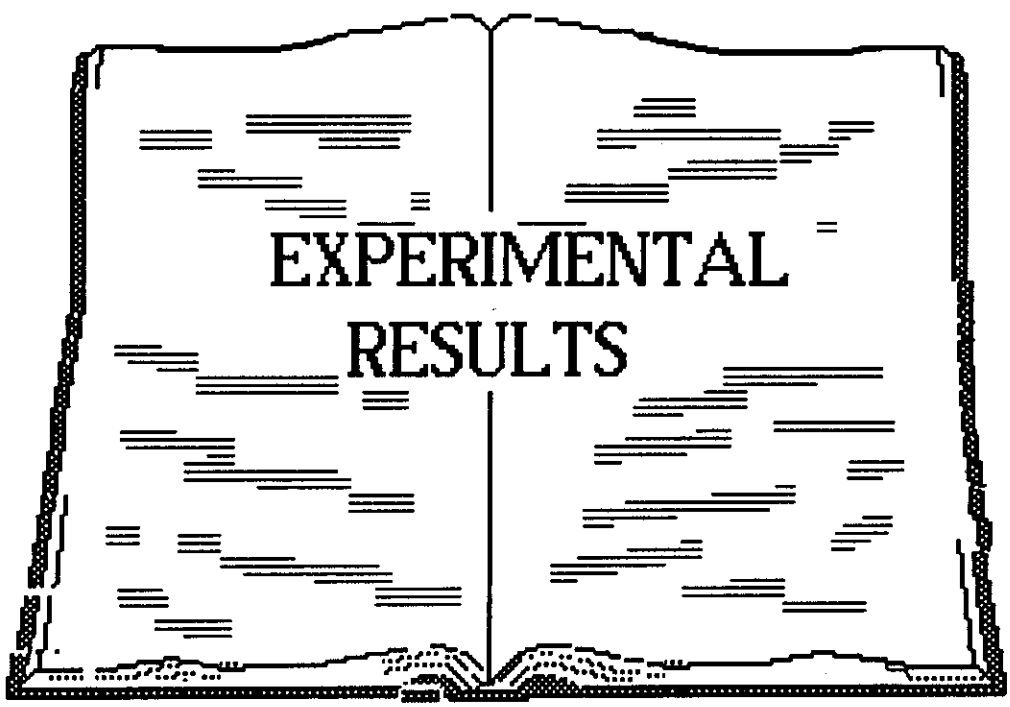
EXPERIMENTAL
RESULTS

PART I
DESILICATION AND DELIGNIFICANTION OF BALCK LIQUOR

A- Desilication of black liquor

Difficults encountered in obtaining black liquor sampels from RAKTA

Because of certain technical problems it was difficult to obtain fresh black liquor samples directly from the digestors at RAKTA. The draining system of the black liquor is a closed circuit. The only possible sample available were from stored stocks. Storing resulted in a sharp drop in the pH of the black liquor and the precipitation of silica. According by the obtained samples of black liquor was of low silica content which made studies on desilication very difficult when these samples were used. For this reason black liquor for desilication studies were prepared at the laboratory. As it was difficult to use rice straw for preparation of black liquor, rice hulls were used instead of.

Preparation of black liquor from rice hulls for desilication studies:

In 20 liter capacity flasks one kilogram of rice hulls + 7 litres of water + 175 grams of solid NaOH pellets were prepared. Flasks were plugged with cotton and autoclaved at 1.5 atomspher for 1 hr. The black liquor was filterd from the rice hulls and used for desilication. About 5 liter were obtained from 1 kg of rice hulls. pH of the black liquor $\simeq$ 11.

NEW NONCONVENTIONAL METHOD OF FRACTIONATION OF BLACK LIQUOR

Introduction :-

The black liquor contains three main components : (a) silica in the form of sodium silicate, (b) lignin in the form of sodium ligninate, and (c) hemicelluloses, mainly pentozans and hexozans. The fresh black liquor contains a great amount of sodium hydroxide, which renders the black liquor highly alkaline (pH 10.5-11).

The main cause of the pollution caused by black liquor is the hemicelluloses, as these compounds are very suitable nutrients for many microbes that propagate and lead to the creation of nutritive chains, which results in the propagation of undesired biota.

Our plan aims not only at the removal of components but at making use of these substances. The first step in our plan for fractionation of black liquor is the extraction of silica, then lignin and at last hemicelluloses.

Difficulties encountered in the extraction of silica

Silica is highly soluble in the highly alkaline black liquor. The conventional methods of the desilications of black liquor rely on the neutralization of black liqour using mineral acids as sulphuric or hydrochloric - to a pH of 9-8, where silica is converted to silicic acid, silica gel settles down in a gel form. The main disadvantages of such methods are :

1 - High cost as great amounts of mineral acids are needed for neutralization.

2 - Gelation of silica which leads to the adsorption of a lot of lignin and hemicelluloses.

3 - Difficulty of purification of the impure silica and need for tedious and expensive methods of purification.

Aim of the new nonconventional method of desilication

Our research work was directed towards working out a scheme for desilication avoiding the above mentioned three difficulties. For working out a scheme that could realize the desired aims an intensive consultation of the published literature on the behaviour of silicates in solution, colloid formation, gelation and coagulation was carried out. A short summary of the main bases on which our scheme relied is given below.

SOME SCIENTIFIC BASES OF SILICATE BEHAVIOUR IN SOLUTION AND COAGULATION

1 - The behaviour of silicates in solution is governed by two interdependent sets of equilibria :

$\longrightarrow$ Si - OH + H_2O $\rightleftharpoons$ Si - O^- + H_3O^-

monomer

$\longrightarrow$ Si- OH + H^- Si $\rightleftharpoons$ Si - O - Si + H_2O

$\longrightarrow$ Si - OH + O^- - Si $\rightleftharpoons$ Si - O - Si + OH^-

i.e polymerization $\rightleftharpoons$ depolymerization

2 - There are three stages in the formation of silicates gels:

a - polymerization of monomer to form polymer particles.

b - growth of particles.

c - linking of particles together to form colloid particles.

3 - The formd gel particles posses physical adsorptive characteristics, Vander Wall's adsorption. In the dispersion medium ions capable of being polarized are adsorbed on colloid particle surfaces. Particles bearing a definite charge adsorb opposite charged ions forming electric double layer.

4 - Molecules of SiO_2, which are on the surface of silica particles interact with the dispersion medium, are hydrated and form silicic acid which can be ionized :

H_2SiO_3 $\rightleftharpoons$ SiO_3^{2-} + $2H^+$

In this case, the silicate ion remains on the surface of the particles so that it is charged negatively, while hydrogen ions pass over to the solution.

5 - Colloidal systems may coagulate under the action of several factors:

a) aging of a system.

b) change in the concentration of the dispersed phase.

c) addition of electrolytes.

d) cations coagulate sols having negatively charged particles and anaions, sols, having positively charged particles.

6 - Rules of coagulation by electrolytes :

a) the coagulation power of anaions is the greater, the higher its valency, this is called schultz and Hardy valency rule.

b) the coagulation powers of ions of the same valency grows with the ionic radius.

c) certain minium concentration of an electrolyte in a sol must be reached for coagulation to begin. This quantity is known as the coagulation threshold and is usually experssed in m mol\L or mg-equiv/1.

7 - The series of ions compared according to their ability to interact with the medium are known as the lyotropic series of Hofmeister series. Monovalent cations can be put in the following series according to their adsorbability

Li + > Na+ > K+ > Rb+ > Cs+

USE OF DIFFERENT ELECTROLYTES FOR SEPARATION OF SILICA FROM BLACK LIQUOR

Aim : The aim of the following trial was, working out a procedure of silica separation that avoids the use of much acid for silica separation and prevention of gelation of separated silica.

I - USE OF ELECTROLYTES WITH MONOVALENT CATIONS

1 - Use of NaCl :

It was found that the addition of only NaCl at all the tested concentrations did not cause the precipitation of silica from black liquor. Consequently, the black liquor variants with different NaCl concentrations were titrated using 10% HCL. The acid was added on shaking, till the onset of silica preciptation.

The data given in table (1) and fig (6) show that the addition of NaCl to black liquor in concentrations from 4.5% to 20% led to the following :

Firstly : The precipitation of silica in the form of an amorphous precipitate and not in gel form, on addition of HCl.

Table (1): Effect of use of different concentrations of NaCl for precipiation of silica.

NaCl conc. %	Volum of 10 % HCl needed for silica precipitation ml.	Gel formation	Weight of precipitated silica (g)
20	4.2	no	1.9856
18	4.2	no	2.0136
16	4.2	no	2.0296
14	4.2	no	2.0716
12	4.2	no	2.0119
10	4.2	no	2.0248
8	4.2	no	1.9555
6	4.2	no	2.0893
5	4.2	no	1.9646
4.5	4.2	no	1.9558
4	4.5	Gel formed	2.0253
3.5	4.8	Gel formed	1.9755
3	5.2	Gel formed	1.9214
2.5	5.8	Gel formed	1.9254
2.0	6.8	Gel formed	1.9089
1.5	7.8	Gel formed	2.1004
1.0	8.8	Gel formed	2.0145
0.5	9.0	Gel formed	2.1035
0.0	11.5	Gel formed	1.9952

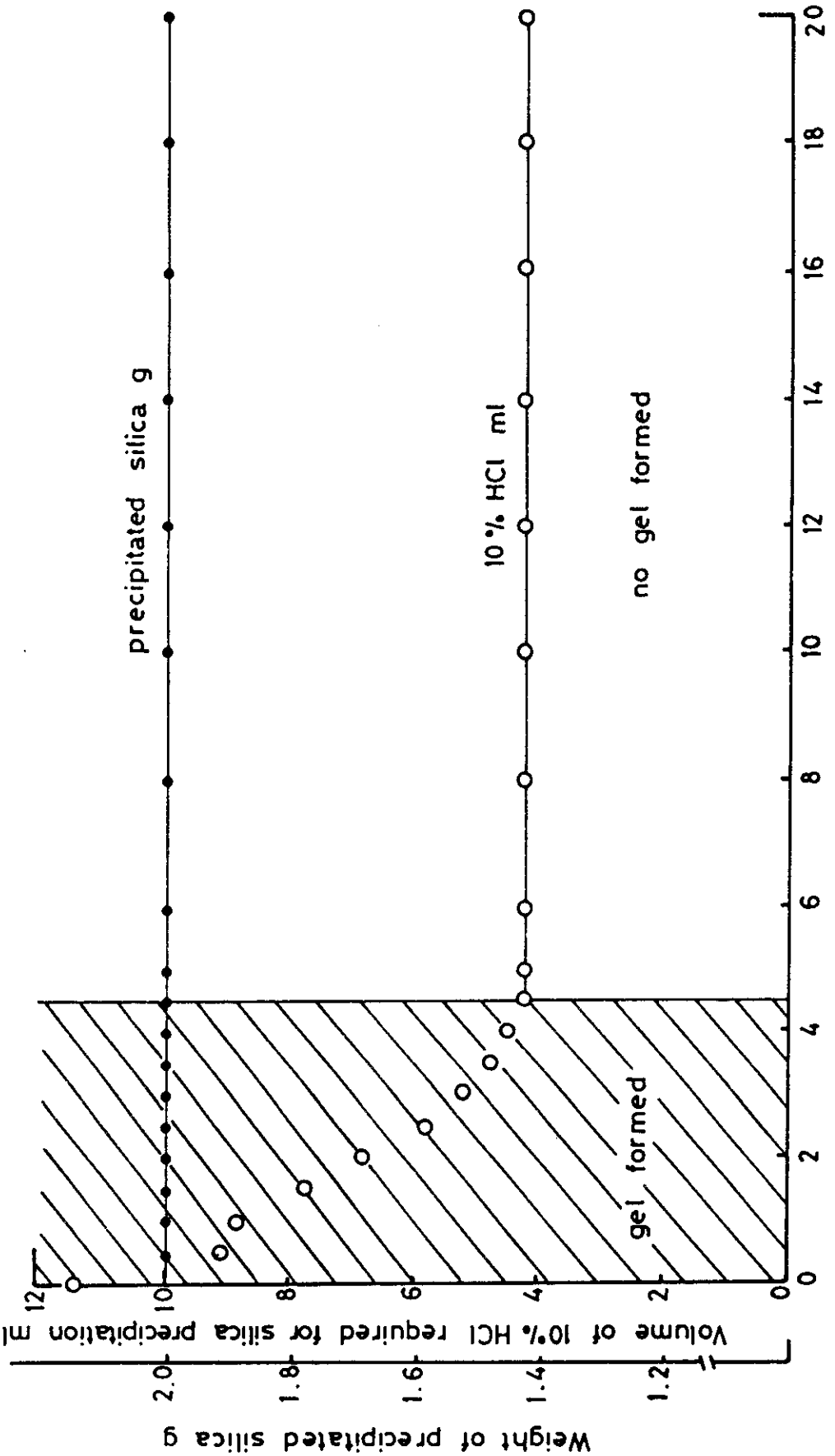

Fig.(6) Use of NaCl for silica precipitation from black liquor.

Secondly : All variants of black liquor with NaCl in concetrations 4.5- 20% required for silica precipitation the same amount of 10 % HCL.

Thirdly : The addition of NaCl in concentrations of 4.5 - 20 % decreased the required amount of HCl to almost 1/3, compared to the case without NaCl.

Fourthly : Silica preciptated at much higher pH value ($\simeq$ 10) compared to the variant without NaCl.

Fifthly : Silica obtained as precipitate was easily separated, washed and obtained as snow white preparation.

For variants of black liquor having lower than 4.5% NaCl the following was recorded :

Firstly : Silica did not precipitate in the form of amorphous precipitate but in gel form.

Secondly : The amount of 10% HCl required for silica precipitation increased with the decrease of NaCl % in balck liquor.

Thirdly : The pH at which silica precipitated was $\simeq$ 9

Fourthly : The obtained silica was difficulty purified as being in gel form and adsorbed much lignin and hemicelluloses.

It might be concluded that 4.5% NaCl is a threshold NaCl concentration for coagulation of silica and that the coagulating effect of NaCl is pH dependent and it is most exhibited when the pH of the

black liquor was lowered to $\simeq$ 11, the value attained due to the addition of 4.2 ml 10% HCl to 100 ml black liquor.

It is very important that the amount of silica preciptiated in the above mentioned treatments were the same for all variants. This means that all silica is separated as precipitate in the variants with NaCl concentrations > 4.5 %.

Conclusion :

The addition of NaCl in concentrations > 4.5% to black liquor followed by acid neutralization realized easy and more economic separation of silica. NaCl addition in % > 4.5 prevented gelation and serverd as good coagulant enabling obtaining highly pure snow white amorphous preparation of silica. This achievement will be presented for patenting.

2 - Use of different mineral acids for the desilication of black liqour

Different acids were used for the desilication of black liquor. These were HCl, HNO_3, H_2SO_4 and H_3PO_4. One normal solutions of these acids were prepared and used for the titration of black liquor till the complete precipitation of the silica from black liquor. The data given in table (2) show that using 1 N of either HCl, HNO_3 or H_2SO_4, equivalent volumes of these 1N acids were required for the desilication of black liquor, while using H_3PO_4 quite great volume was used. This could be attributed to the dissociation coefficient of these acids, while HCl, HNO_3 and H_2SO_4 are of high dissociation coefficient, H_3PO_4 are of low dissociation coefficient.

Table (2) : Volumes of different mineral acids used for the desilication of 50 ml. of black liquor

Acid	Volume of 1 N needed for desilication of 50 ml. black liquor
HCl	2.9
HNO_3	2.9
H2SO4	2.9
H_3PO_4	4.2

The obtained data confirms that it is the H+ cation that of the main role in the process of desilication, while the anion (Cl^- , NO_3^- or SO_4^{--}) are of no role.

3 - Use of sulfuric acid for the desilication of black liquor containing different NaCl concentrations.

Titrations using 5% H_2SO_4 was carried out for black liquor samples (50 ml.) having different concentrations of NaCl.

The data given in table (3) and Fig. (7) show that as it was the case using HCl the following can be concluded :

Firstly : The preciptation of silica in the form of an amorphous precipitate and not in gel form in the variants of black liquor having NaCl concentrations from 4.5 to 20%.

Secondly : All variants of black liquor with NaCl concentrations from 4.5 to 20 % required for silica preciptation, the same volume of 5% H_2SO_4

Thirdly : The addition of NaCl in concentrations from 4.5 to 20% decreased greatly the required amount of H_2SO_4 needed for desilication compared to the case without NaCl.

Fourthly : Silica precipitated at much higher pH value (10.0) compared to the variant without NaCl.

Fifthly : Silica obtained as amorphous precipitate was easly separated, washed and obtained as snow white preparation.

For variants of black liquor having NaCl lower than 4.5% NaCl, the following was recorded :

Table (3): Effect of use of different concentrations of NaCl for silica precipiation using $H_2 SO_4$

NaCl conc. %	Volum of 5% $H_2 SO_4$ needed for silica precipitation ml.	Gel formation	Weight precipitated of silica
20	0.2	no	1.950
18	0.2	no	1.945
16	0.2	no	1.946
14	0.2	no	1.948
12	0.2	no	1.950
10	0.2	no	1.951
8	0.2	no	1.949
6	0.2	no	1.948
5	0.2	no	1.949
4.5	0.2	no	1.950
4	0.25	Gel formed	1.948
3.5	0.3	Gel formed	1.949
3	0.7	Gel formed	1.948
1.0	1.9	Gel formed	1.900
0.5	2.2	Gel formed	1.850
0.0	2.3	Gel formed	1.880

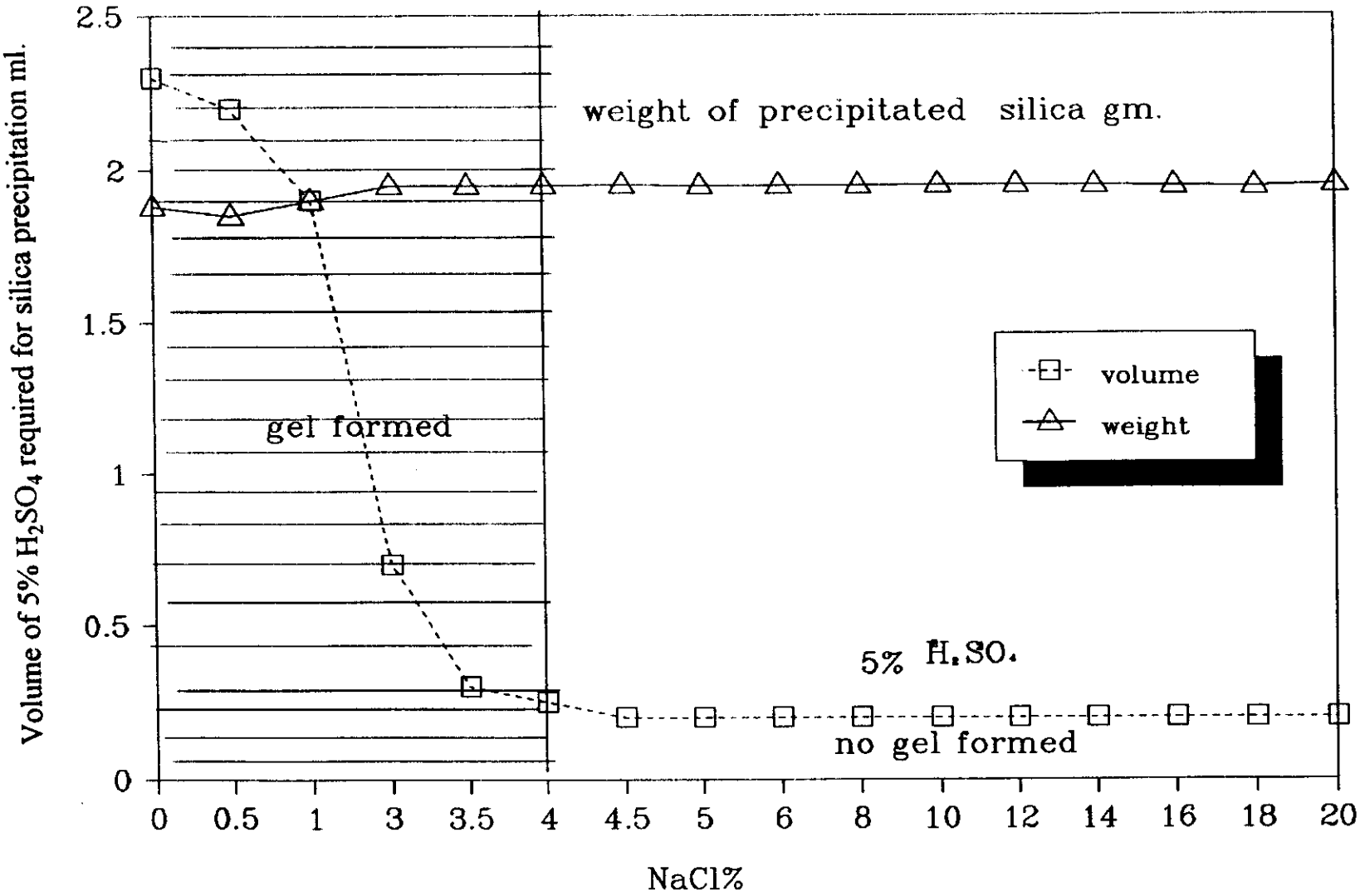

Fig. (7): Use of H_2SO_4 for silica precipitation from black liquor

Firstly : Silica did not precipitate in the form of amorphous precipitate but in gel form.

Secondly : The amount of 5% H_2SO_4 for silica precipitation increased with the decrease of NaCl concentration in black liquor.

Thirdly: The pH at which silica precipitated was $\simeq$ 9.0

Fourthly : The obtained silica was difficultly to be purified as being in gel form and absorbing much lignin and hemicelluloses.

It can be concluded that for H_2SO_4 as it was for HCl 4.5% NaCl is a threshold concentration for coagulation of silica and that the coagulating effect of NaCl is pH dependent and it is most exhibited when the pH of the black liquor was lowered to $\simeq$ 11.0 the value attained due to the addition of 4.2 ml, 10% HCl or 0.4 ml 5% H_2SO_4 to 100 ml black liquor.

It is very important to note that the amount of silica precipitated using either 10% HCl. or H_2SO_4 were the same from the variants with NaCl concentration > 4.5%.

Conclusion :

The use of H_2SO_4 for silica precipitation from black liquor variants containing > 4.5% NaCl showed the same results as when HCl is used.

As H_2SO_4 is cheaper than HCl it can be recommended for desilication of black liquor than HCl.

4- Use of other sodium salts

The success encountered using NaCl for the desilication of black liquor encouraged us to study the possibility of using other sodium salts for such desilication. For this purpose some monovalent soduim salts (NaI, NaBr and $NaNO_3$), divalent (Na_2SO_4 and Na_2Co_3) and trivalent (Na_3PO_4) were used. Series of 100 ml aliquots of black liquor were prepared. The mentioned salts were added in weights containing the same sodium content as in 5.0g NaCl. After complete soulbilization of the salts, titration using 5% HCl was carried out till the complete precipitation of silica. The volume of HCl was recorded.

The data given in table (4) show that except for Na_2CO_3, the use of monovalent salts (NaI, NaBr and $NaNo_3$) divalent (Na_2SO_4) or trivalent ((Na_3PO_4) required the same volume of 5% HCl (4.8 ml) for silica precipitation. For sodium carbonate 6.0 ml. of 5% HCl were required, moreover gel was formed. This can be attributed to the low dissociation coefficient of Na_2CO_3 as it is a weak electrolyte.

Table (4) Use of different sodium salts for the desilication of black liquor.

Sodium salt	Molecular weight	Equisodium weight equivalent to 5.0 g. Na	Volume of 5% HCl needed for precipitation of silica from. 50 ml. black liquor ml.
Nacl	58.45	5.000	4.8
NaI	149.92	12.821	4.8
NaBr	102.91	8.501	4.8
$NaNo_3$	85.01	7.022	4.8
Na_2SO_4	142.00	11.730	4.8
Na_3PO_4	380.16	31.404	4.8
Na_2CO_3	106.00	8.756	*6.0

* Gel is formed.

The obtained data indicate that it is the Na^+ (Sodium ion) which is responsible for the neutralization of the negative charge on the colloidal particles of silicate ions leading to their easy and quite precipitation. However, from the data given in table (4), about the weights needed from the used salts, it is clear that the least weight is that of NaCl.

Furthermore this salt is the cheapest of all these salts as indicated from the data given in table (5). Sodium chloride is far the cheapest salt for desilication of black liquor.

Table (5): Best price of used sodium salts for desilication of black liquor.

Sodium salt	price of one kg.
Nacl	6.50
NaI	120.50
NaBr	14.00
$NaNo_3$	40.00
Na_2SO_4	10.00
Na_3PO_4	10.00
Na_2CO_3	10.00

II - USE OF OTHER MONOVALENT CATIONS FOR DESILICATION OF BLACK LIQUOR

As it is well known for the same anion, the thresholds of coagulation of equivalent cations are not the same and for monovalent cations they are arranged as follows : $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. This series is called liotropic series. In view of the fact that Li, Rb and Cs are very expensive the present studies were restricted to K^+ and NH_4^+.

a - Use of potassium chloride for desilication of black liquor

The data given in table (6) and Fig (8) show that KCl showed that almost the same effect in precipitating silica from black liquor. KCl when added alone to black liquor did not cause the precipitation of silica; however, similar to NaCl its presence in concentrations > 4.5% led to the decrease of the amount of 10% HCl needed for the precipitation of silica.

However, KCl showed lower coagulating potentiality than NaCl. While the coagulating threshold of NaCl is 4.5% yet for KCl it was shown to be 6% . With lower concentrations of KCl (< 6%) gel was formed. The amount of precipitated silica, using KCl, was almost the same using NaCl.

Table (6) : Effect of the use of different electrolytes with monovalent cations.

