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Effect of Sugars Replacement on the Growth of *Aspergillus niger* and *Neurospora crassa* under Submerged Cultivation

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The genus *Aspergillus* and *Neurospora* are exceedingly useful fungal groups utilized in both solid and liquid waste management for maintaining the ecological balance. In fungal system it is very difficult to measure the growth due to their filamentous and non homogeneous nature. In the present comparative study we examine the cell growth in terms of cell dry weight (g/l) of *Aspergillus niger* and *Neurospora crassa*. Batch experiments were performed with taking 50 ml pure potato dextrose broth (PDB) and M_2 broth growth media alongwith replaced potato and M_2 broth media containing lactose, xylose, sucrose and maltose, separately rather than dextrose as carbon source, at 30^{0}C and 180 rpm. Samples were taken at every 6 hr intervals till 90 hr.The maximum cell dry weight attained by microbes in PDB, M_2 and replaced media were also investigated and evaluate the effect of various sugars in the growth of respective microbes.It has been observed that xylose, maltose and sucrose were found as consumable sugars as glucose by *Aspergillus niger* strain compared to lactose sugar, whereas strain of *Neurospora* consumes maltose and sucrose much easier like glucose compared to lactose and xylose. This kinetic study may be helpful in knowing the growth rate, growth characteristic and the growth pattern of individual microorganisms under different conditions for better industrial and environmental applications.

Key words: *Aspergillus, Neurospora,* Cell dry weight, Pelleted, Pulpy.

Aspergillus and *Neurospora* plays a vital and significant role in environmental applications. *Aspergillus* and *Neurospora* have also been used in a wide range of commercial enzyme productions, namely, cellulases, hemicellulases, proteases, and β-1,3-glucanase. The ability of fungi to secrete large amount of proteins and the ability to invade substrate has motivated their extensive use for the production of industrial enzymes[1]. *Aspergillus* sp are also known to be a good producers of β-glucosidase and widely used in the fermentation industry[2] *Neurospora crassa* (the common pink, red bread mould and mesophilic fungus) is a filamentous ascomycete and true cellulolytic producer, secrete high levels of all the three enzymes components involved in the cellulose degradation[3]. Enzymes produced by filamentous fungus have a high industrial and environmental interest. Therefore it is necessary to study the growth and morphological pattern of these crucial microbes. Objective of the present paper is to evaluate the growth rate and pattern of *Aspergillus* and *Neurospora* on PDB and M_2 medium respectively and also to investigate the effect of various sugars such as lactose, sucrose, maltose

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and xylose, used as carbon source rather than dextrose in the potato and M_2 broth based cultivation media on the growth of respective microbes.

Background information

In the present section we are trying to point out the fungal growth as well as role of various sugars utilized in the experimental process. Owing to the filamentous and non homogenous nature of the moulds growth, the analysis of the growth characteristic and the growth curve is very difficult. Although determining the rate of colony extension will provide us, measure of fungal growth but it will not necessarily be equivalent to the increase in the biomass of the fungus because hyphae will be growing down through the agar medium as well as across its surface[4]. Due to all these complications, it is better to estimate fungal growth (cell biomass) in terms of cell dry weight. The fungal morphological forms varies from mycelial pulpy to pelleted structure.As the literature reported that filamentous growth characteristic creates a number of process engineering problems attributed to the morphological change accounted during the fermentation process in large scales. Excessive hydrodynamic shear stresses are known to damage mycelial hyphae and form pellets. In such fermentations, the mass transfer of oxygen and nutrients is considerably better and the subsequent separation of the pellets from the medium is simpler. There are variety of sugars have been incorporated in the pure cultivation media such as maltose, sucrose, lactose and xylose. Maltose or malt sugar is a disaccharide formed from two units of glucose jointed with α^{1-4} linkage. The addition of another glucose unit yields maltotriose further additions will produce dextrins and eventually starch. Maltose can be broken down into two glucose molecules by hydrolysis. Plant maltose has a sweet taste, about half as sweet as glucose and 1/6 as sweet as fructose[5]. Sucrose is also one of the vital sugar sometimes called sacchrose. It is a disaccharides derived from glucose and fructose. It is a non reducing sugar since it not contains anomeric hydroxyl group[6] Whereas lactose consists of galactose and glucose fragments bonded through β-1-4 glycosidic linkage. The glucose fragment can be either in the α or β-pyranose form, while the galactose fragment can only have the β-pyranose form[7]. Xylose($C_5H_{10}O_5$) is an aldopentose type of monosaccharide containing five carbon atoms and including an aldehyde forming group. It is also a reducing sugar with free carbonyl group[5].

MATERIAL AND METHODS

Aspergillus niger NCIM 777 and *Neurospora crassa* NCIM 1021 strains were procured from National Chemical Laboratory (NCL),Pune. Fungal spores from a stock, kept at 4°C in 20% (v/v) glycerol. *Aspergillus* and *Neurospora* cultures were grown on potato dextrose agar (PDA) slant and M_2 agar slant at 28° C for 4 days respectively. Slants were maintained at 4°C and subcultured about monthly intervals. For the growth and morphological study, two separate set of experiments have been performed with each culture.