Electrolyte conc. %	NaCl			KCl		
	Volume of 10 % HCL needed for silica ppt. ml.	Gel formation	Weight of ppt. silica g	Volume of 10 % HCL needed for silica ppt. ml.	Gel formation	Weight of ppt. silica g
6.0	6.2	no	1.75	6.2	no	1.75
5.0	6.2	no	1.75	6.2	Gel formed	1.75
4.5	6.2	no	1.75	6.2	Gel formed	1.75
4.0	7.0	Gel formed	1.65	7.0	Gel formed	1.65
3.5	7.2	Gel formed	1.65	7.2	Gel formed	1.65
3.0	7.6	Gel formed	1.75	7.6	Gel formed	1.80
2.0	8.8	Gel formed	2.00	8.8	Gel formed	2.10
1.0	10.5	Gel formed	2.00	10.5	Gel formed	2.10
0.5	11.0	Gel formed	2.00	11.0	Gel formed	2.00
0.0	12.0	Gel formed	2.00	12.0	Gel formed	2.00

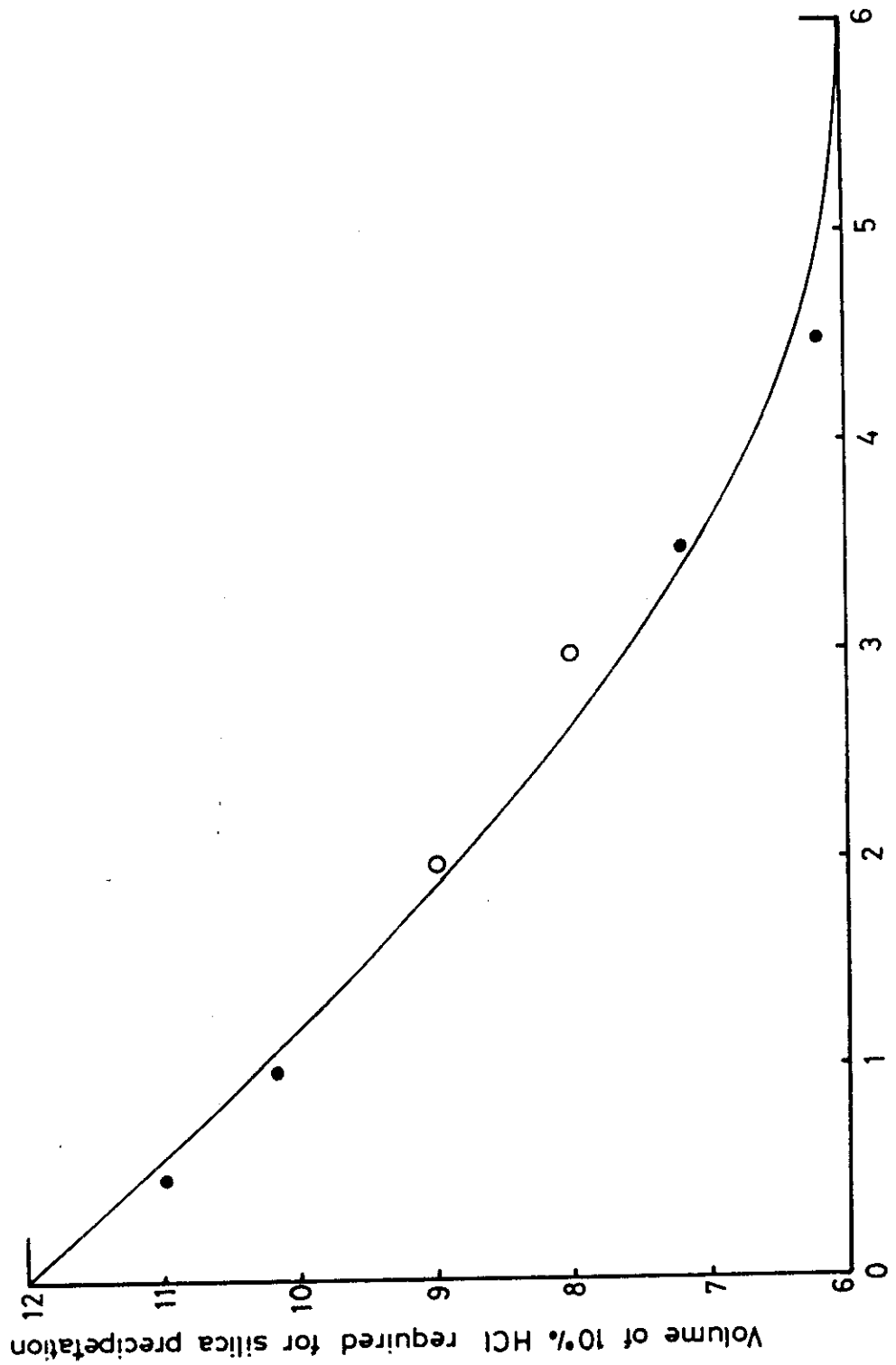

Fig.(8) Effect of the use of different electrolytes with monovalent cations for silica precipetation from black liquor.

Conclusion :

The use of NaCl is preferable than KCl for precipitation of silica because of its higher coagulative potentiality.

b - Use of ammonium chloride for desilication of black liquor

The addition of ammonium chloride alone - led to the coagulation and preciptation of silica. However, the type of the fomed silica precipitate depended on the concentration of ammonium in balck liquor.

As it is clear form the data given in table (7) and fig (9) on adding NH4Cl in amounts of 5 g/100 ml of black liquor spontanous formation of silica precipitate took place. The precipitate consisted of very fine particles and the colour of the black liquor solution turned cream to pale tan. On adding 4.5 g/100 black liquor the formed precipitate conisted of courser particles, while the coluor of black liquor turned plae brown. On using 4.0 g NH_4Cl/100 ml of black liquor the precipitated silica particles became courser and the remaining black liquor became light brown. using 3.2 g NH_4Cl, the formed precipitate silica particles become relatively bigger and the black liquor became red brown. On further decrease of the amount of added NH_4Cl to 3.0 2.0 g/100 ml. black liquor the precipitated silica particles grew bigger and the black liquor became deeper red brown.

On adding NH_4Cl in less amounts. i.e. 1.0 or 0.5 g, silica did not precipitate at once a weak gel was formed after settling of black liquor for 2 days (on adding 1.0 g NH_4Cl) while a more visocus gel was

Table (7): Effect of use of NH_4Cl for fractionation of black liquor into its components.

NH_4CL conc. %	Time required for ppt.	Size of pptated silica particles	Colour of ppt. and treated black liquor	Weight of silica ppt. g
5	at once	Fine particle	Cream ppt.& black liquor	1.89
4.5	at once	coarse particle	Cream ppt. pale brown bl.liq.	1.87
4	at once	coarse particles	Cream ppt. & brown bl.liq.	1.86
3.5	at once	big particle	white ppt. & red brown bl. liq.	1.95
3	at once	big particle	white ppt. & red brown bl.liq.	1.94
2	at once	bigger particles	white ppt. & deep red brown bl.liq.	2.01
1	Gel formed in 2 days	weak gel	weak gel	1.68
0	Gel formed in 5 days	viscous gel	viscous gel	1.64

bl.liq. = black liquor

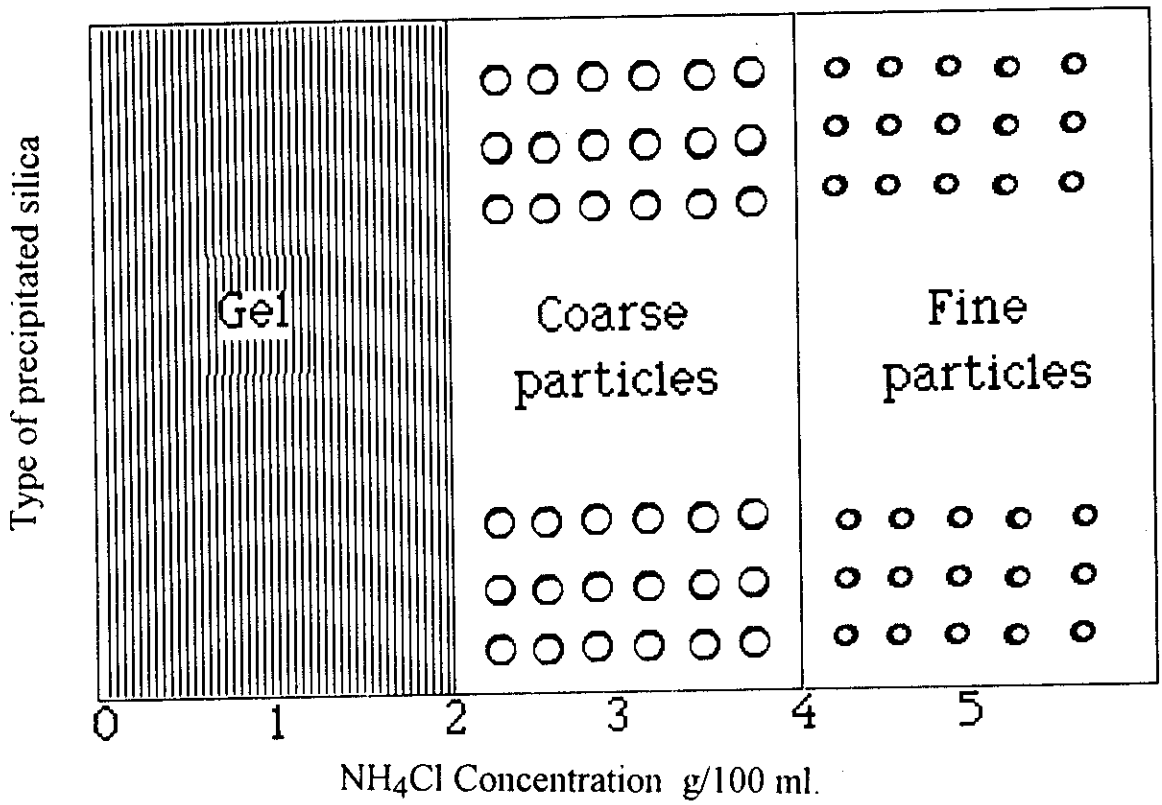

Fig. (9) : Type of preciptated silica using different concentration of NH_4Cl

formed after 5 days on adding 0.5 NH_4Cl. In both cases the colour of black liquor (Gel) was cream.

The following can be concluded :

1 - For NH_4Cl precipitation of silica was achieved without adding HCl, which means far stronger coagulating potentialities than of NaCl or KCl.

2 - NH_4Cl can precipitate silica form black liquor at its initial high pH.

3 - Threshold of NH_4Cl concentration for coagulation seems to be 2.0 g/100 ml of black liquor.

4 - The effect of the addition of NH_4Cl to black liquor was differentiated into three types depending on the used concentration :

a) using concentrations > 3.5 g/100 ml black liquor both silica and lignin precipitated leading to the formation of cream coloured precipitate. Lignin precipitates as cream follocules.

b) Using concentrations > 2.0 g/100 ml. broth, only silica precipitates in coarse particle, while lignin remains dissolved in balck liquor. Dissolved lignin has red brown colour.

c) Using concentrations < 2.0 g/100 ml broth gel is formed.

5 - Threshold of NH_4Cl concentration for silica precipitation is 2.0g/100 ml of broth.

6 - The amount of precipitated silica was almost the same within the range of NH_4Cl concentration from 2.0 - 5.0 g/100 ml. borth.

These important data offer great possibilites of easy sequential

factionation of black liquor using one and the same electrolyte but in different concentrations. This could be as follows :

a - Treating raw black liquor with 2.0 g/100 ml. broth silica precipitates and can be removed.

b - Treatment of desilicated black liquor with additional amounts of NH_4Cl to attain a concentration of 4% black liquor lignin precipitates and can be removed by centrifugation.

c - The desilicated and deligninated liquor contains hemicelluloses which can freed from NH_4Cl on heating. Hemicellulose can serve as a good substrate for single cell protein by appropriate microbial fermentation.

Gerneral Conclusion :

The above mentioned achievement of the successful fractionation of black liquor using NH_4Cl has the following advantages :

1 - Cheap fractionation as there is no need for using expensive lots of mineral acids.

2 - Easy extraction of silica as an easily harvested precipitate.

3 - Extraction of lignin by simple technology.

4 - Sound possibility of making several uses of hemicelluloses.

The above mentioned achivements will be presented for patenting

c- Use of other ammonium salts

Desilication of black liquor was tried using different ammonium salts. These salts were : ammonium hydroxide, ammonium chloride, ammonium nitrate, ammonium sulphate, ammonium oxalate and ammonium tartarate. The salts were added in 5 g/100 ml of black liquor. The data given in table (8) show that the highest amount of precipitated silica was on using NH_4Cl followed by ammonium nitrate and ammonium sulphate then comes ammonium oxalate, while least amount of precipitated silica was on using ammonium tartarate.

Table (8) : Desilication of black liquor using different ammonium salts

Ammonium salts	Weight of precipitated silica g
ammonium hydroxide	0.8116
ammonium chloride	1.5062
ammonium nitrate	1.3735
ammonium sulphate	1.3704
ammonium oxalate	1.3220
ammonium tartarate	1.9063

It can be concluded that out of the tested ammonium salts, ammonium chloride proved the best for desilication of black liquor.

III - USE OF DI -OR TRIVALENT CATIONS FOR DESILICATION OF BLACK LIQUOR

Different di - or trivalent cations were used for the desilication of black liquor. These were of the following salts : $CaCl_2$, $BaCl_2$, $MgCl_2$, $CuCl_2$, $FeCl_2$, $ZnCl_2$, $NiCl_2$; $FeCl_3$, and $AlCl_3$. These salts were added in different concentrations ranging from 10 to 1000 mg%.

The use of these salts revealed that they when added to the black liquor formed insoluble hydroxides or hydroxide gels because of the high NaOH content of the black liquor. For this reason high amounts of these salts were required firstly to neutralize the NaOH of the balck liquor and then further to neutralize the negative charge on the silicate anaions. Most of these salts formed gels when added to the black liquor.

The data given in table (9) show that great amounts of these di or tri cations are required for the desilication of black liquor. Thus 6.0 ml. of 1 mol solution of either $CaCl_2$, $BaCl_2$, $AlCl_3$ or $FeCl_3$ are needed for the desilication of black liquor. Using $AlCl_3$ or $FeCl_3$ gel is formed which makes the process of desilication very difficult.

Moreover, these salts form insoluble hydroxides which precipitate together with silica. This is clearly demonstrated from the data giving in table (10) for the cases of using $CaCl_2$ or $BaCl_2$. The weight of the

Table (9) : Desilication of black liquor using some di and trivalent salts

Salts	Volume needed of 1 mol. of the salt for the desilication of 50 ml. black liquor ml.	pH of black liquor solution
$CaCl_2$	6.0	10.84
$BaCl_2$	6.0	11.23
$AlCl_3$	6.0 Gelling	6.10
$FeCl_3$	6.0 Gelling	8.70

Initial pH of black liquor = 11.57

Table (10): Desilication of black liquor using some di and trivalent cations

Concentration used g/100 black liquor	Weight of precipitate using different salts g		
	$CaCl_2$	$BaCl_2$	$AlCl_3$
6	4.6830	5.0666	5.5315
5	3.9215	5.1860	5.2225
4	3.9019	5.0151	4.9826
3	3.4100	3.8744	3.8291
2	2.8618	2.5400	2.8100
1	2.1068	0.9944	2.2010
0.5	1.1921	0.4290	1.2340

formed precipitate exceeded that of the contained silica proving the formation of insoluble hydroxide.

It can be concluded that for desilication of black liquor the use of either divalent or trivalent cations did not facilitate desilication but on the contrary led to serious complications.

B- DELIGNIFICATION OF BLACK LIQUOR

Lignin is a very complex polymer which is almost undergradable by microorganisms, except those of wood rot fungi. It may be toxic for many microorganisms and thus delignification is very important when the microbial utilization of hemicelluloses of the black liquor is aimed at. However, lignin can be considered as a feed stock for many complex polymers such as polyphenols and may be hydrocracked into vanilin. For this reasons some experiments for the delignification of black liquor were carried out.

1 - Delignification using mineral acids

Trials were made for the delignification of desilicated black liquor (containing 5% NaCl). The results showed that the addition of either HCl, HNO_3 or H_2SO_4 led to the precipition of lignin in the form of light brown follecules or light precipitate. This takes place using the same amounts of equivalent solutions of HCl, HNO_3 or H_2So_4. Lignin preciptiation takes place at a considerable high pH value $\simeq$ pH 9.88 (table 11) using these mineral acids.

It is well known that without the addition of NaCl lignin precipitation takes place at pH 4.0. This means that the addition of NaCl to black liquor favoured ligning precipitation possibly by neutralizing the negative charge on the ligninate anaion.

Tablle (11): Delignification of desilicated black liquor using HCl or NH_4Cl.

Substance	Volume used			
	for desilication of black liquor		dilignification of desilicated of black liquor	
	vol.	pH	vol.	pH
HCl 5%	4.2 *	10.66	4.0 *	9.88
NH_4Cl 1 mol.	3.0	11.5	9.0*	10.1

* in presence of 5% NaCl.
used volume of black liquor was 50 ml.

2 - Delignification using ammonium chloride

Since NH_4Cl helped the desilication of black liquor, its use for delignification of desilicated black liquor was tried. The data given in table (11) show that using 1 mol solution of NH_4Cl about 3 ml for the desilication then other 9 ml. NH_4Cl were needed to bring out delignification which took place at a high pH value $\simeq$ pH 10.1. The relatively greater amount of NH_4Cl needed for delignification (9ml.) compared to that needed for desilication may suggest that the role of NH_4^+ in desilication may be different from that in delignification. It is worthy to note that almost equal amounts of HCl are needed for desilication and delignification, which is not the case on using NH_4Cl.

3 - Delignification using di or trivalent salts

The data given in table (12) show that amolst equal amounts of molar solutions of $CaCl_2$, $BaCl_2$, $AlCl_3$, and $FeCl_3$ were needed for the delignification of desilicated black liquor. Delignification took place with $CaCl_2$ and $BaCl_2$ at high pH value 10.0, while, though using the same amounts of the molar solution, it took place at far lower pH values 5.7 using $AlCl_3$ and $FeCl_3$.

It was found also that using these trivalent salts gel is formed. Consequently, the use of $CaCl_2$, $BaCl_2$, $AlCl_3$ or $FeCl_3$ for delignification is not recommended.

Table (12) Delignification of desilicated black liquor using some di and trivalent salts.

Salts	Volume needed of 1 mol of the salts for the delignification of desilication black liquor ml.	pH of black liquor solution
$CaCl_2$	3.0	10.16
$BaCl_2$	3.0	10.50
$AlCl_3$	3.0 Gelling	5.77
$FeCl_3$	3.0 Gelling	5.64

Initial pH of delignified black liquor = 10.66
Blacl liquor contains 5% NaCl.

In conclusion, for delignification of desilicated black liquor the use of either mineral acids or NH_4Cl is far better than using di or trivalent salts. However, in view of the less amounts of mineral acids needed for delignification compared to using NH_4Cl, the use of mineral acids for delignification is recommended.

PATR II

BIOCONVERSION OF BLACK LIQUOR HEMICELLUOSES INTO SINGLE CELL PROTEIN USING FUNGI

Isolation of Black Liquor Hemicelluloses Consuming fungi :

Eight soil samples were collected from Governorate El-Qalubia. Samples were collected from cultivated fields the places of soil sample collection are given in table (13) on the count of black liquor consuming fungi in the used soil samples. It is clear that the black liquor consuming fungi were more abundant in soils of Toukh, Moshtohor, Kafr El-Haddeden (A) and Benha than soils of Arab Saleh, El-Dier, Kafr El-Haddadeen (B) or Asneet.

Table (13): Count of fungi isolated on black liquor from different soil samples.

Place of collection of soil sample	Total count (Thousand g soil)
Kafr El-Haddadeen (A)	42
Kafr El-Haddadeen (B)	38
El- Dier	26
Arab Saleh	21
Asneet	40
Toukh	56
Moshtoher	48
Benha	42

Differentiation of Ioslates Into Genera

The obtained fungal isolates were differentiated into the following eight genera :

	Number of isolates
1- Aspergillus	84
2- Penicillium	13
3- Paecilomyces	37
4- Fusarium	22
5- Alternaria	67
6- Monilia	49
7- Botryotrichum	19
8- Scopulariopsis	19

It is clear that the most abundant black liquor consuming fungal genus is *Aspergillus,* followed by *Alternaria, Monilia,* then *Paecilomyces.* Genera of less abundance were *Fusarium, Botryotrichum, Scopulariopsis* then *Penicillium.*

Taxonomical Identification of Fungal Species

Why The Need For Fungal Identification

As we aim at the bioconversion of the hemicelluloses of the black liquor into single cell protein via microorganisms it is necessary to use harmless, nontoxic microorganisms. The taxonomic identificaiton of isolates hepls in the exclusion of possibly known hazardous or harmful

microbes. In additions, it supplies useful informations about useful products, which can be produced by the selected microbes, such as organic acids, solvents, antibiotics, vitamins, steroids or hormones.

The obtained fungal isolates were differentiatied into 14 fungal species as follows :

Aspergillus flavus :

Colonies widely spreading., Condial areas sea-foam yellow to citron green, reverse and media uncoloured or buff. Condiophores arise separately from substratum, conidia pyriform to globose, sclerotia white then brown. Phialides in double series.

Aspergillus fumigatus :

Colonies velvety, green to dark green to almost black, reverse and substratum colorless to yellow. Conidiophores short, densely crowded, Chains of conidia form solid columns, conidia dark green in mass , globose, phialides in one series.

Aspergillus clavatus :

Colonies gray-green to dark green, reverse and substratum brownish. Condiophores with small walls. Phialides in a single series. Conidia elleptical, green, smooth. Cleistothecia are found. Colonies enlarged at the apex.

Penicillium notatumu :

Colonies bright blue-green later dark, reverse yellow. Condiophores long and broad, fructification in three stages. Conidia globose to oval.

***Paecilomyces silvatica* :**

Colonies brown, substratum and media brown, conidiophores erect, rarely branching, alternate, simple or forked at tips, long phialides, conidia in long chains, elleptical or oblong .

***Paecilomyces divaricata* :**

Colonies brown, substratum brown. Conidiophores freely and irregularly branched, fructification in two stages, conidiophore branches carry terminal verticil of divergent metulae with divergent phialides. Conidial chains very long divergent. Conidia elleptical, smooth.

***Fusarium oxysporum* :**

Stroma brownish-white to violet, sclerotia hard bodies. Chlamydospores, globose, smooth, one-celled seldom two celled. Macroconidia usually three seldom four to five septate. Microcondia, stroma extended smooth or sclerotial scrumpent, pale green to dark blue.

Alternaria humicola :

Colonies white to grayish white. Conidia variable in shape, cylindrical, obclavate, oblong, at first hyaline later honey coloured finally black green . Three to seven times septate, muriform, in advanced age dense and very finely roughened, slightly or non-constricted at the septa.

Alternaria tenuis :

Conidiophores short, septate, unbranched or branched, brown-green conidia in chains, muriform with three to five cross walls, constricted at the outer walls, conidia smooth.

Alternaria geophila :

Colonies dark brown, turf gray, composed of sterile prostrate filaments, brown formed of short parts, filled with oil droplets. Each cell of the spore contains a fat globule.

Monilia grisea :

Mycelium dark brwon, forming a dark gray turf, Hyphae fasciculate, branching, septate. Conidia terminal in chains globose.

Monilia acremonium :

Colonies spreading, floccose, white sterile hyphae creeping, hyaline, sparsely septate, with oil drops. Conidiophore erect, united in bundles with numerous septa, bearing the condial chains terminaly. Conidia ovate-pyriforous, somewhat truncate at the base.

Botryotrichum piluliferum :

Colonies dark gray, reverse yellow - brown, sterile hyphae turf - like, slightly curved, smooth or somewhat roughened, slightly thickened at the base. Conidia terminal, globose, hyaline, fertile hyphae branched, growing between the sterile hyphae.

Scopulariopsis brevicaulis :

Colonies white at first later yellowish brown or chocolate, consisting & short crowded condiophores making powdery areas overgrown by loose trailing floccose hyphae. Conidial fructifications either simple chains, terminating unbranched or sparingly branched conidiophore in young conidia. Phialides continuous with conidiophores. Conidia pear-shaped, slightly tuberculate at the apex with broad base.

Choice of Most Active Black Liquor Consuming Fungal Isolates :

The potentiality of isolates to consume black liquor was determined indirectly by two parameters :

a) growth rate on solid BLS medium and this was expressed as linear growth of the tested fungal species colonies.

b) Biomass production in liquid shaken culture of BLS expressed as grams of biomass produced in 8 days.

A- Linear growth test :

The data given in table (14) and fig. (10) show that the used 14 fungal isolates differed in their growth rate when cultivated on BLS. Highest growth rate was recorded for *Fusarium oxysporum* followed by *Botryotrichum piluliferum* then *Scopulariopsis brevicaulis* followed by *Alternaria humicola* and *Alternaria tenuis.* Slower growth rates were shown by *Monilia acremonium* > *Asperigllus fumigatus* > *Alternaria geophila* > *Aspergillus clavatus.* Very slow growth rates were recorded for *Aspergillus flavus* > *Penicillium notatum* > *Monilia grisea* > *Pacilomyces divaricata* > *Paecilomyces silvatica.*

B- Biomass Production Test :

Having in mind the low orgainc mater content of the black liquor, it was necessary to determine the efficiency of the potentiality of the used fungi to bioconvert the orgainc matter of black liquor into biomass, i.e. single cell protein, using concentrated black liquor. Two concentration-5 folds & 10 folds-were used.

As it clear from the data given in table (15) and graphically represented in figs. (11 & 12), the used fungi were differentiated by their bioconversion potentially into 3 types :

Type A: This included seven fungal species : *Fusarium oxysporum, Paecilomyces silvatica, Alternaria geophila, Alternaria humicola, Paecilomyces divaricata, Aspergillus fumigatus* and

Table (14) : Linear growth rates of isolated Fungal species on black liqor salts agar (cm).

Fungal species	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Fuserium oxysporum	3.10	4.30	5.20	6.30	7.20	8.10	8.60	9.00				
Botryotrichum piluliferum	2.90	4.00	4.76	5.70	6.60	7.30	8.20	8.70	9.00			
Scopulariopsis brevicaulis	1.90	2.60	3.40	4.00	4.56	5.20	5.76	6.20	6.80	7.20	8.10	9.00
Alternaria humicola	1.86	2.56	3.00	3.80	4.20	4.80	5.30	5.80	6.30	6.60	7.20	8.00
Alternaria tenuis	1.20	2.00	2.80	3.10	3.80	4.20	5.00	5.20	5.80	6.10	7.10	7.66
Monilia acremonium	1.60	2.26	2.70	3.36	3.76	4.30	4.66	5.30	5.70	6.00	6.50	7.00
Aspergillus fumigatus	1.16	2.10	2.50	3.00	3.60	4.06	4.40	4.90	5.10	5.60	5.80	6.30
Alterneria geophila	0.50	1.86	2.10	3.00	3.60	4.00	4.70	4.70	5.06	5.50	5.76	5.80
Aspergillus clavatus	2.00	2.56	2.90	3.40	3.56	3.80	4.20	4.36	4.50	4.66	4.80	5.00
Aspergillus flavus	1.20	1.60	2.00	2.20	2.60	3.00	3.50	5.70	4.00	4.20	4.30	4.40
Penicillium notetum	0.90	1.50	1.60	1.70	1.90	2.10	2.40	2.56	2.96	3.10	3.20	3.20
Monilia grisea	0.90	1.20	1.40	1.50	1.60	2.00	2.10	2.20	2.40	2.60	2.80	3.20
Paecilomyces divaricata	1.20	1.46	1.60	1.86	2.00	2.10	2.40	2.60	2.76	3.00	3.00	3.00
Paecilomyces silvatica	1.20	1.30	1.50	1.50	1.60	1.70	1.90	2.00	2.20	2.40	2.50	2.50

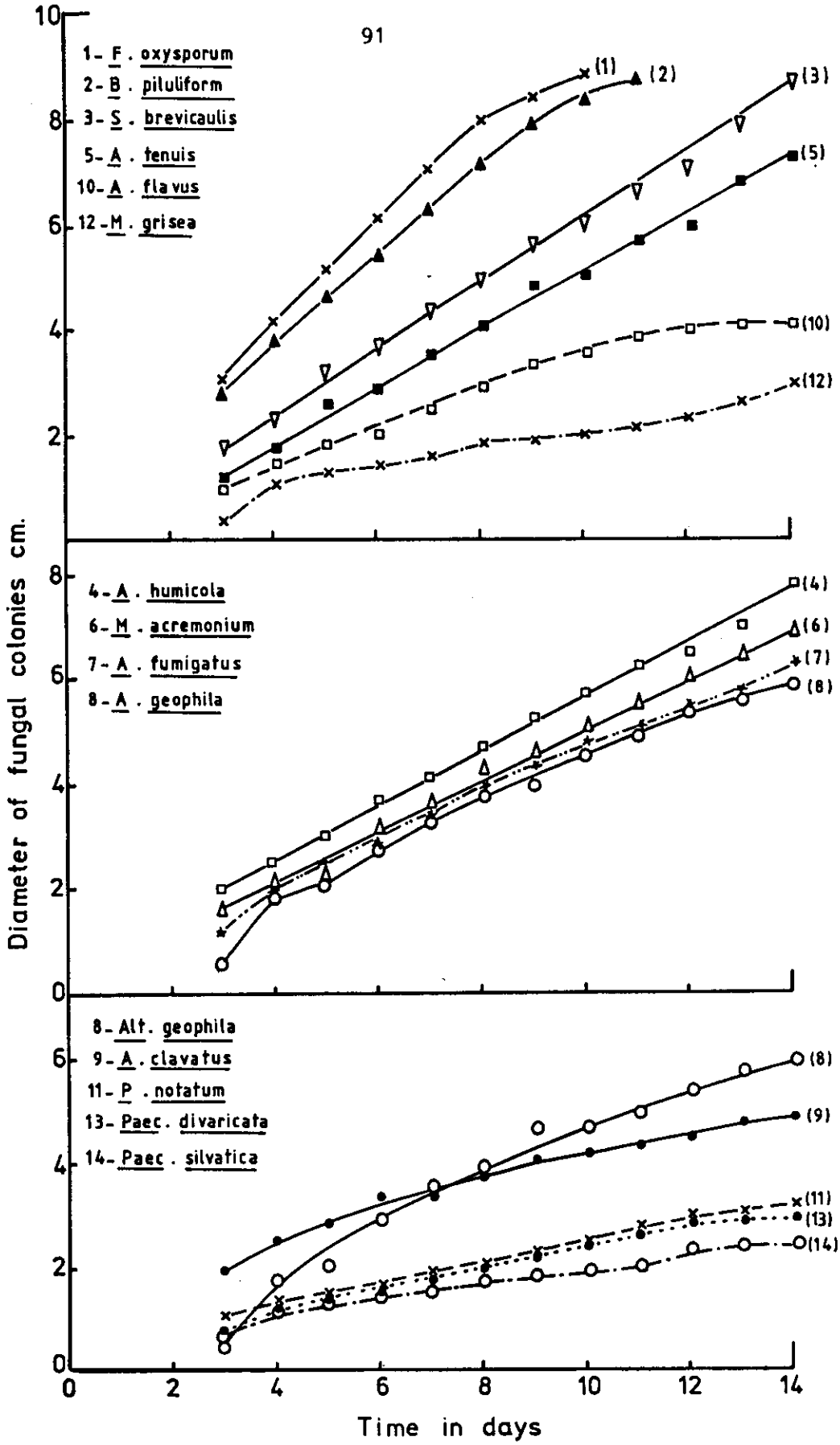

Fig.110 Linear growth rates of isolated fungal species on black liquor salts agar.

Table (15) : Biomass production by isolated fungal species on black liquor of different concentrations.

Isolated fungal species	Concentration folds of black liquor		
	1	5	10
Fusarium oxysporum	0.05	0.18	0.825
Paecilomyces silvatica	0.04	0.25	0.785
Alternaria geophila	0.08	0.20	0.825
Alternaria humicola	0.07	0.17	0.815
Paecilomyces divaricata	0.06	0.155	0.870
Aspregillus fumigatus	0.02	0.155	0.765
Aspregilus flavus	0.02	0.10	0.790
Botryotrichum piluliferum	0.0625	0.4320	0.5414
Penicillium notatum	0.0281	0.5740	0.5706
Scopulariopsis brevicaulis	0.11075	0.5818	0.6628
Monilia grisea	0.0636	0.7887	0.7144
Monilia acremonium	0.0454	1.1727	0.4047
Aspergillus clavatus	0.01975	1.1528	1.25515
Alternaria tenuis	0.02495	1.2622	0.6628

Figurs = dry weight of produced biomass (in grams)

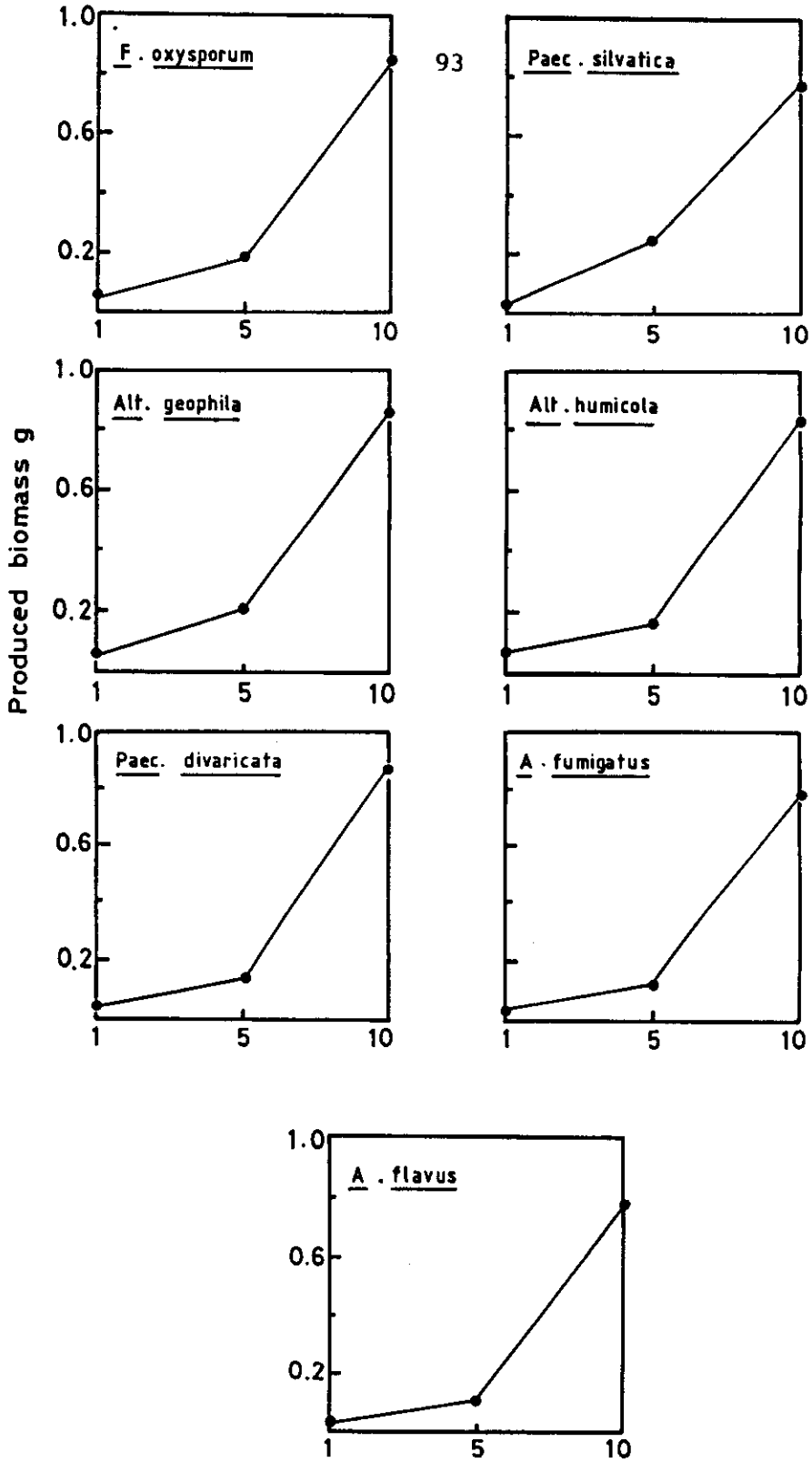

Fig.(|) Biomass production of isolated fungal species on black liquor of different concentrations .

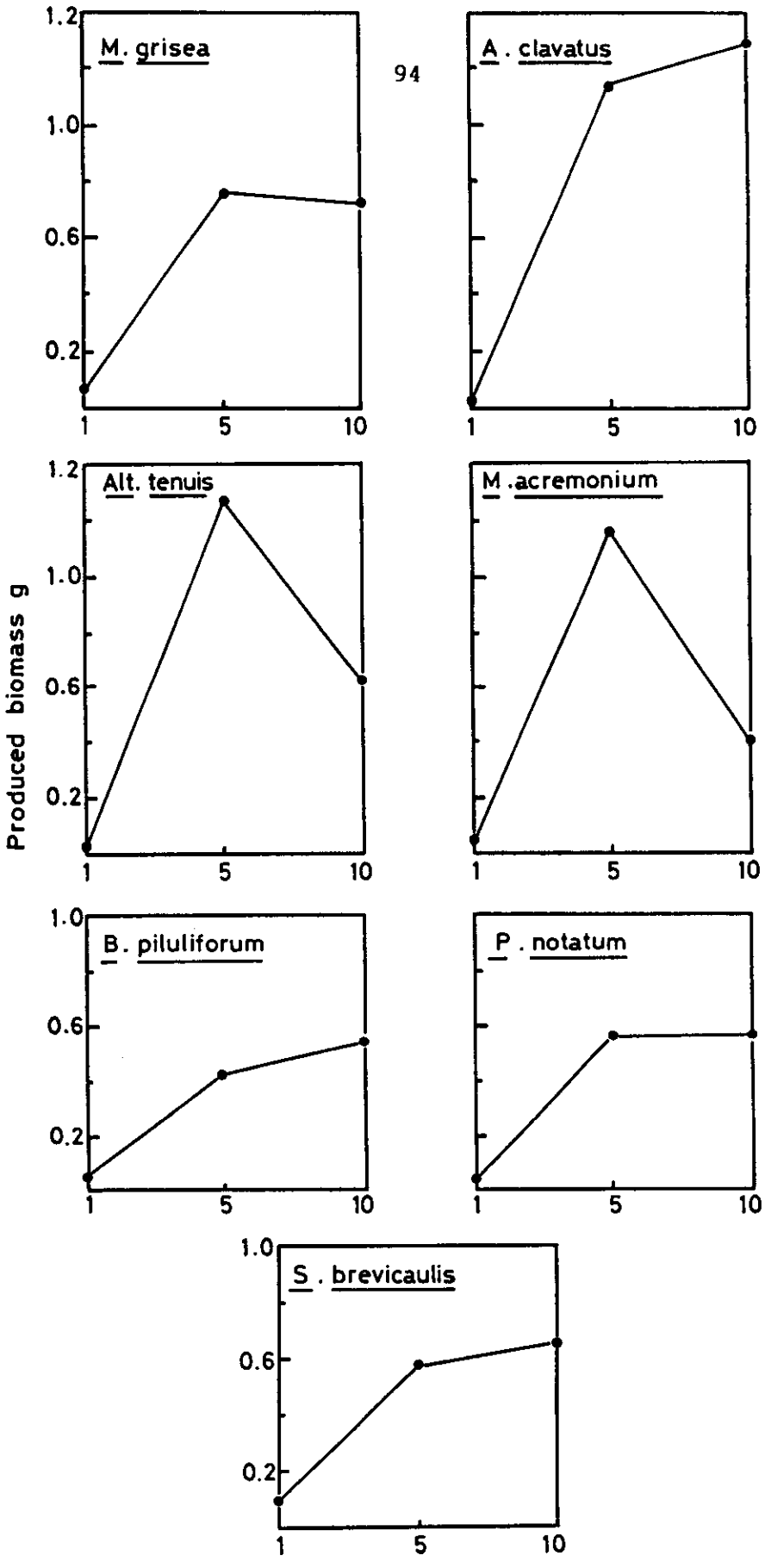

Fig.(12) Biomass production of isolated fungal species on black liquor of different concentrations.

Aspergillus flavus. This type is characterized by very low mass producetion on the initial black liquor,low biomass production on 5 folds concentrated black liquor but high biomass on 10 folds concentrated black liquor.

Type B: This includes three fungal species : *Botryotrichum piluliferum, Penicillium notatum* and *Scopulariopsis brevicaulis.* This type is characterized by low biomass production on the initial black liquor, moderate biomass prouction on 5 folds concentrated black liquor but more or less the same level of biomass production on 10 folds black liquor. This shows that for isolates of this type the increase of black liquor concentration from 5 to 10 folds did not cause significant increase in biomass production.

Type C: This includes four fungal species : *Monilia grisea, Aspergillus clavatus, Alternaria tenuis* and *Monilia acremonium.* This type is characterized by low biomass poduction on the initial blak liquor but very high biomass production at 5 folds black liquor; however, on using 10 folds black liquor the aforementioned four fungal species were differentiated into two sub-types :

Sub type 1- This includes two fungal species : *M. grisea* and *A. clavatus.* These two fungal species show slight variation-either slight increase or slight decrease- in biomass production using 10 folds black liquor concentration compared to the case using 5 folds.

Sub type 2- This includes two fungal species : *Alternaria tenuis* and *Monillia acremonium*. Both species showed sharp decrease in biomass production when 10 folds black liquor was used compared to the case with 5 folds.

PART III

PATTERN OF CONSUMPTION OF BLACK LIQUOR SUGARS AND SOME ENZYMATIC ACTIVITIES BY SELECTED SPECIES OF THE STUDIED FUNGI

A - Pattern of Consumption of Black Liquor Sugars by Selected Species of The Studied Fungi

Sugar consumption by ten selected species of the used fungi, that showed promising growth producing higher biomass, was studied on black liquor along a period of ten days. The amounts of consumed carbohydrates, reducing sugars and hexosamines were determined along this period.

1- Consumption Course of Black Liquor Sugars by *Fusarium oxysporum*

1- Consumption course of carbohydrates :

The consumption of carbohydrates by *Fusarium oxysporum* took place at a very rapid rate as shown from table (16) and fig (13) the consumption reached a level of 25.93% on the fourth day, 62.96% on the sixth day and showed a slight increase to reach a maximum level of 66.67% on the eigth day of incubation after which no further increase in the consumption of carbohydrates was detected.

Table (16) Consumption course of black liquor carbohydrates by *Fusarium oxysporum*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.20	3428.5702	3.4285	25.93
4	0.14	2399.9991	2.3999	48.15
6	0.10	1714.2851	1.7142	62.96
8	0.09	1542.8565	1.5428	66.67
10	0.09	1542.8565	1.5428	66.67

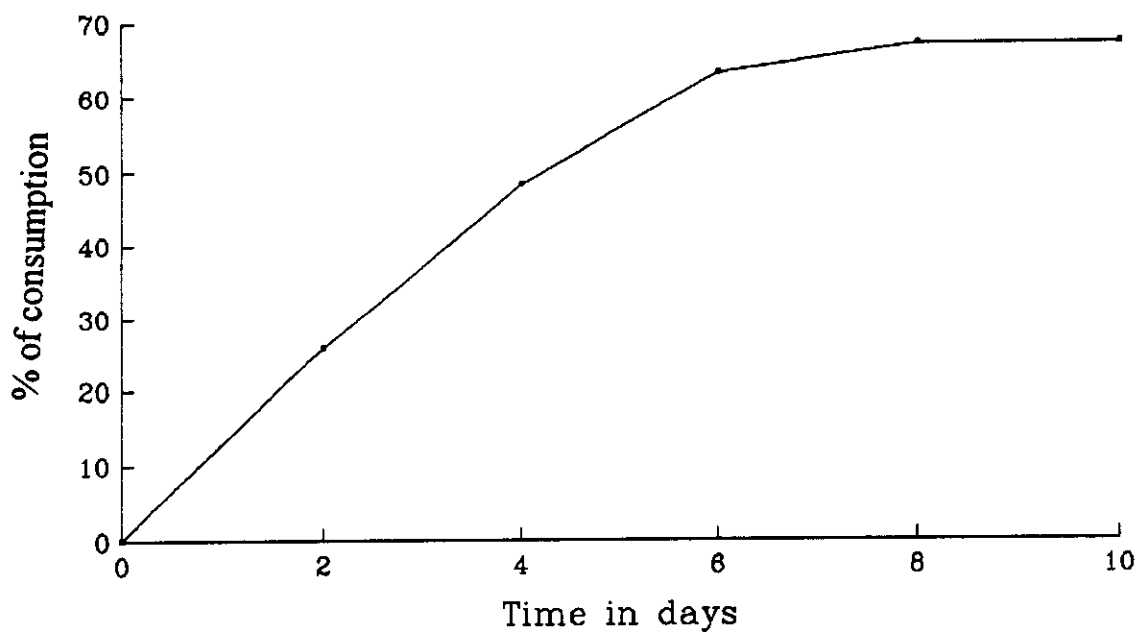

Fig. (13): Consumption course of black liquor carbohydrates by *Fusarium oxysporum*

2- Consumption course of reducting sugars :

The data given in table (17) and fig (14) show *Fusarium oxysporum* started at a low rate being 12.20% on the second day of incubation then it sharply increased to reach a level of 39.02% on the fourth day, 58.54% on the sixth day and slightly increased to reach a level of 68.29 % on the eighth day of incubation after which no increase in the consumption of reducing sugars was detected.

3- Consumption course of hexosamines :

As it is clear from the data given in table (18) and fig (15) the consumption of hexosamines took place very rapidly and early. It was 36.37% on the second day, increased to 54.50 % on the fourth day and 81.81% on the sixth day of incubation. On further incubation no consumption was recorded.

It can be concluded that *Fusarium oxysporum* showed maximum consumption of carbohydrates at the rate of 66.67%, reducing sugars at the rate of 68.29% while hexosamines at the rate of 81.81%.

Table (17) Consumption course of black liquor reducing sugars by *Fusarium oxysporum*

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.36	3.6	3.6	12.20
4	0.25	2.5	2.5	39.02
6	0.17	1.7	1.7	58.54
8	0.13	1.3	1.3	68.29
10	0.13	1.3	1.3	68.29

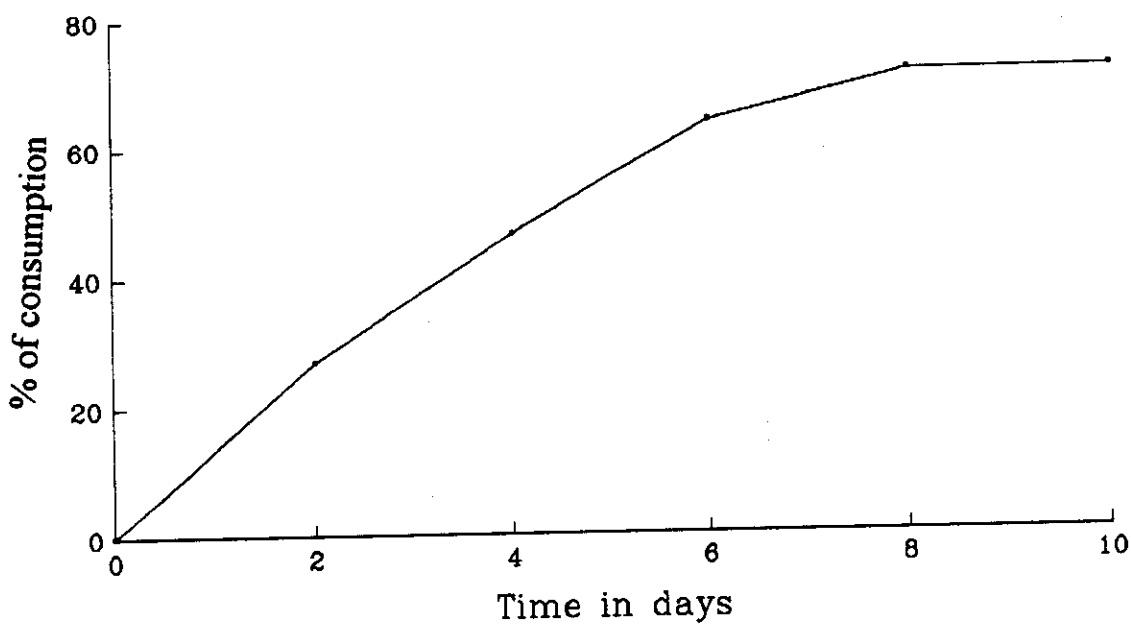

Fig. (14): Consumption course of black liquor reducing sugars by *Fusarium oxysporum*

Table (18) Consumption course of black liquor hexosamines by *Fusarium oxysporum*

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.07	49.9997	0.04999	36.38
4	0.05	35.7140	0.03571	54.50
6	0.02	14.2856	0.01428	81.81
8	0.02	14.2856	0.01428	81.81
10	0.02	14.2856	0.01428	81.81

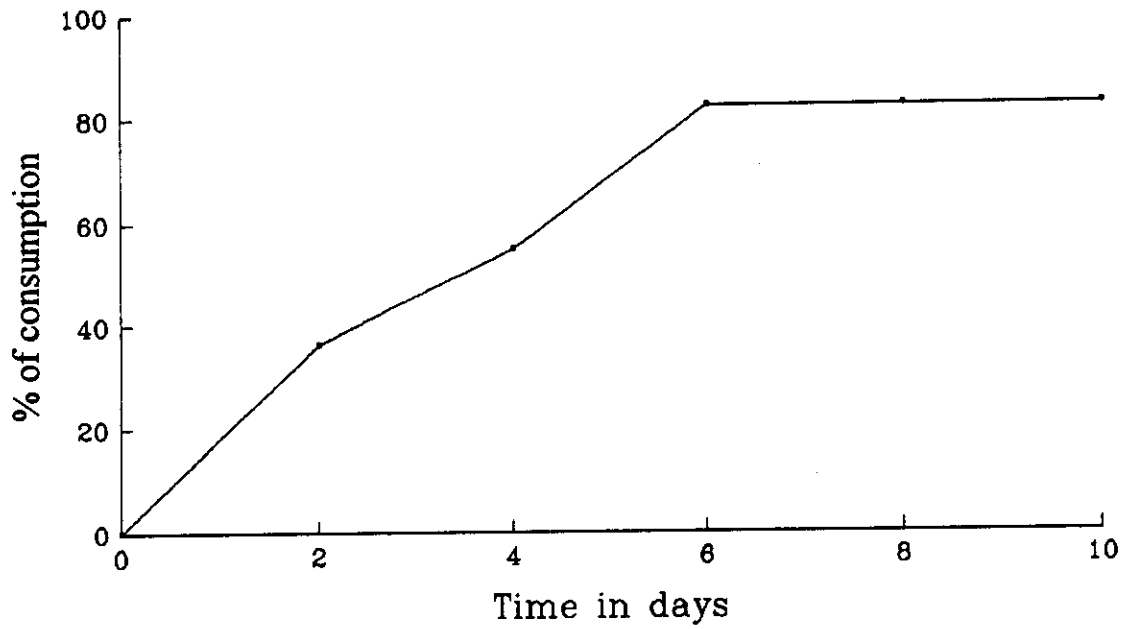

Fig. (15): Consumption course of black liquor hexosamines by *Fusarium oxysporum*

2- Consumption Course of Black Liquor Sugars by *Alternaria humicola*

1- Consumption course of carbohydrates :

The consumption rate of carbohydrates by *Alternaria humicola* took place very early and rapidly as shown from table (19) and fig(16) It reached a level of 22.22% on the second day of incubation then highly increased to reach 55.56% on the fourth day and increased to a maximum level of 70.37% on the sixth day of incubation after which no further increase in the consumption of carbohydrates was detected.

2- Consumption course of reducing sugars :

Consumption rate of reducing sugars reached a level of 34.14% on the second day of incubation, 51.22% on the fourth day, then showed a slight increase to reach a level of 56.10% on the sixth day and reached a maximum level of 63.41% on the eighth day of incubation after whch no increase in the consumption of reducing sugars was detected, (table 20 and fig 17).

3- Consumption course of hexosamine :

The data given in tabe (21) and fig (18) showed that the consumption of hexosamines started at a low rate being 27.28% on the second day of incubation, then it increased to a level of 45.46% on the fourth day, 63.64% on the sixth day and increased to a maximum rate

Table (19) Consumption course of black liquor carbohydrates by *Alternaria humicola*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.21	3599.9987	3.5999	22.22
4	0.12	2057.1421	2.0571	55.56
6	0.08	1371.428	1.3714	70.37
8	0.08	1371.428	1.3714	70.37
10	0.08	1371.428	1.3714	70.37

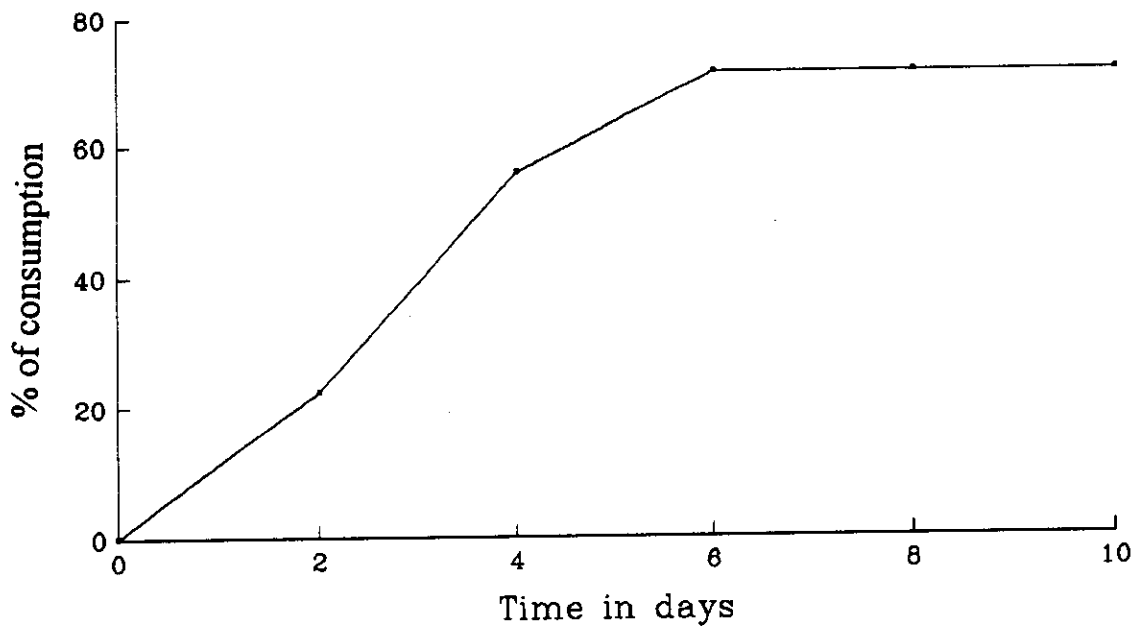

Fig. (16): Consumption course of black liquor carbohydrates by *Alternaria humicola*

Table (20) Consumption course of black liquor reducing sugars by *Alternaria humicola*

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.27	2.7	2.7	34.15
4	0.20	2.0	2.0	51.22
6	0.18	1.8	1.8	56.10
8	0.15	1.5	1.5	63.41
10	0.15	1.5	1.5	63.41

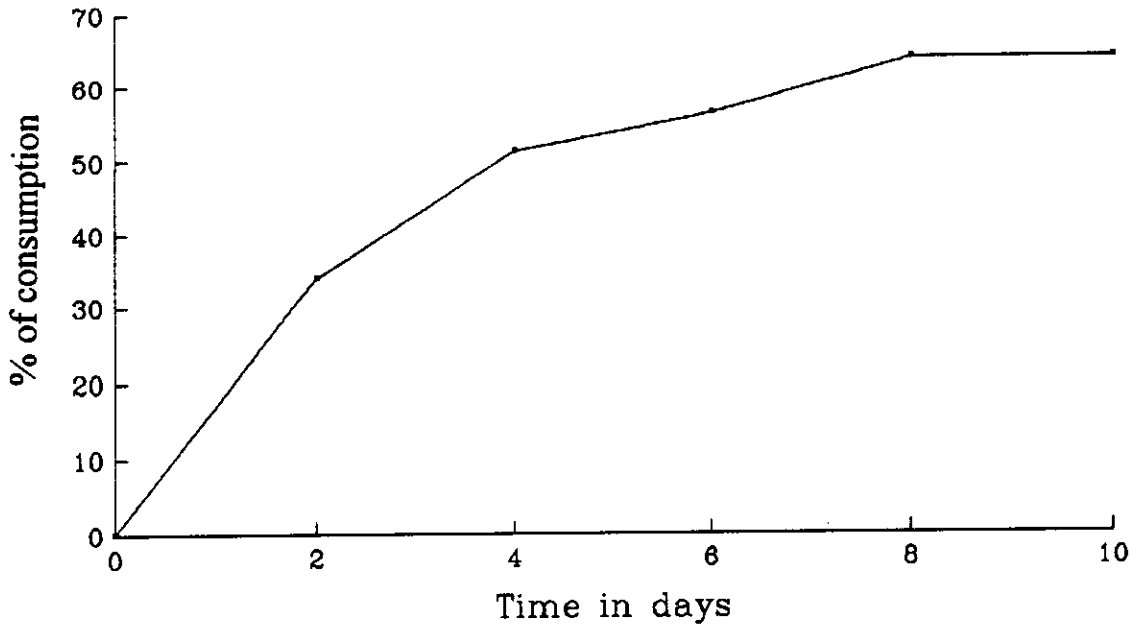

Fig. (17): Consumption course of black liquor reducing sugars by *Alternaria humicola*

Table (21) Consumption course of black liquor hexosamines by *Alternaria humicola*

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.08	57.1425	0.05714	27.28
4	0.06	42.8569	0.04285	45.46
6	0.04	28.5712	0.02857	63.64
8	0.02	14.2856	0.01428	81.81
10	0.02	14.2856	0.01428	81.81

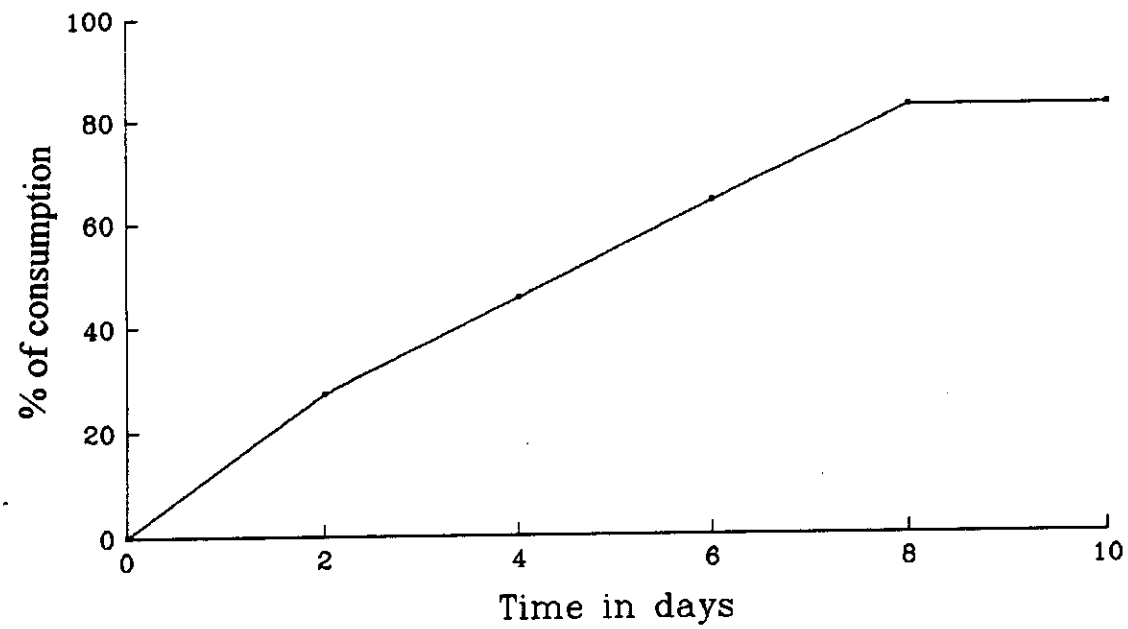

Fig. (18): Consumption course of black liquor hexosamines by *Alternaria humicola*

of 81.81% on the eighth day without any further increase on further incubation.

It can be concluded that *Alternaria humicola* showed maximum consumption of carbohdrates at the rate of 70.37%, reducing sugars at the rate of 63.41% while hexosamines at the rate of 81.81.

3- Consumption Course of Black Liquor Sugars by *Botryotrichum piluliferum*

1- Consumption course of carbohydrates :

The data given in table (22) and fig (19) show that the consumption of carbohydrates by ***Botryotrichum piluliferum*** was on the second day at a rate of 14.81%, then slightly increased to a level of 29.63% on the fourth day, 44.44% on the sixth day and increased to a maximum of level 62.96% on the eighth day of incubation . On further incubation no further incubation of carbohydrates was detected.

2- Consumption course of reducing sugars :

From the data given in table (23) and fig (20), it is clear that reducing sugars were consumed at a low rate of 9.76% on the seccond day of incubation, then slight increased to a level of 21.95% on the fourth day, 39.02% on the sixth day and increased to a maximum level of 53.66% on the eighth day of incubation after which no consumption was detected.

3- Consumption course of hexosamines :

The data given in table (24) and fig (21) show that the consumption of hexosamines started at a low rate being 18.19% on the second day of incubation then it sharply increased to a rate of 45.46% on the fourth day and highly increased to a maximum of level 81.83% on the sixth day of incubation with no further increase at later periods.

Table (22): Consumption course of black liquor carbohydrates by *Botryotrichum piluliforum*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.23	3942.8557	3.9428	14.81
4	0.19	3257.1416	3.2571	29.63
6	0.15	2571.4276	2.5714	44.44
8	0.10	1714.2851	1.7142	62.96
10	0.10	1714.2851	1.7142	62.96

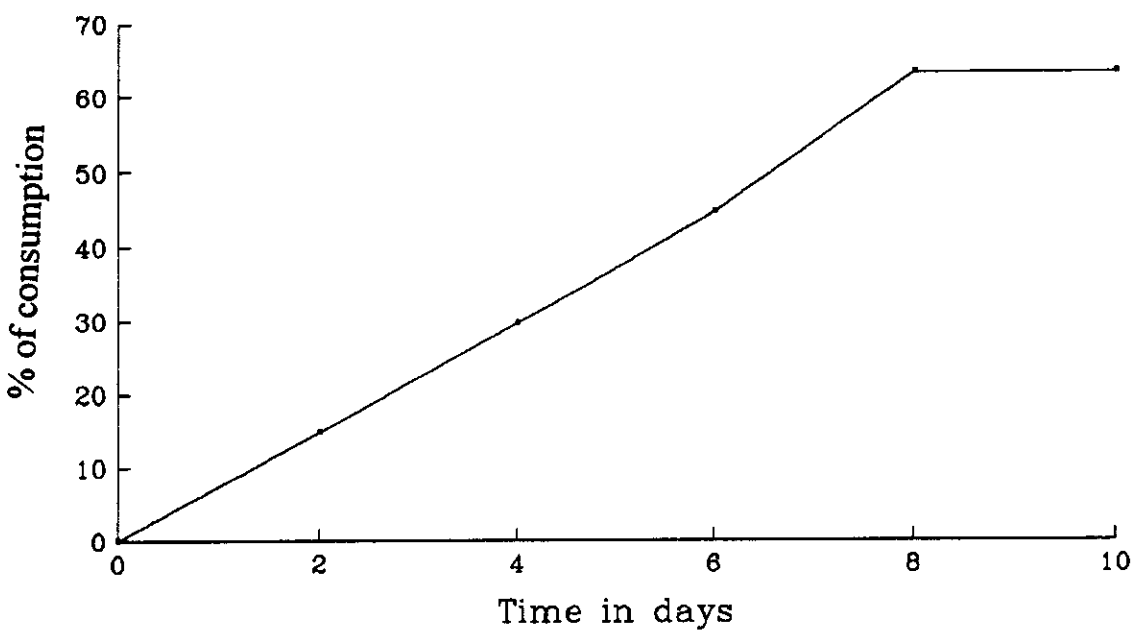

Fig. (19): Consumption course of black liquor carbohydrates by *Botryotrichum piluliferum*

Table (23): Consumption course of black liquor reducing sugars by *Botryotrichum piluliforum*

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.37	3.7	3.7	9.76
4	0.32	3.2	3.2	21.95
6	0.25	2.5	2.5	39.02
8	0.19	1.9	1.9	53.66
10	0.19	1.9	1.9	53.66

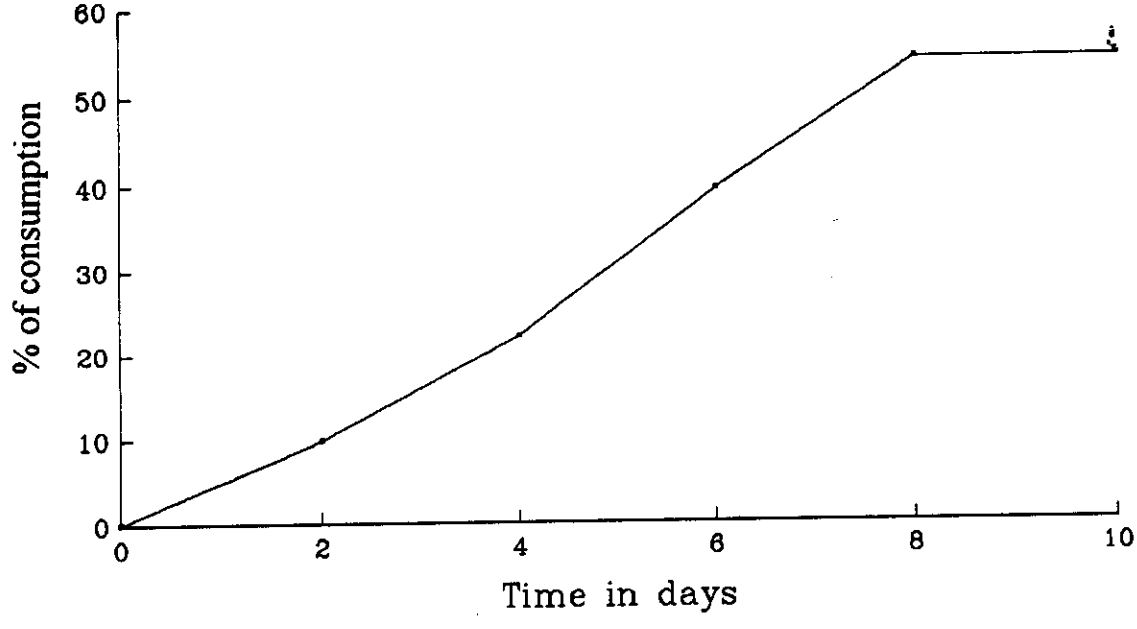

Fig. (20): Consumption course of black liquor reducing sugars by *Botryotrichum Piluliferum*

Table (24): Consumption course of black liquor hexosamines by *Botryotrichum piluliforum*

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.09	64.2852	0.06428	18.19
4	0.06	42.8569	0.04285	45.46
6	0.02	14.2856	0.01428	81.83
8	0.02	14.2856	0.01428	81.83
10	0.02	14.2856	0.01428	81.83

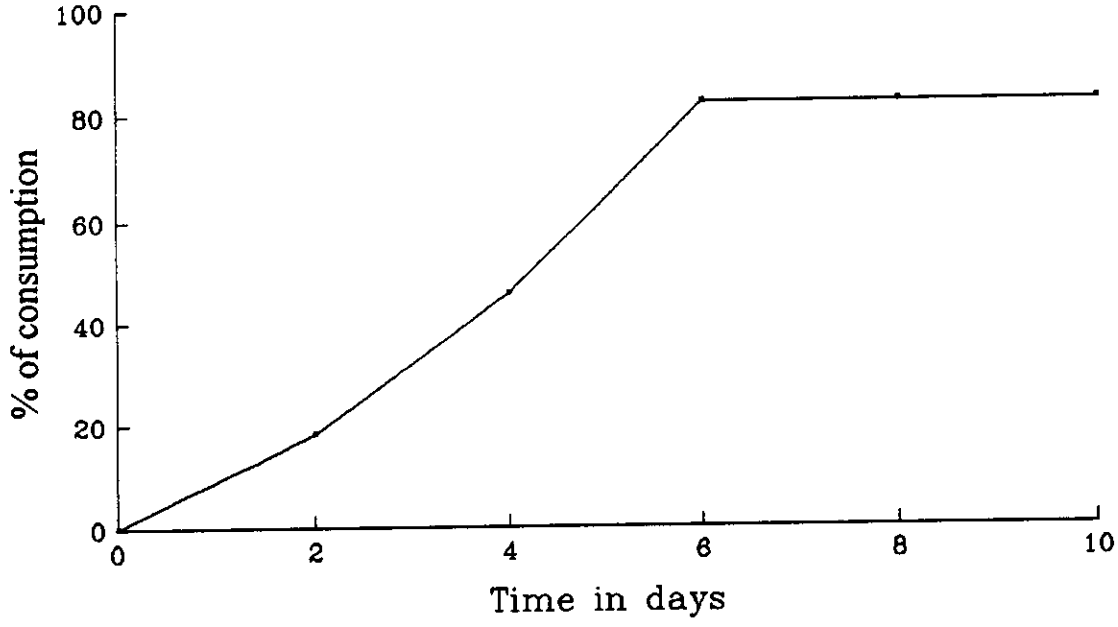

Fig. (21): Consumption course of black liquor hexosamines by *Botryotrichum piluliferum*

It can be conculded that *Botryotrichum piluliferum* showed maximum consumption of carbohydrates at the rate of 62.96%, reducing sugars at the rate of 53.66%, while hexosamines at a rate of 81.83%.

4- Consumption Course of Black Liquor Sugars by *Scopulariopsis brevicaulis*

1- Consumption course of carbohydrates :

The consumption of carbohydrates by *Scopulariopsis brevicaulis* took place early and started at a rate of 25.93% on the second day of incubation as shown from table (25) and fig (22). It reached a level of 44.44% on the fourth day and highly increased to a maximum level of 70.37% on the sixth day of incubation after which no further increase in the consumption of carbohydrates was detected.

2- Consumption course of reducing sugars :

The data given in table (26) and fig (23) show that the consumption of reducing sugars started at a low rate being 9.76% on the second day of incubation then it sharply increased to a rate of 31.71% on the fourth day, 53.66% on sixth day and reached a maximum rate of 63.41% on the eighth day of incubation with no further increase at later periods.

3- Consumption course of hexosamines :

As it clear from the data given in table (27) and fig (24) the consumption of hexosamines started at a low rate of 18.19% on the second day then it sharply increased to a rate of 54.50% on the fourth day, 81.81% on the sixth day and reached a maximum level of 90.90%

Table (25): Consumption course of black liquor carbohydrates by *Scopulariopsis brevicaulis*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.20	3428.5702	3.4285	25.93
4	0.15	2571.4276	2.5714	44.44
6	0.08	1371.428	1.3714	70.37
8	0.08	1371.428	1.3714	70.37
10	0.08	1371.428	1.3714	70.37

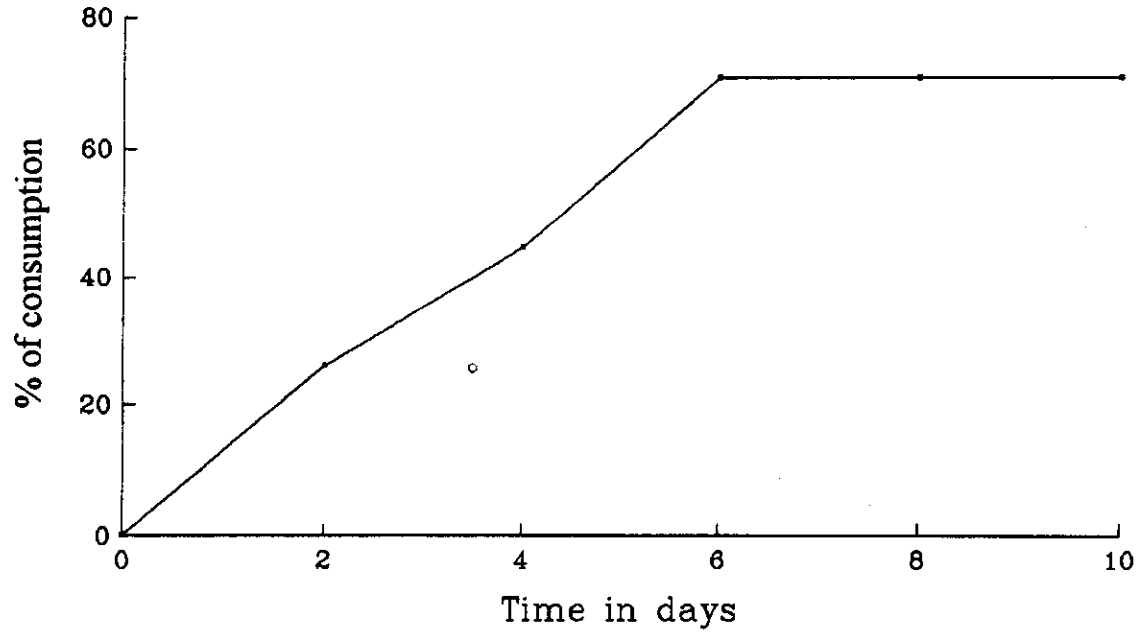

Fig. (22): Consumption course of black liquor carbohydrates by *Scopulariopsis brevicaulis*

Table (26): Consumption course of black liquor reducing sugars by *Scopulariopsis brevicaulis*

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.37	3.7	3.7	9.76
4	0.28	2.8	2.8	31.71
6	0.19	1.9	1.9	53.66
8	0.15	1.5	1.5	63.41
10	0.15	1.5	1.5	63.41

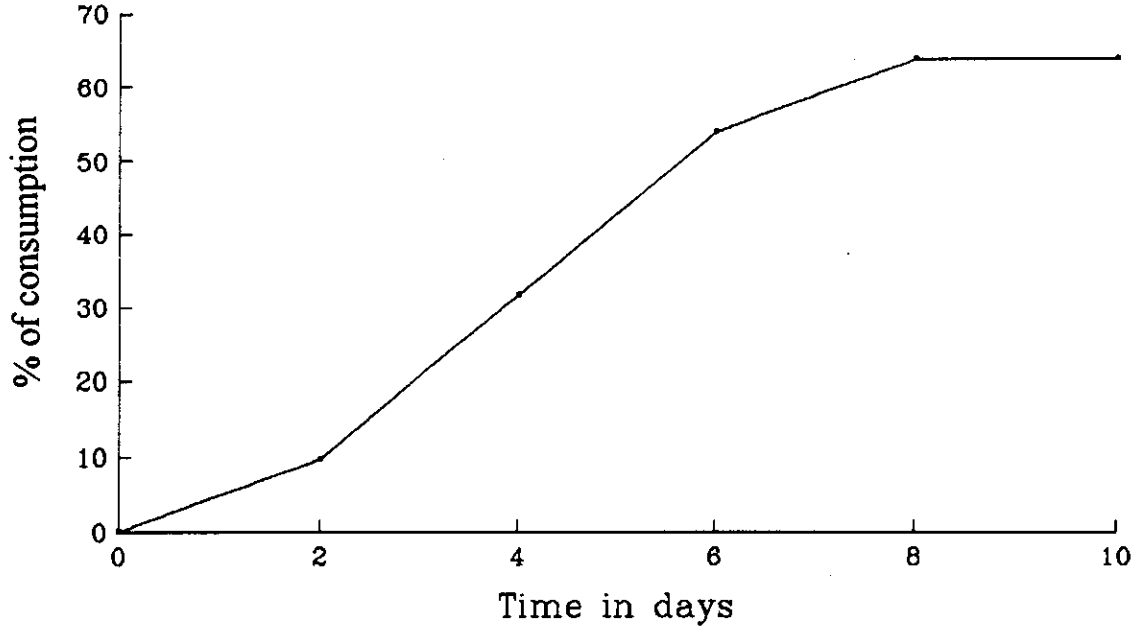

Fig. (23): Consumption course of black liquor reducing sugars by *Scopulariopsis brevicaulis*

Table (27): Consumption course of black liquor hexosamines by *Scopulariopsis brevicaulis*

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.09	64.2852	0.06428	18.19
4	0.05	35.7140	0.03571	54.50
6	0.02	14.2856	0.1428	81.81
8	0.01	7.1428	0.00714	90.90
10	0.01	7.1428	0.0714	90.90

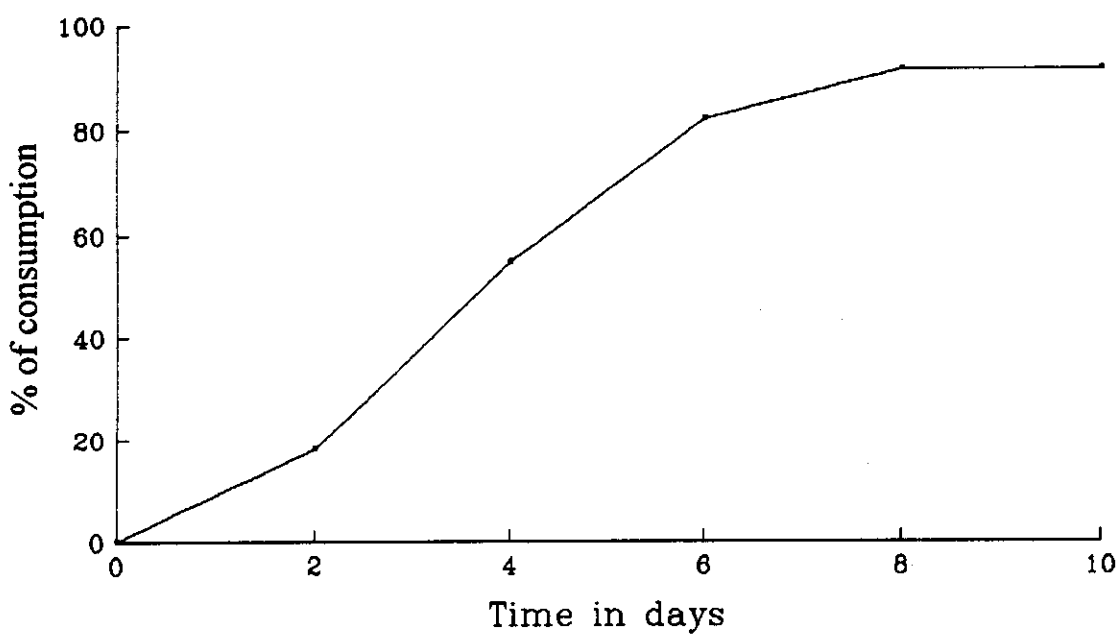

Fig. (24): Consumption course of black liquor hexosamines by *Scopulariopsis brevicaulis*

on the eighth day of incubation after which no consumption was detected.

It can be concluded that *Scopulariopsis brevicaulis* showed maximum consumption of carbohydrates at the rate of 70.37%, reducing sugars at the rate of 63.41% while hexosamines at the rate of 90.90%.

5- Consumption Course of Black Liquor Sugars by *Monilia acremonium*

1- Consumption course of carbohydrates :

The data given in table (28) and fig (25) show that the consumption of carbohydrates by *Monilia acremonium* took place at more or less low rate. Consumption rate was 14.81%on the second day of incubation then increased to 25.93% on the fourth day, 48.15% on the sixth day and to 59.26% on the eighth day after which no further increase in the consumption of carbohdrates was detected.

2- Consumption course of reducing sugars :

As it is clear from the data given in table (29) and fig (26), the consumption of reducing sugars started at a very low rate being 7.32% on the secod day of incubation then it increased to reach a level of 26.63% on the fourth day, 39.02% on the sixth day and to 51.22% on the eighth day of incubation with no further increase at later periods.

3- Consumption course of hexosamines :

The consumption of hexosamines as shown from table (30) and fig (27) reached a level of 27.28% on the second day then increased to 54.50% on the fourth day and reached a level of 72.71% on the sixth day without any further increase on further incubation.

Table (28): Consumption course of black liquor carbohydrates by *Monilia acremonium*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.23	3942.8557	3.9428	14.81
4	0.20	3428.5702	3.4285	25.93
6	0.14	2399.9991	2.3999	48.15
8	0.11	1885.7136	1.8857	59.26
10	0.11	1885.7136	1.8857	59.26

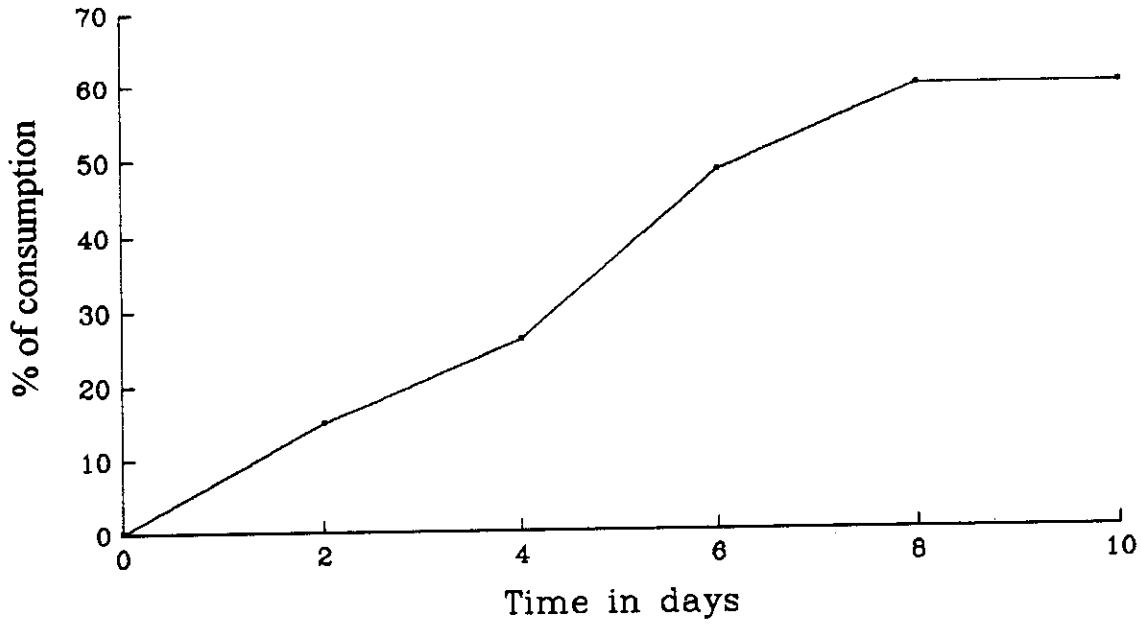

Fig. (25): Consumption course of black liquor carbohydrates by *Monilia acremonium*

Table (29): Consumption course of black liquor reducing sugars by *Monilia acremonium*

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.38	3.8	3.8	7.32
4	0.30	3.0	3.0	26.63
6	0.25	2.5	2.5	39.02
8	0.20	2.0	2.0	51.52
10	0.20	2.0	2.0	51.52

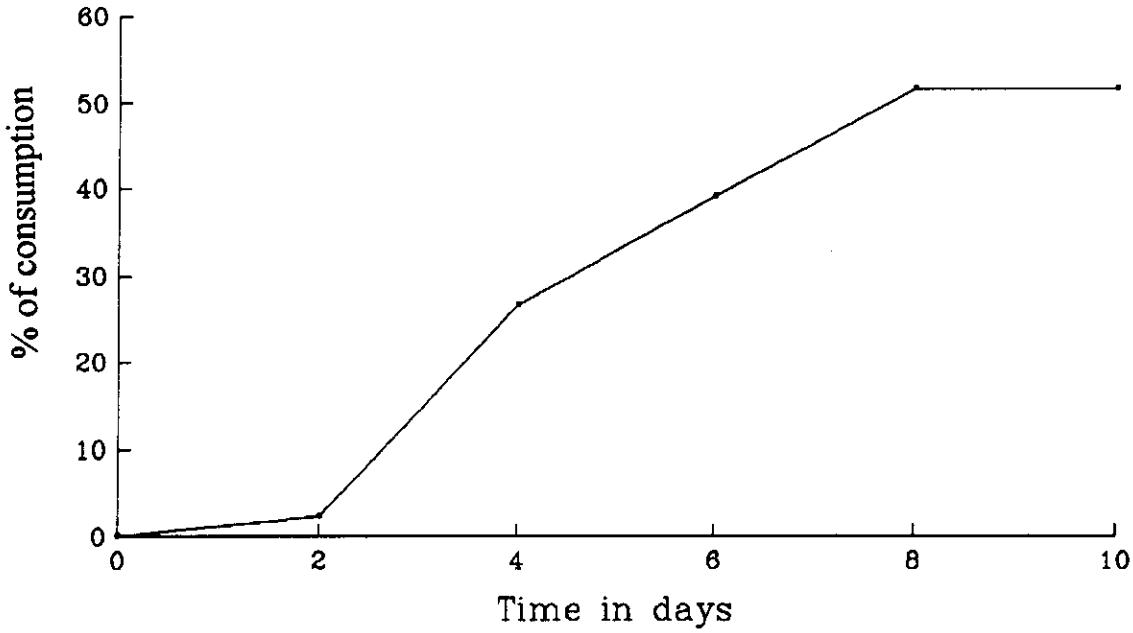

Fig. (26): Consumption course of black liquor reducing sugars by *Monilia acremonium*

Table (30): Consumption course of black liquor hexosamines by *Monilia acremonium*

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.08	57.1425	0.05714	27.28
4	0.05	35.7140	0.03571	54.50
6	0.03	21.4284	0.2142	72.71
8	0.03	21.4284	0.2142	72.71
10	0.03	21.4284	0.2142	72.71

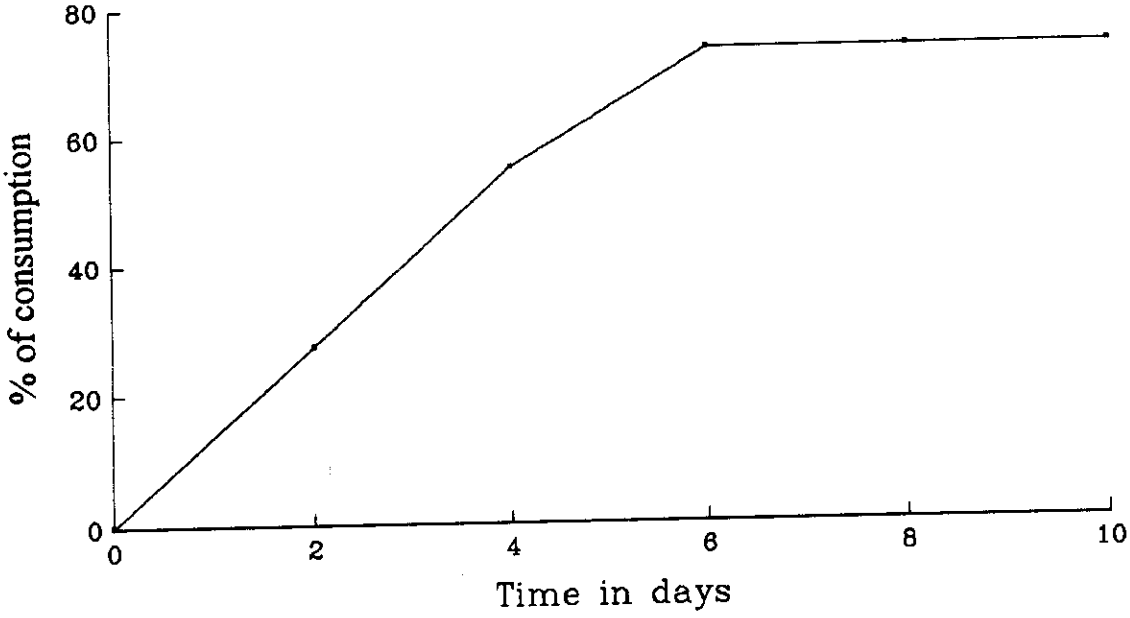

Fig. (27): Consumption course of black liquor hexosamines by *Monilia acremonium*

it can be concluded that *Monilia acremonium* showed maximum consumption of carbohydrates at the rate of 59.26%, reducing sugars at the rate of 51.22% while hexosamines at the rate of 72.71%.

6- Consumption Course of Black liquor Sugars by *Paecilomyces divaricata*

1- Consumption course of carbohydrates :

As it is clear from the data given in table (31) and fig (28), the consumption rate of carbohydrates by *Paecilomyces divaricata* was 14.81% on the second day of incubation then gradually increased to reach a level of 25.93% on the fourth day, 29.63% on the sixth day and increased at later peirod of incubation to reach 44.44% on the eighth day then 48.15% on the tenth day of incubation with further increase at later periods.

2- Consumption course of reducing sugars :

The data given in table (32) and Fig (29) show that the consumptioon of reducing sugars started at a low rate being 9.76% on the second day of incubation then it increased to reach a rate of 26.83% on the fourth day , 43.90% on the sixth day and to 51.22% on the eighth day without any further increase on further incubation.

3- Consumption course of hexosamines :

Consumption rate of hexosamines reached a level 18.18% on the second day then increased to reach a level of 45.46% on the fourth day, 54.50% on the sixth day, 72.71% on the eighth day and increased at the later period to reach a level of 81.81% on the tenth day of incubation with further increase at later periods, (table 33 and fig 30).

Table (31): Consumption course of black liquor carbohydrates by *Paecilomyces divaricata.*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.23	3942.8557	3.9428	14.81
4	0.20	3428.5702	3.4285	25.93
6	0.19	3257.1416	3.2571	29.63
8	0.15	2571.4276	2.5714	44.44
10	0.14	2399.9991	2.3999	48.15

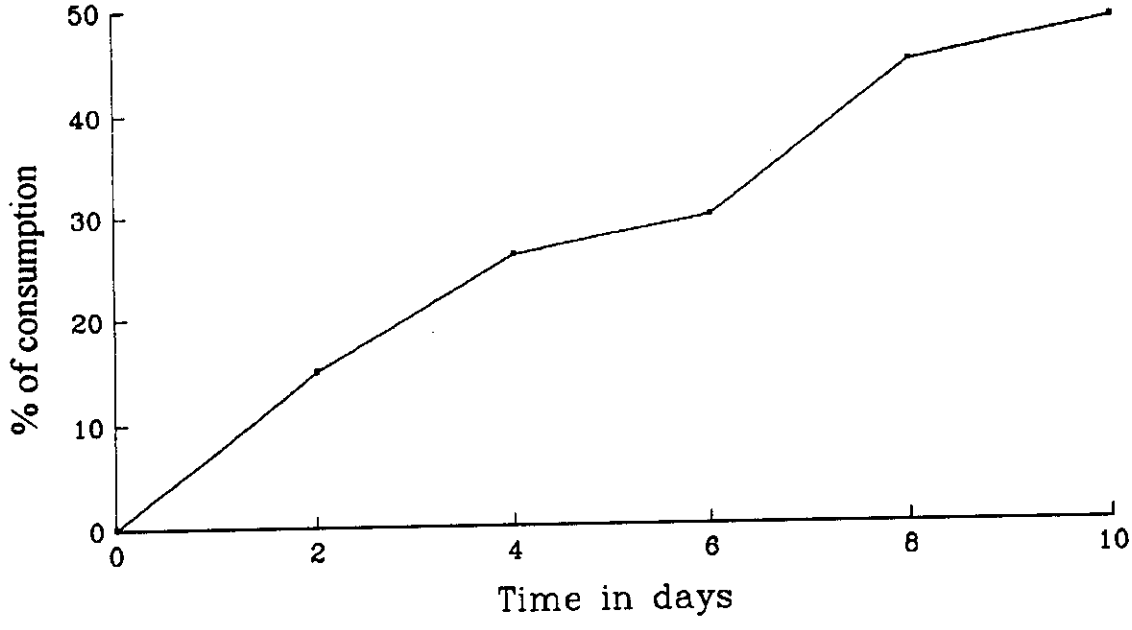

Fig. (28): Consumption course of black liquor carbohydrates by *Paecilomyces divaricata*

Table (32): Consumption course of black liquor reducing sugars by *Paecilomyces divaricata*.

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.37	3.7	3.7	9.76
4	0.30	3.0	3.0	26.83
6	0.23	2.3	2.3	43.90
8	0.20	2.0	2.0	51.22
10	0.20	2.0	2.0	51.22

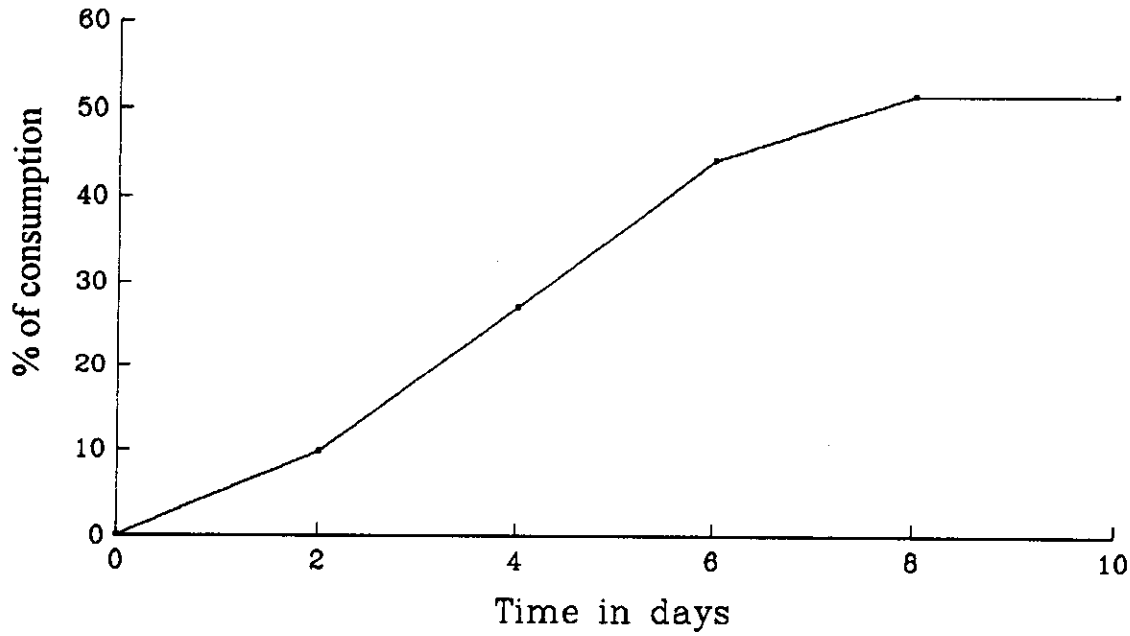

Fig. (29): Consumption course of black liquor reducing sugars by *Paecilomyces divaricata*

Table (33): Consumption course of black liquor hexosamines by *Paecilomyces divaricata.*

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.09	64.2852	0.06428	18.18
4	0.06	42.8569	0.04285	45.46
6	0.05	35.7140	0.03571	54.50
8	0.03	21.4284	0.02142	72.71
10	0.02	14.2856	0.01428	81.81

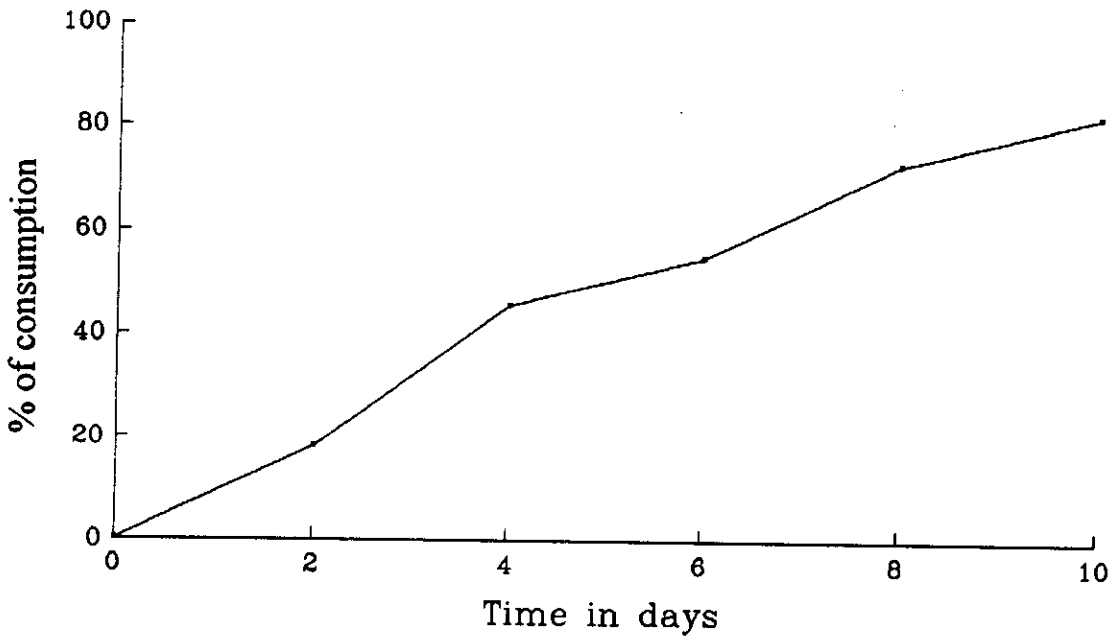

Fig. (30): Consumption course of black liquor hexosamines by *Paecilomyces divaricata*

In can be concluded that *Paecilomyces divaricata* showed maximum consumption of carbohydrates, even at later periods to reach a level of 48.15% and reducing sugars at the rate of 51.22% while hexosamines showed maximum consumption level of 81.81%.

7- Consumption Course of Black Liquor Sugars by *Paecilomyces silvatica*

1- Consumption course of Carbohydrates :

The consumption of carbohydrates by *Paecilomyces silvatica* as shown from table (34) and fig (31) took place at the rate of 11.11% on the second day of incubation then slight increased to a level of 22.22% on the fourth day, 33.33% on the sixth day and increased to a level of 44.44% on the eighth day of incubation. On further incubation no consumption of carbohydrates was detected.

2- Consumption course of reducing sugars :

These sugars were consumed at a low rate. As it is clear from the data given in tabe (35) and fig (32), it reached to a level of 9.76% on the second day of incubation and increased to a level of 24.39% on the fourth day, 41.46% on the sexth day, 46.34% on the eighth day and increased at later period to reach 51.22 on the tenth day of incubation which consumption was detected.

3- Consumption course of hexosamines :

The data given in table (36) and fig (33) show that the consumption rate of hexosamines by *Paecilomyces silvatica* was 9.10% on the second day of incubation then increased to 27.28% on the fourth day, 54.50% on the sixth day and increased at later period of

Table (34): Consumption course of black liquor carbohydrates by *Paecilomyces silvatica.*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.24	4114.2842	4.1142	11.11
4	0.21	3599.9987	3.5999	22.22
6	0.18	3085.1731	3.0851	33.33
8	0.15	2571.4276	2.5714	44.44
10	0.15	2571.4276	2.5714	44.44

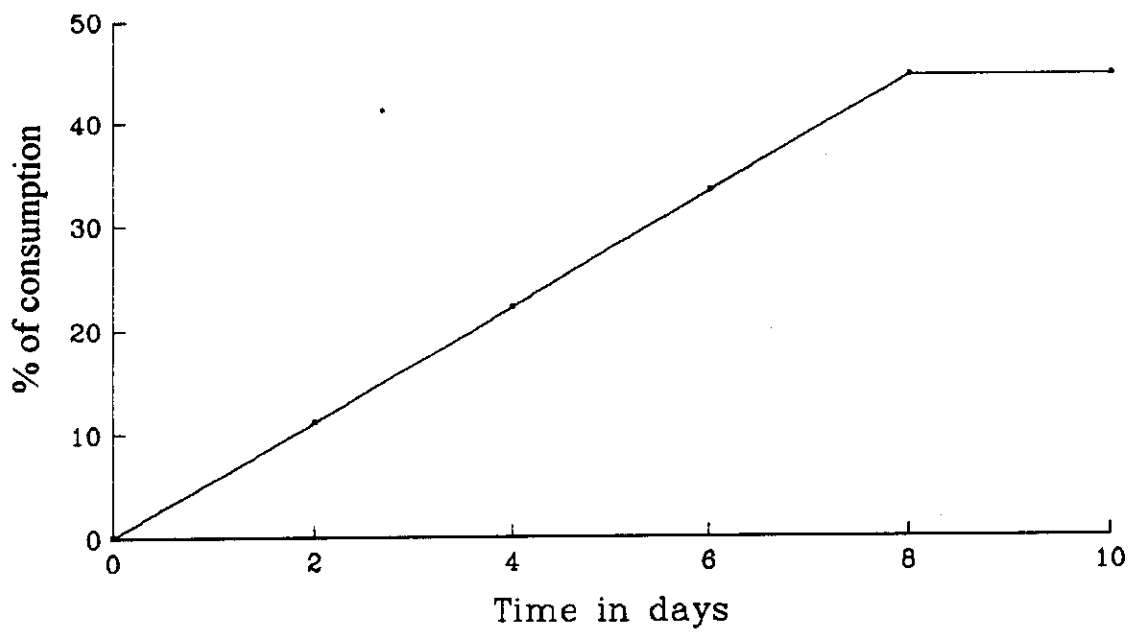

Fig. (31): Consumption course of black liquor carbohydrates by *Paecilomyces silvatica*

Table (35): Consumption course of black liquor reducing sugars by *Paecilomyces Silvatica.*

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.37	3.7	3.7	9.76
4	0.31	3.1	3.1	24.39
6	0.24	2.4	2.4	41.46
8	0.22	2.2	2.2	46.34
10	0.20	2.0	2.0	51.22

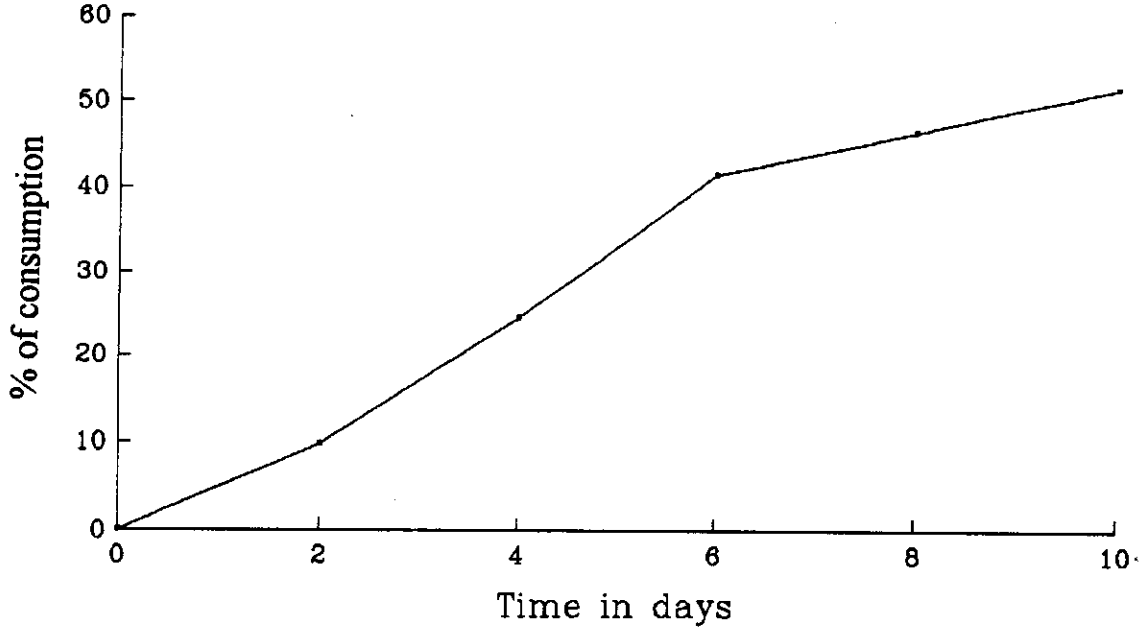

Fig. (32): Consumption course of black liquor reducing sugars by *Paecilomyces silvatica*

Table (36): Consumption course of black liquor hexosamines by *Paecilomyces silvatica*.

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.10	71.4281	0.07142	9.10
4	0.08	57.1425	0.05714	27.28
6	0.05	35.7140	0.03571	54.50
8	0.04	28.5712	0.02857	63.64
10	0.03	21.4284	0.02142	72.74

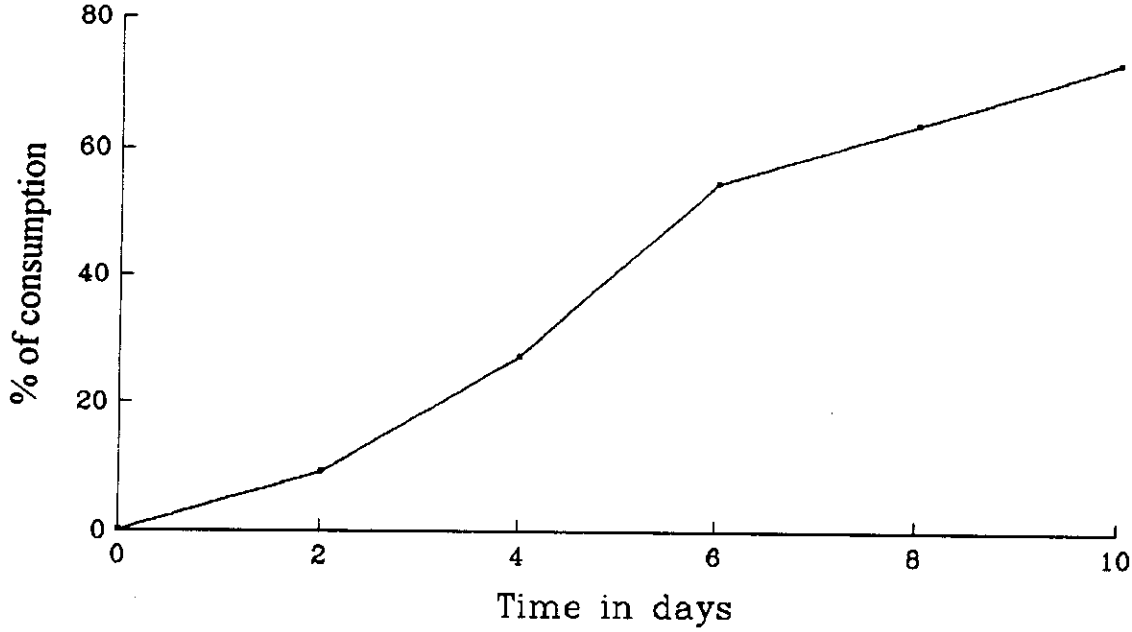

Fig. (33): Consumption course of black liquor hexosamines by *Paecilomyces silvatica*

incubation to reach 63.64% on the eighth day then 72.74% on the tenth day of incubation with further increase at later periods.

It can be concluded that *Paecilomyces silvatica* showed maximum consumption of carbohydrates at a rate of 44.44% and reducing sugars consumed even the later periods at the rate of 51.22%. While hexosamines showed maximum consumption even the later of periods to reach a level of 72.74%.

8- Consumption Course of Black Liquor Sugars by *Aspergillus clavatus*

1- Consumption course of carbohydrates :

The data given in table (37) and fig (34) show that the consumption of carbohydrartes by *Aspergillus* clavatus took place at more or les slow rate consumption rate was 11.11% on the second day of incubation then increased to 22.22% on the foruth day, 40.74% on the sexth day and increased to reach a maximum level of 51.85% on the eighth day after which no further increase in the consumption of cabrolhdrates was detected.

2- Consumption course of reducing sugars :

Consumption rate of reducing sugars reached to a low level of 9.76% on the second day of incubation then slight increased to reach a level of 26.83% on the fourth day, 46.34% on the sixth day and 51.22 on the eighth day of incubtation with no further increase at later periods as shown table (38) and fig (35).

3- Consumption course of hexosamines :

The data given in table (39) and fig (36) show that the consumption of hexosamines by *Aspergillus clavatus,* reached to a level of 27.28% on the second day, 45.46% on the fourth day, 63.64% on the sixth day and to 72.71% on the eighth day of incubation whithout any further increase on further incubation.

Table (37): Consumption course of black liquor carbohydrates by *Aspergillus clavatus*.

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.24	4114.2842	4.1142	11.11
4	0.21	3599.9987	3.5999	22.22
6	0.16	2742.8561	2.7428	40.74
8	0.13	2228.5706	2.2285	51.85
10	0.13	2228.5706	2.2285	51.85

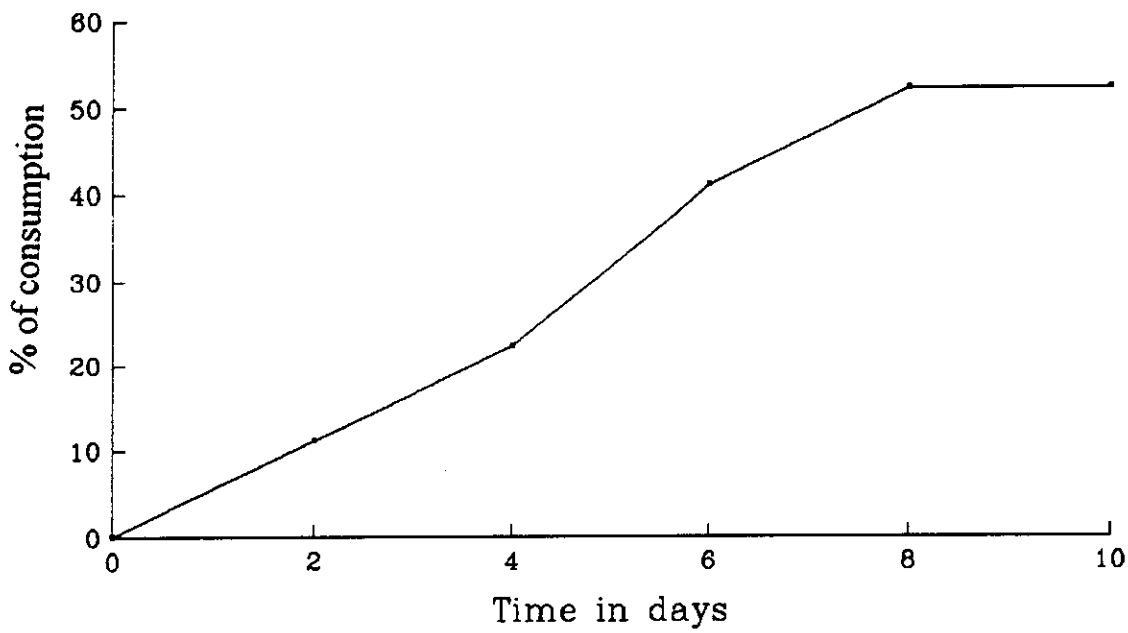

Fig. (34): Consumption course of black liquor carbohydrates by *Aspergillus clavatus*

Table (38): Consumption course of black liquor reducing sugars by *Aspergillus clavatus*.

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.37	3.7	3.7	9.76
4	0.30	3.0	3.0	26.83
6	0.22	2.2	2.2	46.34
8	0.20	2.0	2.0	51.22
10	0.20	2.0	2.0	51.22

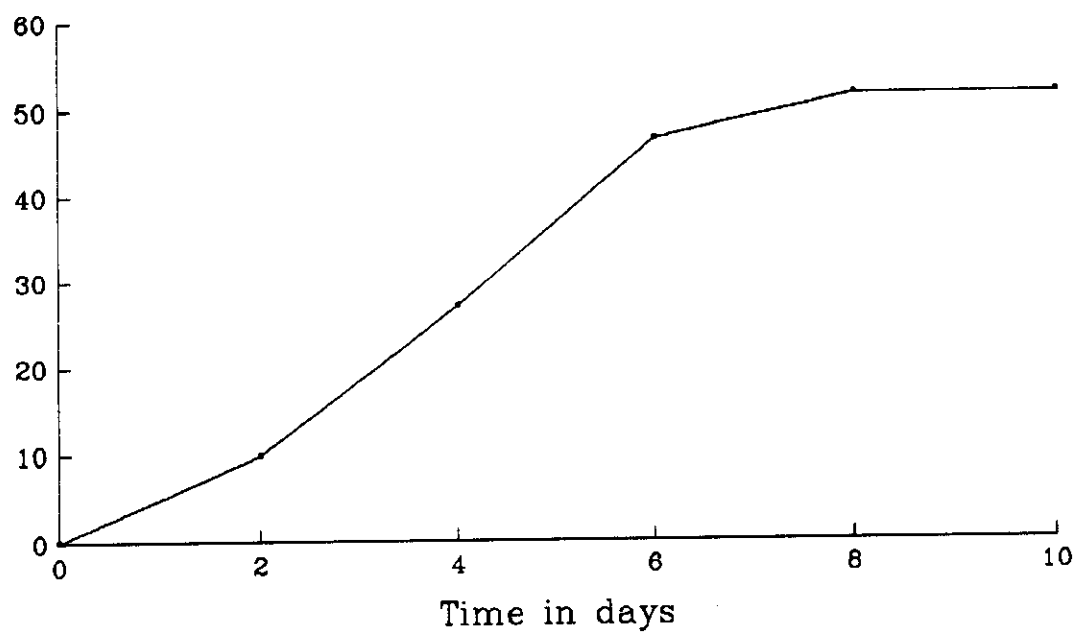

Fig. (35): Consumption course of black liquor reducing sugars by *Aspergillus clavatus*

Table (39): Consumption course of black liquor hexosamines by *Aspergillus clavatus*.

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.08	57.1425	0.05714	27.28
4	0.06	42.8569	0.04285	45.46
6	0.04	28.5712	0.02857	63.64
8	0.03	21.4284	0.02142	72.71
10	0.03	21.4284	0.02142	72.71

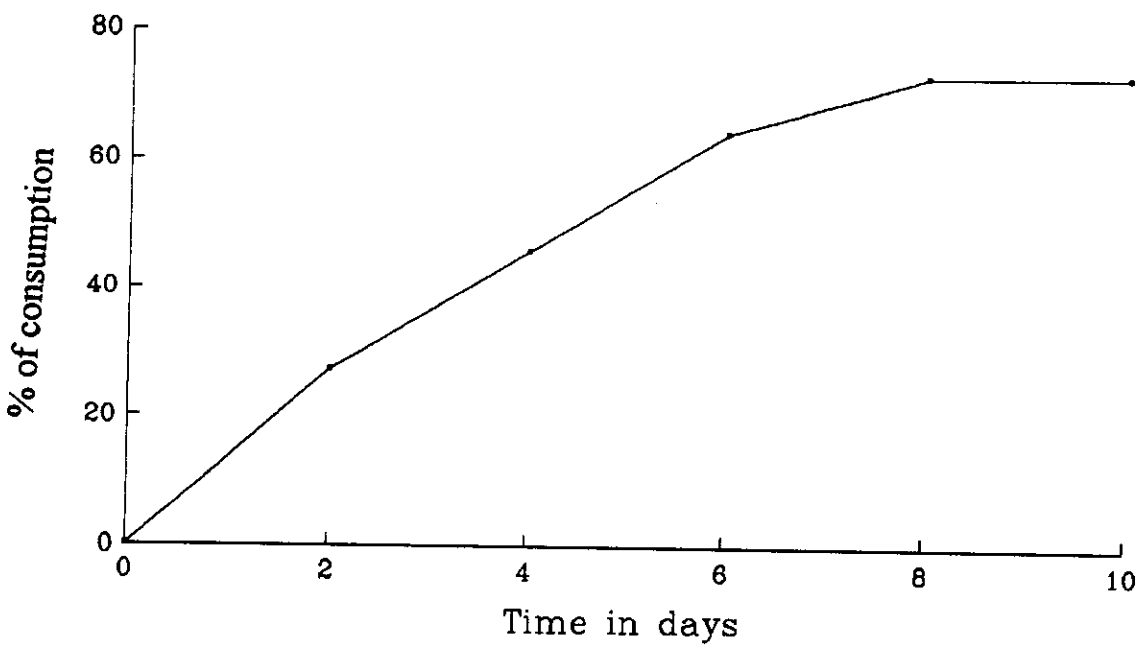

Fig. (38): Consumption course of black liquor hexosamines by *Aspergillus clavatus*

It can be concluded that *Aspergillus clavatus* showed maximum consumption of carbohydrates at the rate of 51.85%, reducing sugars at the rate of 51.22% , while hexosamines at the rate of 72.71%.

9- Consumption Course of Black Liquor Sugars by *Aspergillus flavus*

1- Consumption course of carbohydrates :

The consumption of carbohydrates by *Aspergillus flavus* took place very early and rapidly as shown from table (40) and fig (37). It reached to a level of 14.81% on the second day of incubation, 40.74% on the fourth day and showed increased to reach a maximum level of 55.56% on the sixth day incubation after which no further increase in the consumption of carbohydrates was detected.

2- Consumption course of reducing sugars :

The data given in table (41) fig (38) show that the consumpiton of reducing sugars was on the second day at a rate of 14.63%, 36.59% on the fourth day, 51.22% on the sixth day and increased to reach a level of 63.41% on eighth day of incubation. On further incubation no consumption of reducing sugars was detected.

3- Consumption course of hexosamines :

As it is clear from the data given in table (42) and fig (39), the consumption rate of hexosamines by *Aspergillus flavus* was 27.28% on the second day of incubation then sharply increased to reach a level of 54.50% on the fourth day, 72.71% on the sixth day slight increased on the eighth day reached to a level 81.81% even at later of periods increased to reach a level of 90.91%.

Table (40): Consumption course of black liquor carbohydrates by *Aspergillus flavus*.

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.23	3942.8557	3.9428	14.81
4	0.16	2742.8561	2.7428	40.74
6	0.12	2057.1421	2.0571	55.56
8	0.12	2057.1421	2.0571	55.56
10	0.12	2057.1421	2.0571	55.56

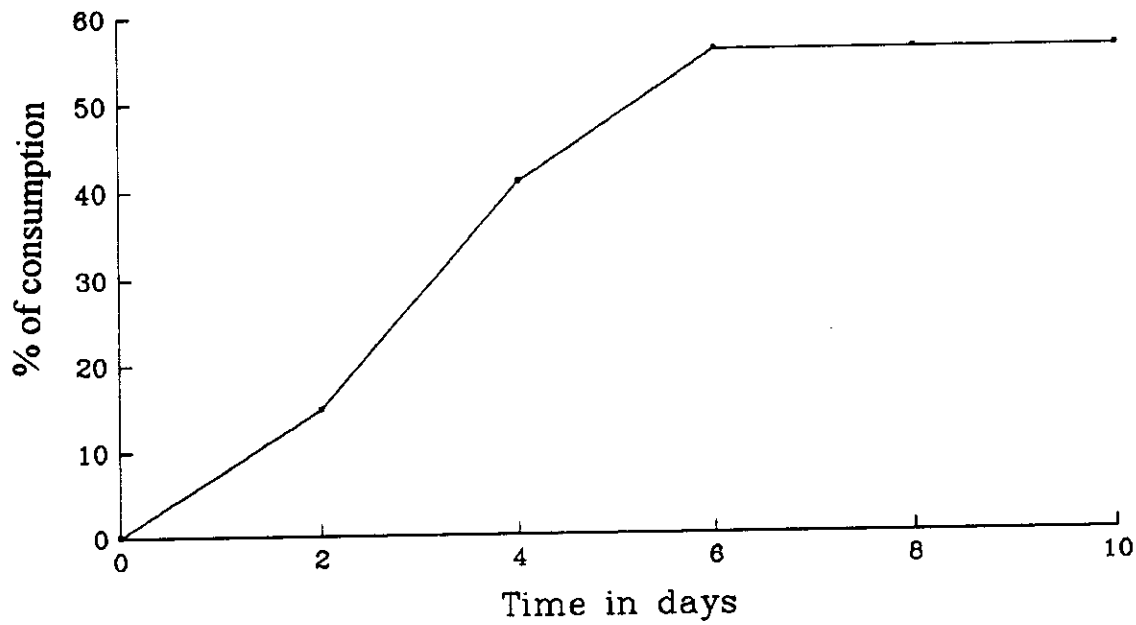

Fig. (37): Consumption course of black liquor carbohydrates by *Aspergillus flavus*

Table (41): Consumption course of black liquor reducing sugars by *Aspergillus Flavus*.

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.35	3.5	3.7	14.63
4	0.26	2.6	2.6	36.59
6	0.20	2.0	2.0	51.22
8	0.15	1.5	1.5	63.41
10	0.15	1.5	1.5	63.41

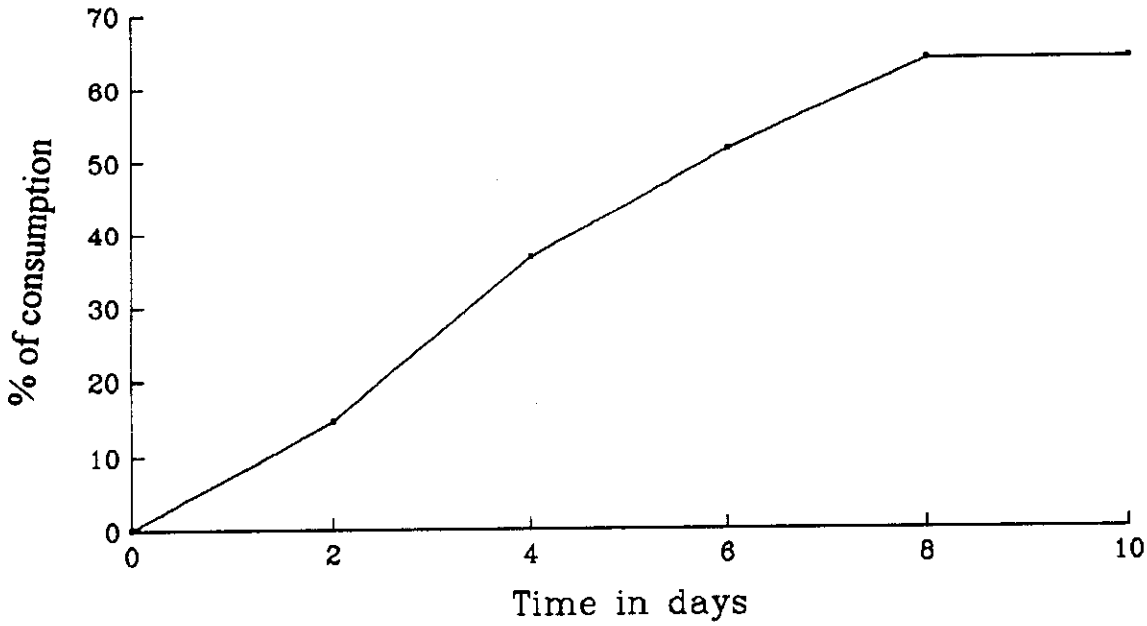

Fig. (38): Consumption course of black liquor reducing sugars by *Aspergillus flavus*

Table (42): Consumption course of black liquor hexosamines by *Aspergillus flavus*.

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.08	57.1425	0.05714	27.28
4	0.05	35.7140	0.03571	54.50
6	0.03	21.4284	0.02142	72.71
8	0.02	14.2856	0.01428	81.81
10	0.01	7.1428	0.00714	90.91

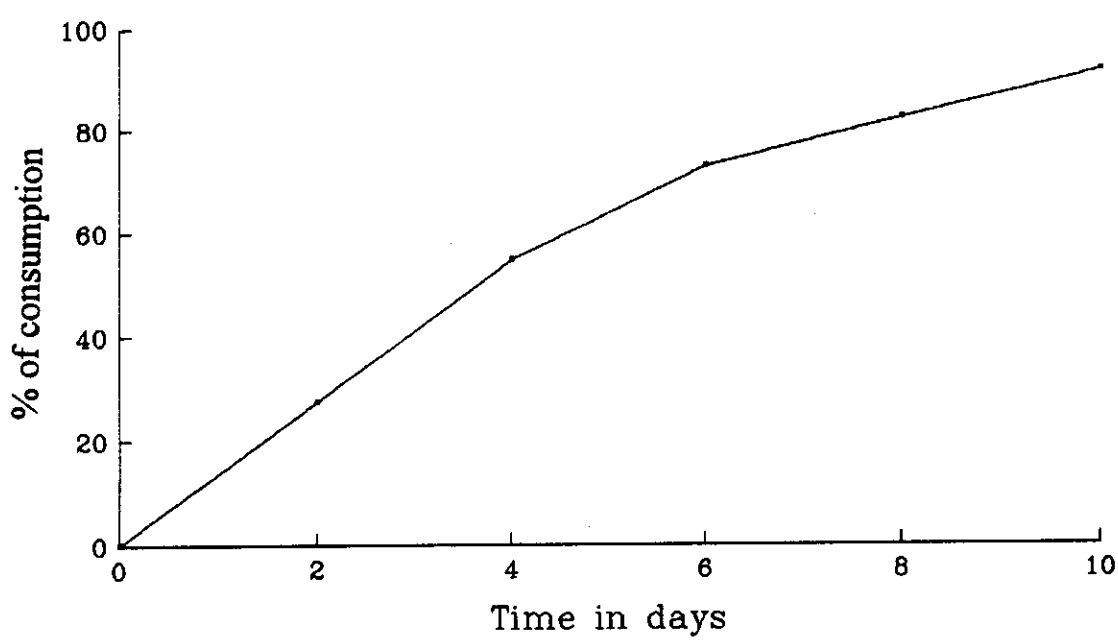

Fig. (39): Consumption course of black liquor hexosamines by *Aspergillus flavus*

It can be concluded that *Aspergillus flavus* showed maximum consumption of carbohydrates at the of 55.56% and reducing sugars at the rate of 63.41%. While hexosamines were consumed at a later periods to reach a level of 90.91%

10 - Consumption Course of Black Liquor Sugars by *Aspergillus Fumigatus*

1- Consumption course of carbohydrates :

The data given in table (43) and fig (40) show that the consumption of carbohydrates by *Aspergillus fumigatus* took place early and rapidly and it reached to a level of 14.81% on the second day of incubation, 44.44% on the fourth day and increased to reach a level of 59.26% on the sixth day of incubation after which no increase in the consumption of carbohydrates was detected.

2- Consumption course of redusing sugars :

The consmption of redusing sugars by ***Aspergillus fumigatus*** took place early and rapidly as shown from table (44) fig (41) . It reached to a level of 17.07% on the second day of incubation, 39.025 on the fourth day and increased to reach a maximum level of 60.98% on the sixth day of incubation with no further increase at later periods.

3 - Consumption course of hexosamines :

The data given in table (45) and fig (42) show that the consumption of hexosamines was on the second day at a rate of 27.28% , 54.50% on the fourth day, 63.64% on the sixth day then showed a slight increase to reach a maximum level of 72.71% on the eighth day of incubation after which no increase in the consumption of hexosamines was detected.

Table (43): Consumption course of black liquor carbohydrates by *Aspergillus fumigatus.*

Time (days)	O.D. 625 nm	ugm of carbohydrates per one ml of broth	gm of carbohydrates per one liter of broth	% of consumption
0	0.27	4628.5697	4.6285	0.00
2	0.23	3942.8557	3.9428	14.81
4	0.15	2571.4276	2.5714	44.44
6	0.11	1885.7136	1.8857	59.26
8	0.11	1885.7136	1.8857	59.26
10	0.11	1885.7136	1.8857	59.26

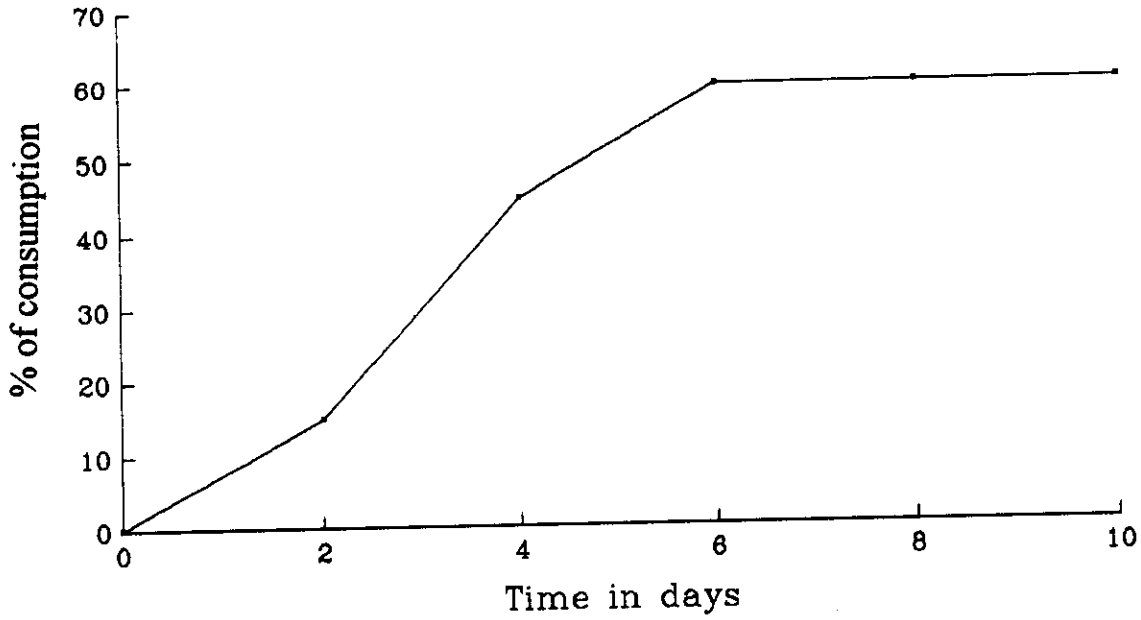

Fig. (40): Consumption course of black liquor carbohydrates by *Aspergillus fumigatus*

Table (44): Consumption course of black liquor reducing sugars by *Aspergillus fumigatus*.

Time (days)	O.D. 530 nm	mgm of reducing sugars per one ml of broth	gm of reducing sugars per one liter of broth	% of consumption
0	0.41	4.1	4.1	0.00
2	0.34	3.4	3.4	17.07
4	0.25	2.5	2.5	39.02
6	0.16	1.6	1.6	60.98
8	0.16	1.6	1.6	60.98
10	0.16	1.6	1.6	60.98

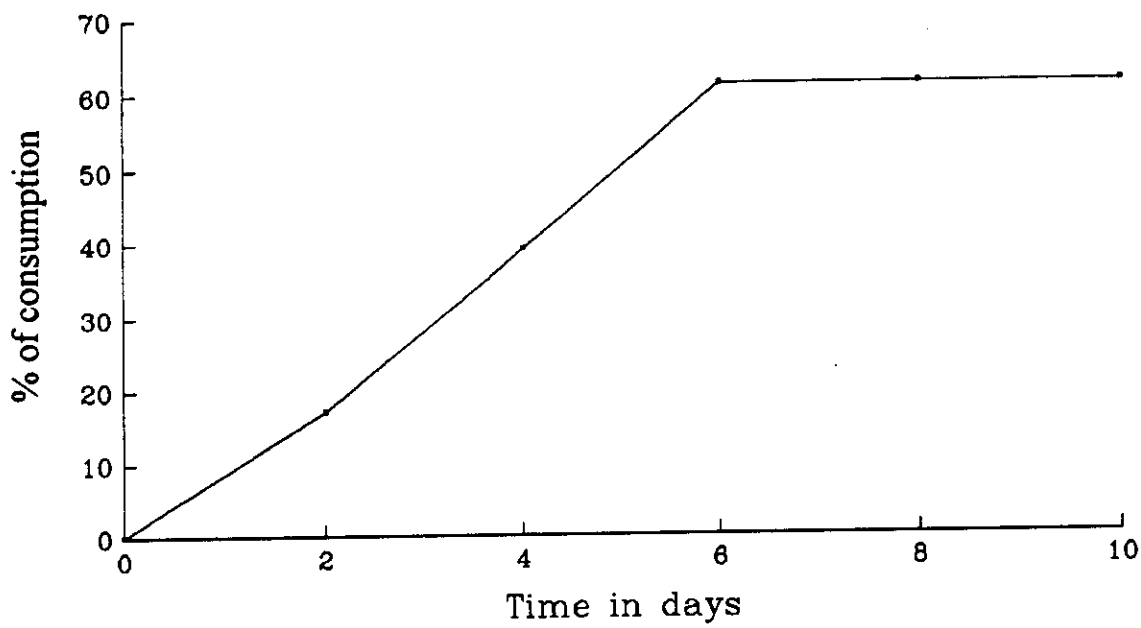

Fig. (41): Consumption course of black liquor reducing sugars by *Aspergillus fumigatus*

Table (45): Consumption course of black liquor hexosamines by *Aspergillus fumigatus*.

Time (days)	O.D. 530 nm	ugm of hexosamine per one ml of broth	gm of hexosamine per one liter of broth	% of consumption
0	0.11	78.5709	0.07857	0.00
2	0.08	57.1425	0.05714	27.28
4	0.05	35.7140	0.03571	54.50
6	0.04	28.5712	0.02857	63.64
8	0.03	21.4284	0.02142	72.71
10	0.03	21.4284	0.02142	72.71

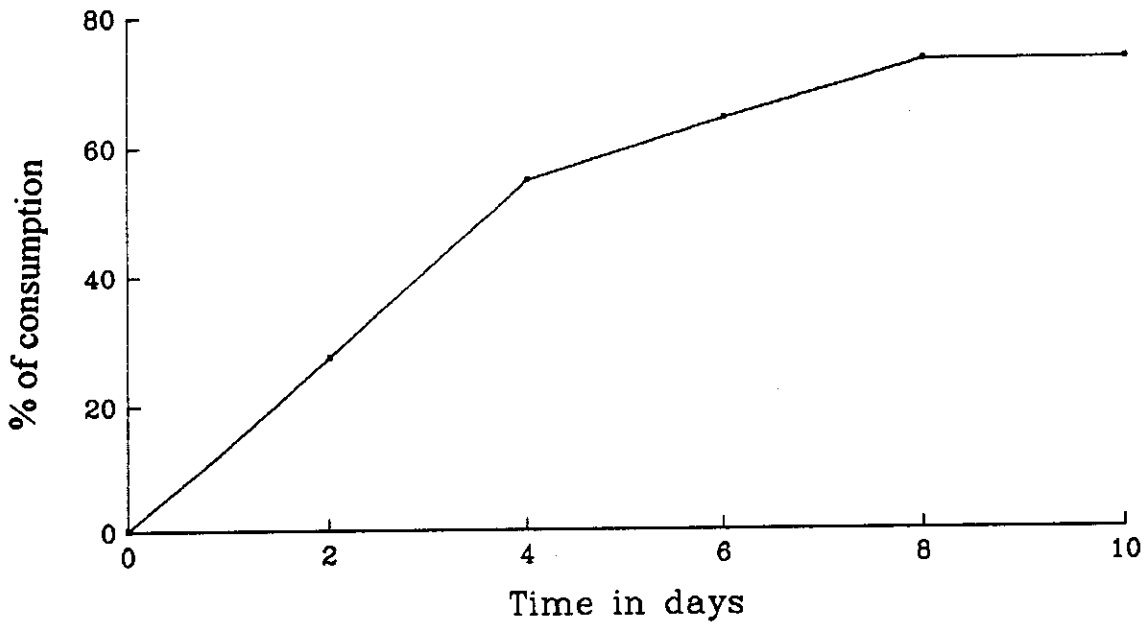

Fig. (42): Consumption course of black liquor hexosamines by *Aspergillus fumigatus*.

It can be concluded that *Aspergillus fumigatus* showed maximum consumption of carbohydrates at the rate of 59.26%, reducing sugars at the rate of 60.98%, while hexosamines at the rate of 72.71%

B- Comparative Production of Enzymes by Black Liquor Sugars Consuming Fungi

1- Comparative Production of Xylanases :

The production of xylanases is quite abundant among the studied fungal species. As it is clear from the data given in table (46) and fig (43), these enzymes were found to be produced by *Fusarium oxysporum* , *Scopulariopsis brevicaulis* ; *Paecilomyces silvatica* ; *Aspergillus flavus* ; *Aspergillus clavatus* and *Aspergillus fumigatus* . *Fusarium oxysporum* and *Asperigllus fumigatus* showed highest production level of 627.42 units/100 ml broth was recorded, while for *Aspergillus flavus* a level of 86.27 units/100 ml of broth (low level) and for *Paecilomyces silvatica* a level of 168.62 units/100 ml of broth was recorded. It is worthy to note that xylanases were produced on the third day of incubation.

2- Comprative Production of Lipases :

Lipase production was found to be very abundant for : *Fusarium oxsporum*, *Alternaria humicola* ; *Scopulariopsis brevicaulis* , *Paecilomyces silvatica* and *Aspergillus flavus.* As it is clear from the data given in table (47) and fig (44) , almost all the studied five speies were found to be active producers of lipases. Moreover the production of these enzyme was detected on the third day. Highest level of lipase production was recorded for *Alternaria humicola* (4.57 mg/ml of broth)

Table (46) : Comparative production of Xylanases enzyme in broth by black liquor sugars consuming fungi

Name of fungus	O.D. 530.nm	u mole reducing sugar per 1 ml of broth	Xylanases activity (units/100 ml of broth)
Fusarium oxysporum	1.60	1129.14	627.42
Alternaria humicola	0.00	0.00	0.00
Botryotrichum piluliferum	0.00	0.00	0.00
Scopulariopsis brevicaulis	0.58	409.31	227.44
Monilia acremonium	0.00	0.00	0.00
Paecilomycis silvatica	0.43	303.46	168.62
Paecilomycis divaricata	0.00	0.00	0.00
Aspergillus clavatus	0.77	5.43	301.95
Aspergillus flavus	0.22	155.26	86.27
Aspergillus fumigatus	1.60	1129.14	627.42

Fig.(43) Comparative production of Xylanases enzyme in broth by black liquor sugars consuming fungi

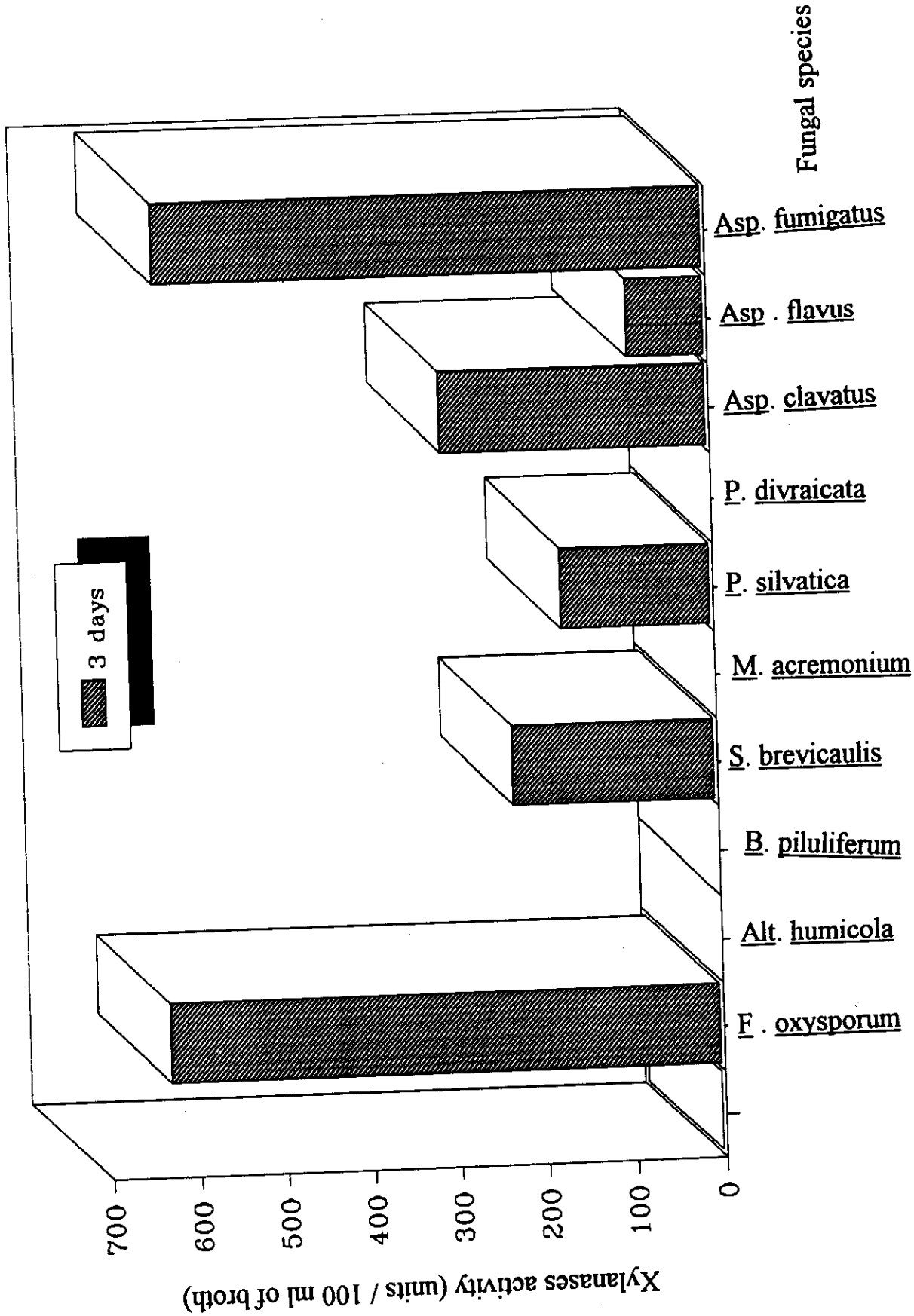

Table (47) : Comparative production of lipases enzyme in broth by black liquor sugars consuming fungi

Name of fungus	diameter of clearing zones (cm)	mg of lipase per 1 ml of broth
Fusarium oxysporum	1.20	3.27
Alternaria humicola	1.40	4.57
Botryotrichum piluliferum	0.00	0.00
Scopulariopsis brevicaulis	1.20	3.92
Monilia acremonium	0.00	0.00
Paecilomycis silvatica	1.00	3.27
Paecilomycis divaricata	0.00	0.00
Aspergillus clavatus	0.00	0.00
Aspergillus flavus	0.80	2.62
Aspergillus fumigatus	0.00	0.00

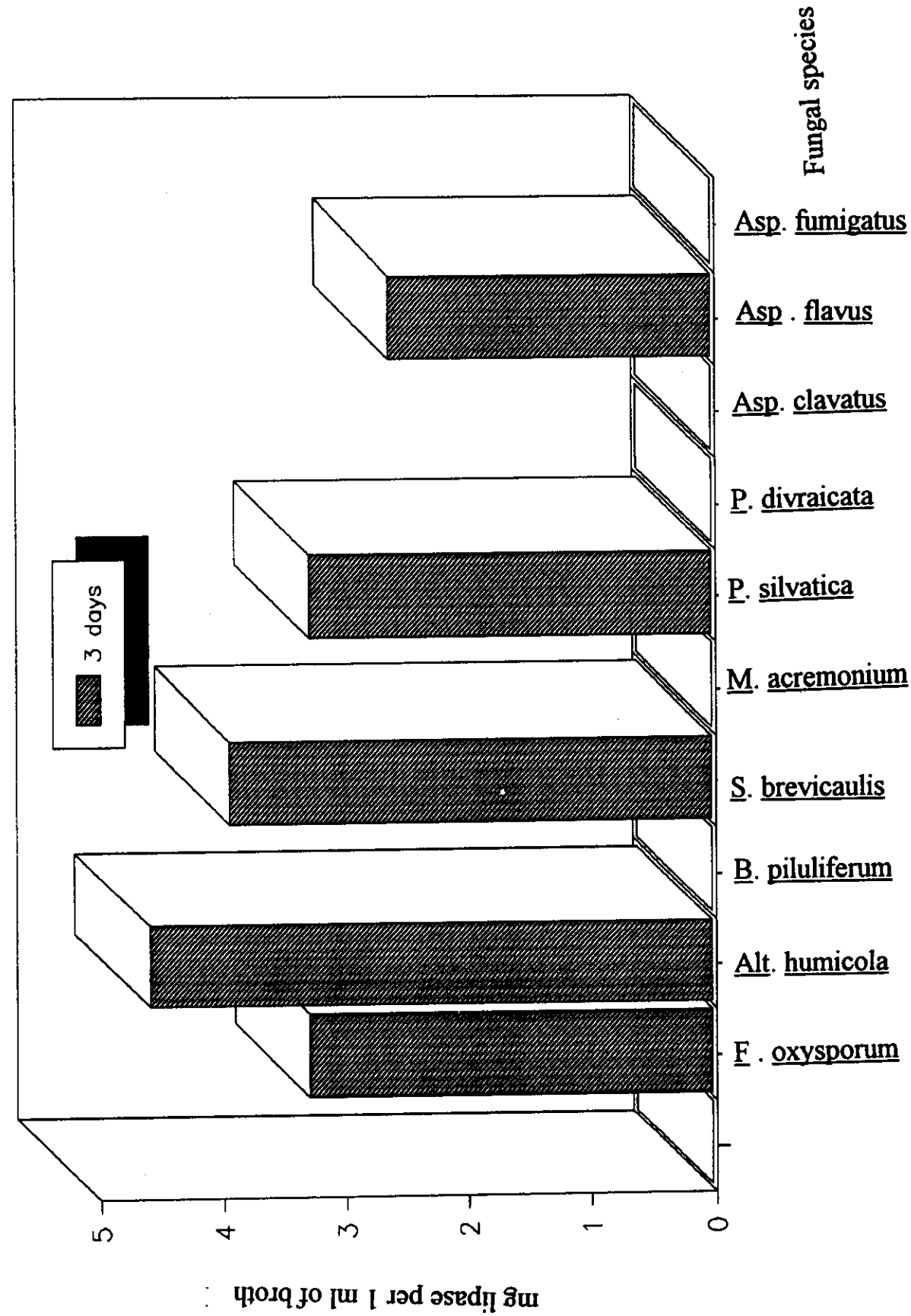

Fig.(44) Comparative production of Lipases enzyme in broth by black liquor sugars consuming fungi.

followed by *Fusarium oxysporum* and *Scopulariopsis brevicaulis* (3.92 mg/ml of broth).

3- Comparative Production of Proteases :

Protease production was found also to be common for the studied fungi species. As it is clear from the data given in table (48) and fig (45), almost all the studied fungal species were found to produce proteases. High level of protease production was recorded for : *Botryotrichum piluliferm*, *Aspergillus clavtus*, and *Aspergillus flavus*. Moderate level of protease production was recorded for *Aspergillus fumigatus* and *Paecilomyces divaricata*. Low level of production was recorded for *Monilia acremonium*.

4- Comparative Production of Keratinases :

Keratinases enzymes are not common in the studied fungi. Only two species namely: *Botryotrichum piluliferm* and *Paecilomyces divaricata* showed very low level of production of keratineases on the third day of incubation.

5- Comparative Production of Cellulases :

Similar to keratinases the production of cellulases was found to be rare in the studied fungi. As it is clear from the data given in table (49) and fig (46). Only three species, *Scopulariopsis brevicaulis* , *Aspergillus clavatus* and *Aspergillus flavus* produced these enzyme which were detected on the third day of incubaiton

Table (48) : Comparative production of Proteases enzyme in broth by black liquor sugar consuming fungi

Name of fungus	O.D. 660.nm	mg starch hydrohysed per 1 ml of broth	Protease activity (units/100 ml of broth)
Fusarium oxysporum	0.00	0.00	0.00
Alternaria humicola	0.00	0.00	0.00
Botryotrichum piluliferum	1.00	0.13	21.22
Scopulariopsis brevicaulis	0.25	0.031	4.40
Monilia acremonium	0.10	0.013	2.12
Paecilomycis silvatica	0.00	0.00	0.00
Paecilomycis divaricata	0.55	0.07	11.67
Aspergillus clavatus	1.00	0.13	21.22
Aspergillus flavus	1.25	0.16	26.52
Aspergillus fumigatus	0.80	0.10	16.98

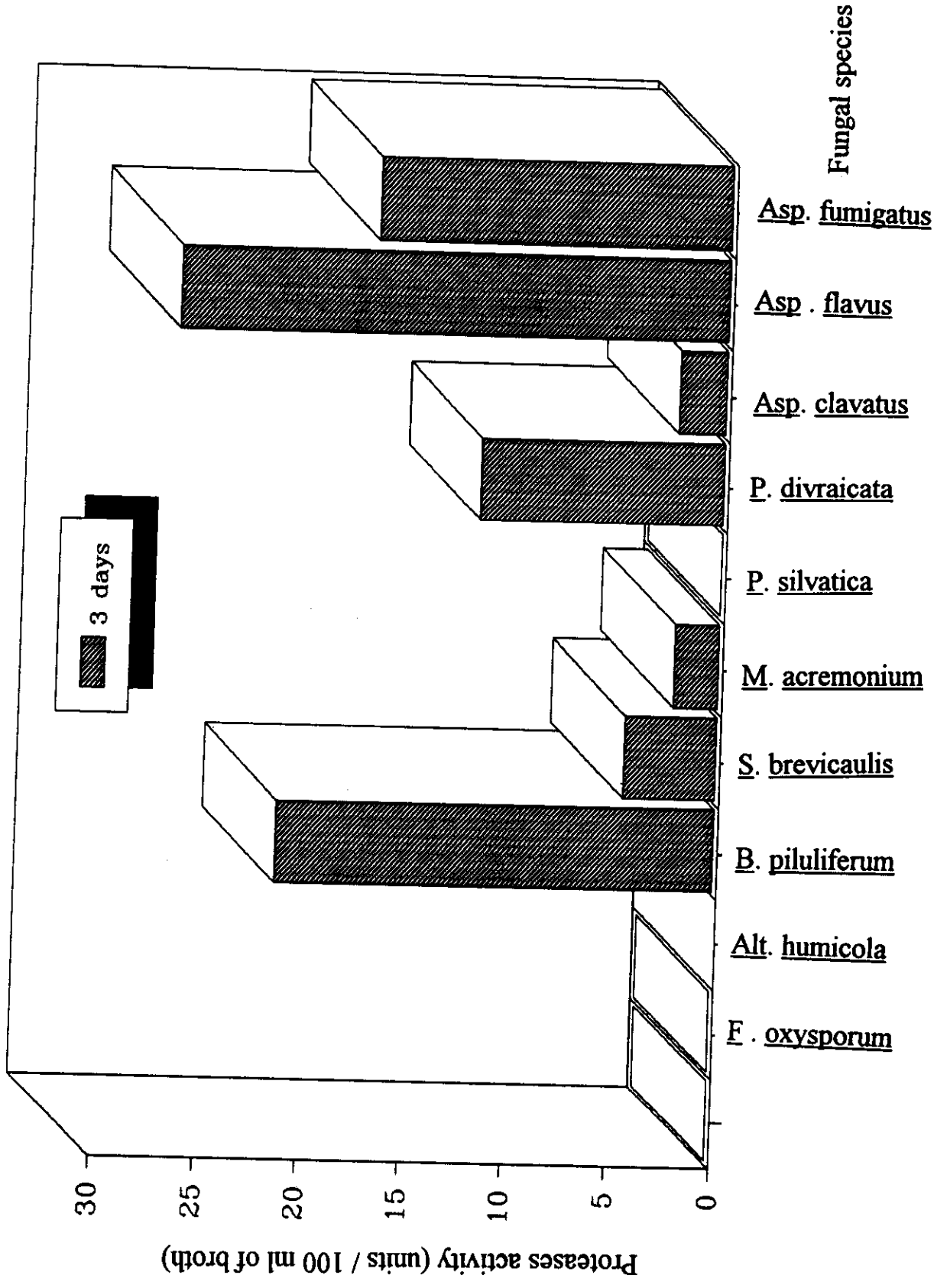

Fig.(45) Comparative production of Proteases enzyme in broth by black liquor sugars consuming fungi.

Table (49) : Comparative production of cellulases enzyme in broth by black liquor sugars consuming fungi

Name of fungus	O.D. 530.nm	mg amino acid per1 ml of broth	Protease activity (units/100 ml of broth)
Fusarium oxysporum	0.00	0.00	0.00
Alternaria humicola	0.00	0.00	0.00
Botryotrichum piluliferum	0.00	0.00	0.00
Scopulariopsis brevicaulis	0.57	1.14	28.5
Monilia acremonium	0.00	0.00	0.00
Paecilomycis silvatica	0.00	0.00	0.00
Paecilomycis divaricata	0.00	0.00	0.00
Aspergillus clavatus	0.65	1.30	32.50
Aspergillus flavus	0.09	0.18	4.50
Aspergillus fumigatus	0.00	0.00	0.00

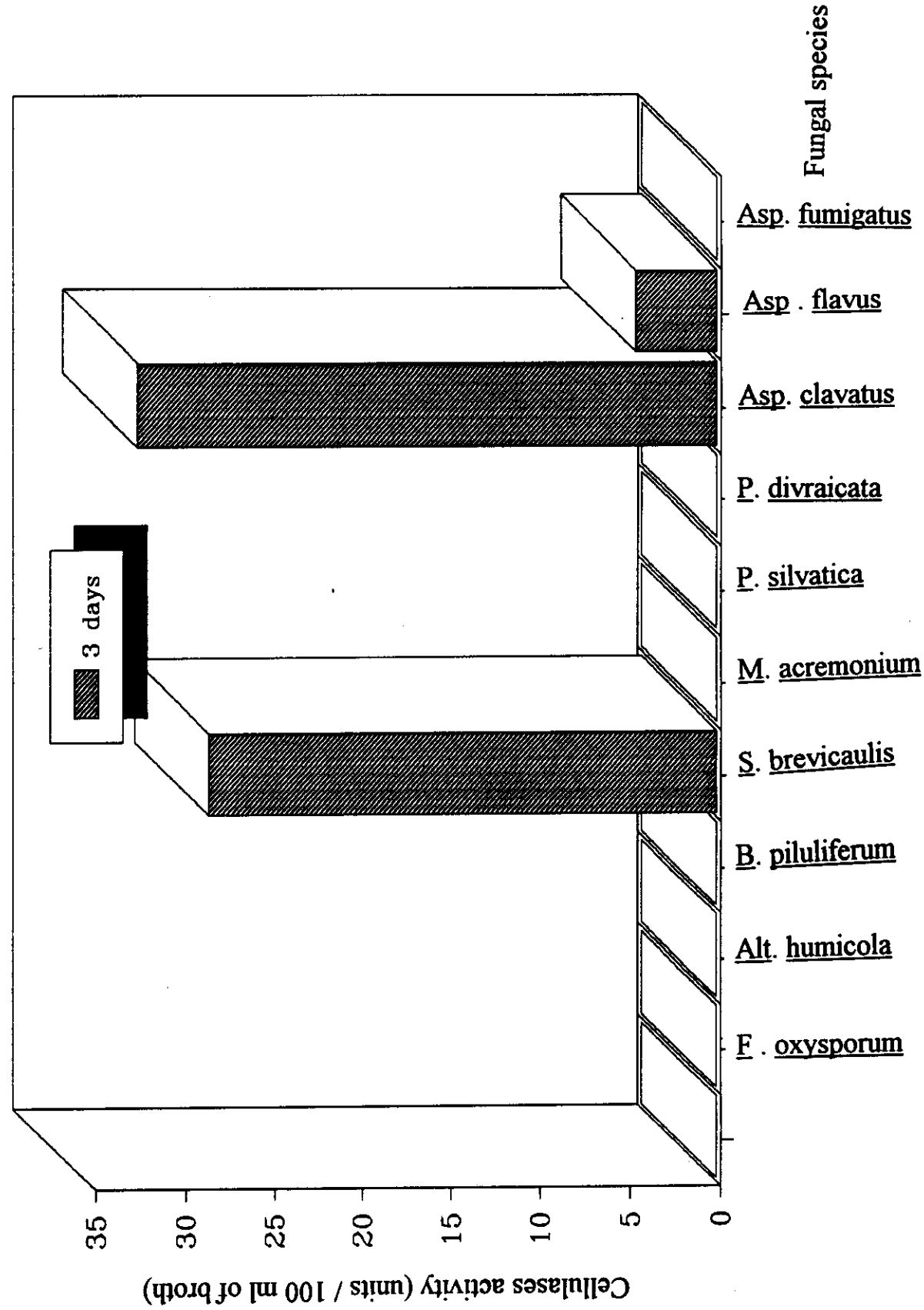

Fig.(46) Comparative production of Cellulases enzyme in broth by black liquor sugars consuming fungi.

6- Production of Amylases :

Amylase enzymes were not proudced by all the studied fungi.

Production of Aflatoxins by The Used Fungi

The study of aflatoxin production by the 10 used fungi revealed that seven of them namely :

Botryotrichum piluliferum

Monilia acremonium

Paecilomyces divaricata

Paecilomyces silivatica

Aspergillus clavatus

Aspergillus flavus

Aspergillus fumigatus

produced aflatoxins . While only three species namly :

Fusarium oxysporum

Scopulariopsis brevicaulis

Alternaria humicola

did not produce aflatoxins. For this reason the study of the bioconversion of black liquor hemicelluloses into single cell proteins was restricted to these three non - aflatoxin producers fungal species.

General conclusion.

In can be concluded that out of all the isolated fungi only three proved to be nonaflatoxin. Of these three fungi ***Fusarium oxysporum*** realized highest bioconversions rate of black liquor hemicelluloses into single cell protein. This fungus can be recommended for the safe bioconversion of black liquor sugars into single cell protein. Biofilter

made of this organism can safely eliminate much of the soluble sugars of black liquor before its disposal and moreover enable usueful use of these sugars by bioconverting them into safe nontoxic singale cell protein.

Single Cell Protein Production by The Selected Fungi

The data given in table (50) show that ***Fusarium oxysporum*** realized highest percentage of total protein of the produced dry weight, this was followed by ***Scopulariopsis brevicaulis*** then ***Alternaria humicola***

Table (50) Amount of single cell protein produced by the selected fungi per 50 ml black liquor.

Name of fungus	biomass dry weight(gm)	total protein %	produced single protein(g
Fusarium oxysporum	0.06562	33.5	0.02198
Scopulariopsis brevicaulis	0.05794	26.9	0.01559
Alternaria humicola	0.06010	21.1	0.01268

Accordingly, it can be concluded that the best organism for single cell protein production is ***Fusarium oxysporum*** as showed - highest biomass production ahd highest total protein content of the produced biomass .

PART IV

BIOCONVERSION OF BLACK LIQUOR HEMICELLULOSES INTO SINGLE CELL PROTEIN USING ACTINOMYCETES

1- Isolation of Black liquor hemicelluloses consuming Actinomycetes.

The previously collected eight soil samples for the isolation of fungi, were used for the isolation of Actinomycetes that can consume hemicelluloses of the black liquor . Soil dilutions were made and used for seeding black liquor basal salts agar plates. To this medium 3g of $CaCO_3$ were added. The developing actinomycete colonies were transferred to slants . Three types of actinomydetes were obtained.

2- Generic identification of isolated actinomycetes.

The taxonomy of actinomycetes is a very hard and tedious task and since the aim of this work is the utilization of black liquor the identification was carried out at the generic level only . Further work will be carried on the identification of these isolates at the species level . The obtained 47 isolated were differentiated into three growth types as follows:

Type (A) number of isolates was 42 . This growth type is characterized by pale yellow aerial mycelium ., Substrate growth pale

yellow without any pigments. Representative isolates of this type were chosen isolates Nos 3,5,6 and 7 . Isolates produce short straight spore chains (Figs 46, 47 and 48) and spores are rod shaped with tappering ends. Spore surface is smooth (Figs. 49, 50 and 51). Isolates of this type were identified as belonging to the genus ***Streptomyces*** and representative isolates were designates as:

Streptomyces No 3

Streptomyces No 5

Streptomyces No 6

Streptomyces No 7

Type (B) Number of isolates was two. This growth type is characterizaed by greenish grey aerial mycelium, substrate mycelium cream colouered, no diffusible pigments. Representative isolates were chosen isolates No 23 & 24 . Isolates produce spiral chains of spores. Spiral are somewhat loose and consist of 3-5 turns : (Figs 52 and 53). The spores have spiny surface (Fig. 54 and 55). Isolates of this type were identified as belonging to the genus ***Streptomyces*** and representative isolates were designated as follows:

Streptomyces No 23.

Streptomyces No 24.

Type (C) Number of isolates was three. This growth type was characterized by pale yellow aerial mycelium, brown substrate mycelium, and brown diffusible pigments. Velvety to cottony growth. The aerial hyphae are very long and fragments into spores. Long empty

intersporal spaces is followed by each spore (Figs 56 and 57) . This indicates the fragmentation method of spore formation. Spores are with smooth surface (Fig 58 and 59) . Isolates of this group were identified as belonging to the genus ***Actinopolyspora*** which produces spores by the whole fragmentation of the aerial hyphae and the representative isolates were designated as:

Actinopolyspora No 43.

Actinopolyspora No 44.

Actinopolyspora No 44.

Fig. (46) : Micromorphology of aerial hypae of ***streptomyces*** (No. 3 X 400) .

Fig. (47) : Micromorphology of aerial hypae of ***streptomyces*** (No. 5 X 400) .

Fig. (48) : Micromorphology of aerial hypae of ***streptomyces*** (No. 7 X 400) .

Fig. (49) : Electron micrograph of spores of ***streptomyces*** (No. 3 X 20,000).

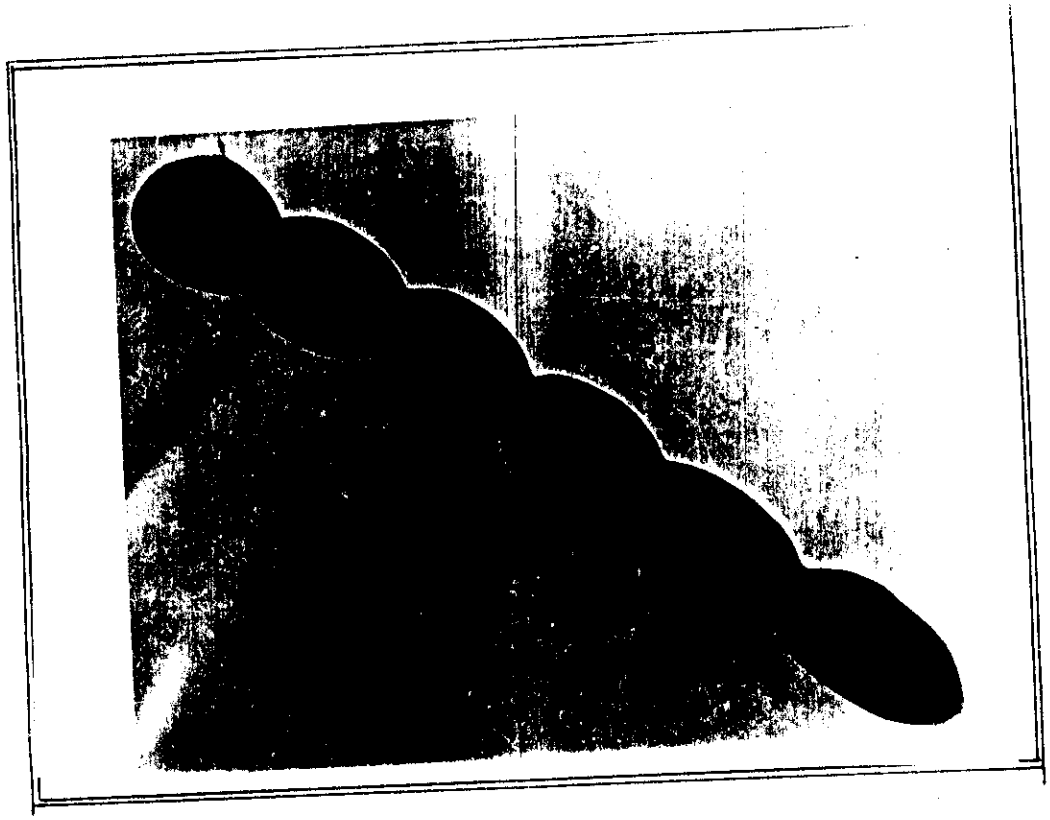

Fig. (50) : Electron micrograph of spores of ***streptomyces*** (No. 5 X 20,000).

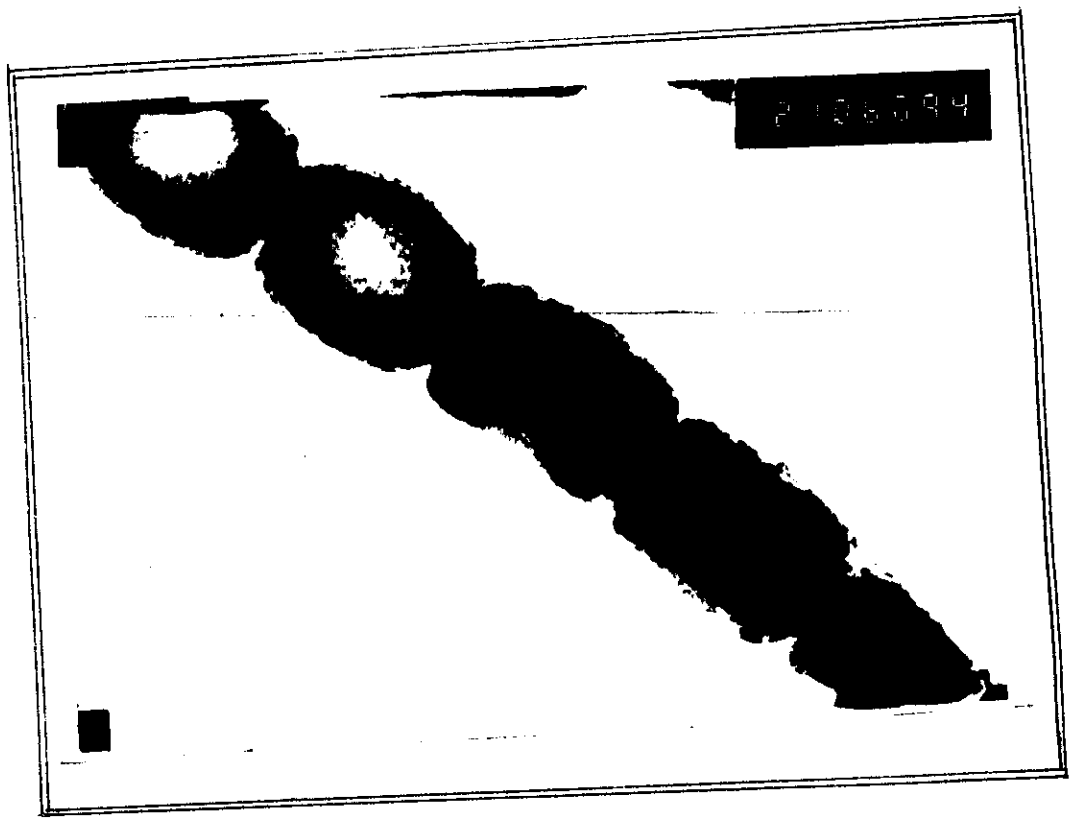

Fig. (51) : Electron micrograph of spores of ***streptomyces*** (No. 6 X 20,000).

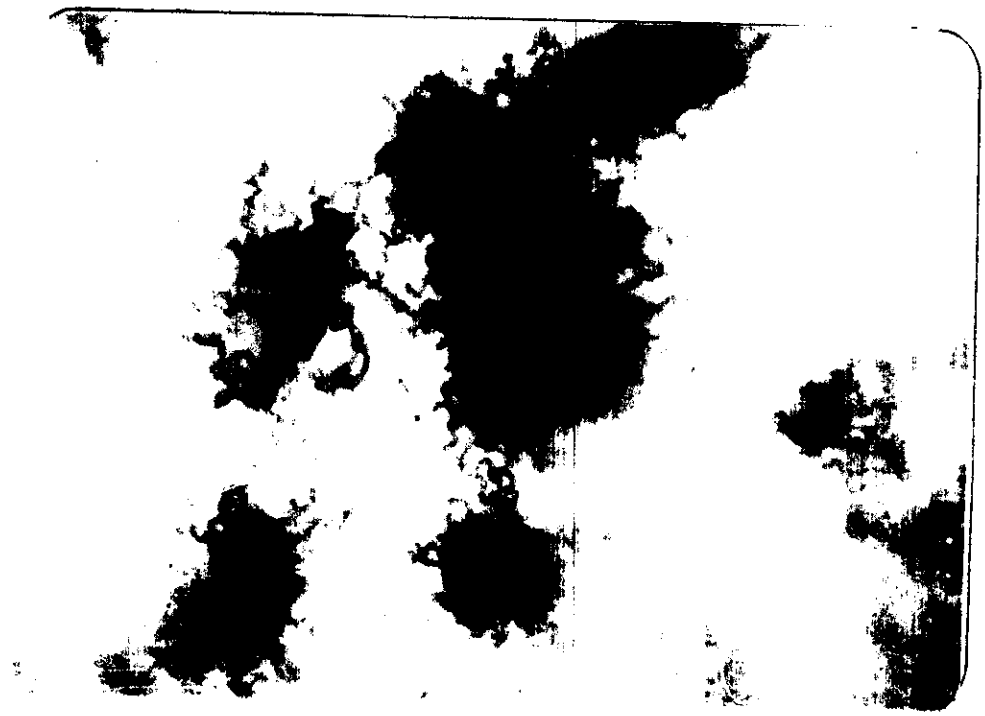

Fig. (52) : Micromorphology of aerial hypae of ***streptomyces*** (No. 23 X 400) .

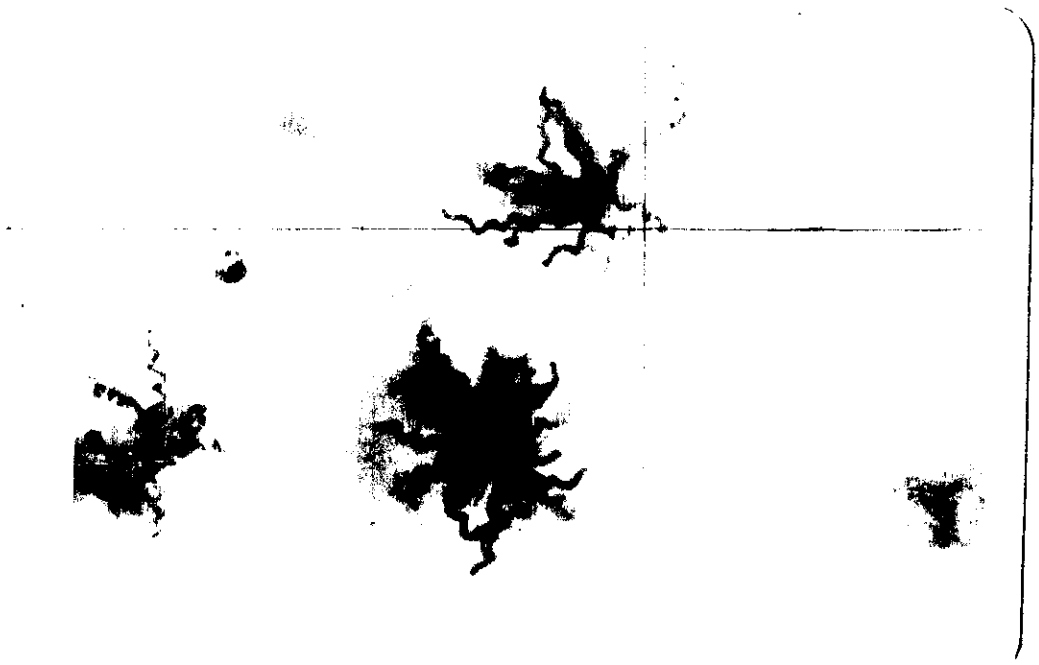

Fig. (53) : Micromorphology of aerial hypae of ***streptomyces*** (No. 24 X 400) .

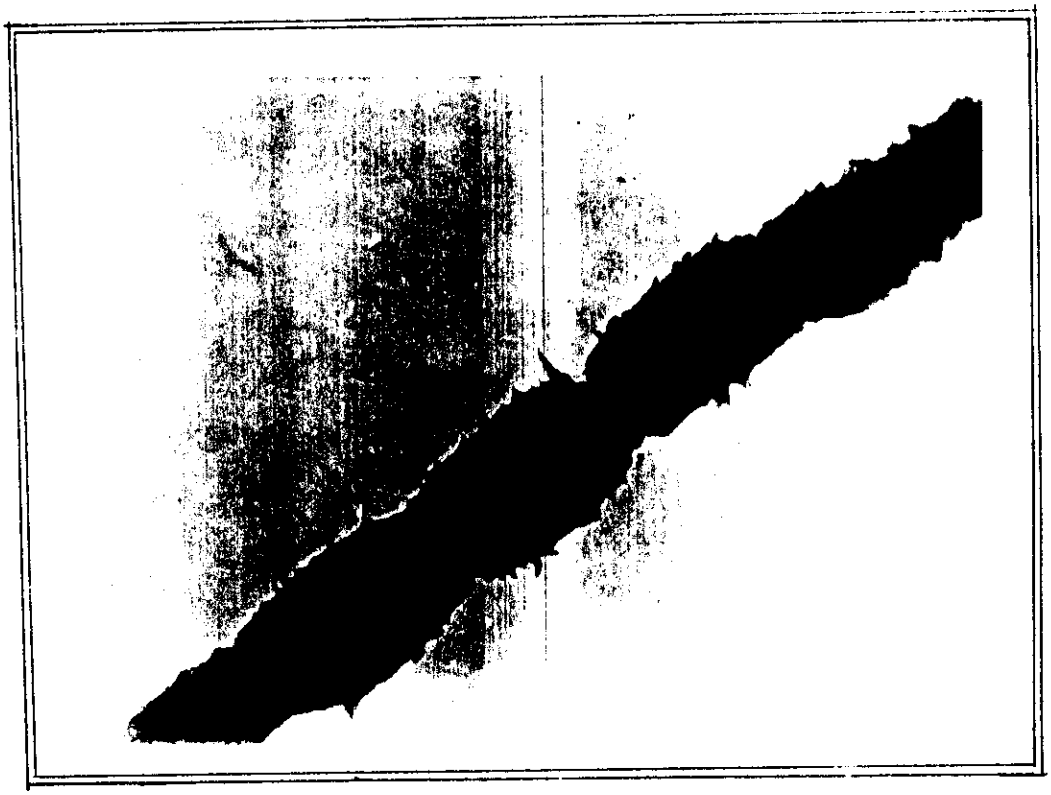

Fig. (54) : Electron micrograph of spores of ***streptomyces*** (No. 23 X 20,000).

Fig. (55) : Electron micrograph of spores of ***streptomyces*** (No. 24 X 20,000).

Fig. (56) : Micromorphology of aerial hypae of ***Actinopolyspora*** (No. 43 X 400) .

Fig. (57) : Micromorphology of aerial hypae of ***Actinopolyspora*** (No. 45 X 400) .

Fig. (58) : Electron micrograph of spores of ***Actinopolyspora*** (No. 43 X 20,000).

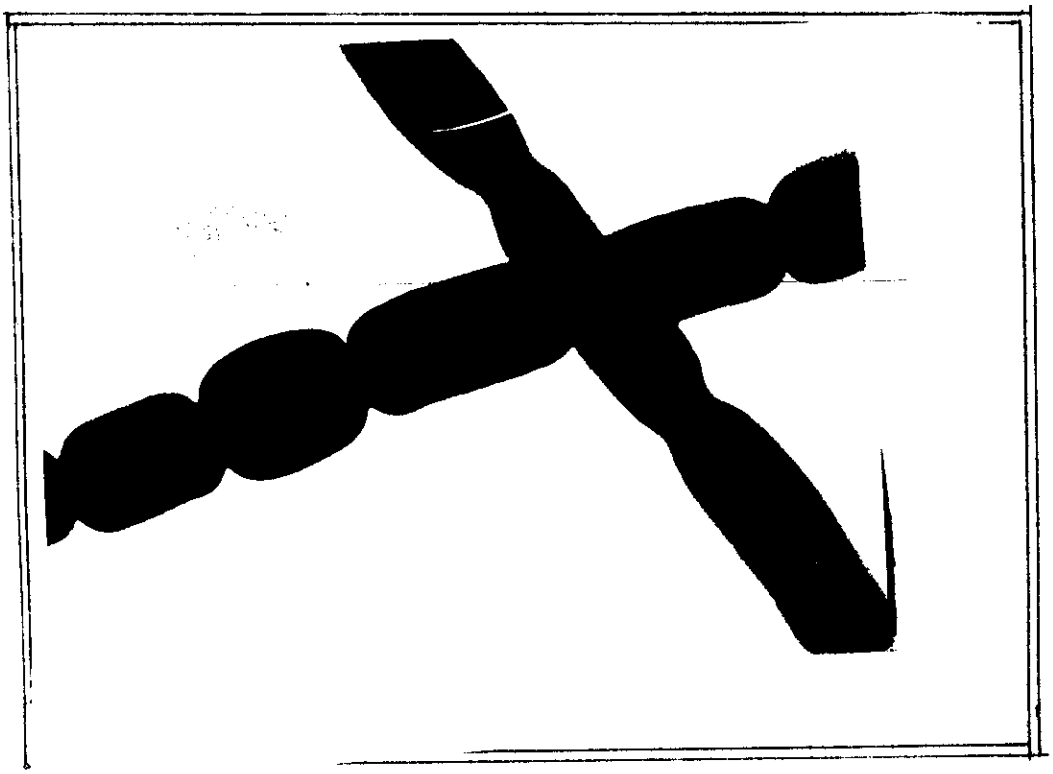

Fig. (59) : Electron micrograph of spores of ***Actinopolyspora*** (No. 45 X 20,000).

Biomass Production by the Used Actinomycetes

Representative isolates of the above mentioned actinomycetes were chosen for the study of the biomass production, when cultivated on black liquor, as follows:

Type A : ***Streptomyces*** No 5 and No 6 .

Type B : ***Streptomyces*** No 23.

Type C : ***Actinopolyspora*** No 45.

The data given in table (51) shows that ***Streptomyces*** No 23 Produced highest biomass when cultivated in black liquor salts shaken medium. This was followed by ***Actinopolyspora*** No 45 and ***Streptomyces*** No 6 then ***Streptomyces*** No 5 .

Table (51) Biomass production by chosen representative of the isolated actinomycetes , when cultivated on black liquor.

Name of actinomycete	Dry weight per 50 ml black liquor
Streptomyces No. 5	0.1648
Streptomyces No. 6	0.1709
Streptomyces No. 23	0.21134
Actinopolyspora No.45	0.1784

Single cell protein Production by the selected Actinomycetes

The data given in table (52) show that the percentage of total protein of the produced biomass was highest (12%) for ***Streptomyces*** No 5 and ***Streptomyces*** No 23, this was followed by ***Actionpolyspora*** No 45 then ***Streptomyces*** No 6.

Table (52) Single cell protein production by the selected actinomycetes.

Name of actinomycetes	Total protein % of dry weight
Streptomyces No. 5	12.0
Streptomyces No. 6	10.3
Streptomyces No. 23	12.0
Actinopolyspora No. 45	11.8

It can be concluded that ***Streptomyces*** No 23 is the best of the selected actinomycetes in the bioconversion of black liquor sugars into single cell protein as it is produces highest biomass and highest percentage of total protein.

Aflatoxin Production by the selected Actinomycetes

None of the selected actinomycetes produced aflatoxin. Accordingly they can be used safely for the bioconversion of black liquor hemicelluloses into single cell protein.