First set of experiments have been carried out in a 250 ml Erlenmeyer flasks containing 150 ml of Potato Dextrose broth (PDB) (In g/l Peeled Potato,200;Dextrose,20; and Yeast extract,0.1) and M_2 medium in which 5 loopfull cultures of fungal spores or mycelial conidia were transferred and shaken at 180 rpm at 30° C in an incubator shaker for 4 days[8]. While other set of batch experiments were performed in 250 ml Erlenmeyer flasks (total 16 flasks for each sugar and each organism) containing 50 ml of pure potato dextrose based (PDB) and M_2 based media separately while in other flasks sugar replaced potato broth and M_2 broth media have been taken, containing lactose, xylose, sucrose and maltose separately used as carbon source rather than dextrose by *Aspergillus* and *Neurospora* respectively at 30°C and 180 rpm. A definite volume of freshly prepared respective cultures, maintaining 0.56 g/l cell dry weight were added to each flask .Samples (As whole flask containing 50 ml, due to nonuniform nature of growth in fungal system) were taken at every 6 hr intervals till 90 hr.

Samples of 50 ml were filtered on a dried and preweighted whatman no -1 filter paper and washed thoroughly with cold distilled water and 50 ml of 0.9% NaCl (saline) Solution. The filter with mycelium and pellets were than dried for 24 h at 105^0 C and weighted .The determination of growth by dry weight were expressed as the mean of 3 independent readings.

RESULTS AND DISCUSSION

For the morphological and the growth kinetic studies separate sets of batch experiments have been performed at 30°C and 180 rpm. *Aspergillus niger* grown in pellatized form of morphology whereas *Neurospora* showed thick mycelial pulpy nature of growth. In submerged culture, a large number of factors contribute to the development of any particular morphological form[9]. As the literature reported that the filamentous growth characteristic creates a number of process engineering problems attributed to the morphological change accounted during the fermentation process in large scales[10]. Due to the thick and pulpy nature of growth morphology organisms suffers mass, heat and O_2 transfer limitation which might be affects the overall productivity of the product produced by fermentation using *Neurospora crassa* ,whereas in pelletized forms heat transfer and mass transfer of nutrients and oxygen is considerably better than other morphological forms[11]. It is evident from Table and Fig. 1, that lag phase observed upto 6 hr in most of the sugars containing cultivation media by *Aspergillus niger* as has been closely reported by Favela-Torres *et al.*, and Bizukojc and Ledakowicz[12,13]. As in the lag phase microbes acclimate to food and nutrients in their new habitat. As the time proceed after 6 hr, exponential phase has been started ,which was almost similar in the glucose ,maltose, lactose and sucrose based potato broth medium although *Aspergillus* growth was faster with higher biomass in case of xylose based culture media. As in this phase substrate conversion and cell mass reached to their maximum values. After 36 hr, steep increment in the growth have been observed till 42 hr, in case of sucrose and xylose based culture media as observed from Table 1. The maximum cell dry weight(g/l) attained by *Aspergillus niger* was 11.26, 11.93, 10.58, 10.36, 9.34 at 78, 42, 54, 42 and 48 hr in glucose, xylose, maltose, sucrose and lactose based culture media respectively, which indicates that growth rate was much faster in xylose, maltose and sucrose based media with respect to glucose and lactose based culture media as shown by Fig 1. Margaris *et al.*,[14] have investigated the *Aspergillus* growth under different carbon source and observed that maltose supported growth substantially more than sucrose as observed by Table 1. The maximum growth has been observed in case of glucose and xylose based

Table 1. Biomass in terms of cell dry weight (g/l) of *Aspergillus niger* and *Neurospora crassa* in PDB and M_2 media alongwith replaced sugars containing potato broth and M_2 broth media respectively

Time	Biomass in terms of cell dry weight(g/l)									
(hr)	Glucose based		Xylose based		Maltose based		Sucrose based		Lactose based	
	A.niger	*N.crassa*	*A.niger*	*N.crassa*	*A.niger*	*N.crassa*	*A.niger*	*N.crassa*	*A.niger*	*N.crassa*
0	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
6	0.87	0.66	0.85	0.58	0.75	0.62	0.72	0.60	0.60	0.58
12	1.68	4.34	2.00	1.02	1.36	4.20	1.30	4.00	1.13	2.32
18	2.02	5.82	3.46	2.10	1.96	5.22	1.93	5.66	1.90	3.86
24	2.52	7.04	4.32	3.12	2.26	6.63	2.60	6.96	2.12	4.00
30	3.86	7.12	5.10	3.26	3.78	6.98	4.21	7.82	3.42	4.13
36	5.12	7.42	7.36	3.28	5.00	7.02	5.98	8.24	4.89	4.45
42	6.64	7.66	11.93	3.16	6.50	7.10	10.36	8.20	6.02	4.80
48	7.78	8.16	11.80	2.98	8.60	7.90	10.20	8.02	9.34	5.00
54	8.50	8.86	11.68	2.93	10.58	8.11	10.00	7.98	9.00	5.25
60	8.94	8.42	11.20	2.83	10.46	8.02	9.96	7.63	8.98	5.20
66	9.60	8.16	11.02	2.76	10.31	7.98	9.88	7.34	8.76	5.02
72	9.94	7.02	10.90	2.59	10.02	7.33	9.72	6.98	8.70	4.98
78	11.26	6.92	10.60	2.53	9.98	6.83	9.42	6.75	8.10	4.93
84	10.91	6.76	10.23	2.41	9.62	6.59	9.30	6.32	8.03	4.83
90	9.42	6.55	9.25	2.40	9.32	6.43	9.21	6.20	7.98	4.80

media. Prathumpai *et al.*, [15] have reported that the activities of the key enzymes for xylose metabolism increased only when the effects of glucose repression had been relieved.Chiang *et al.*,[16] also stated that *A.niger* preferentially utilized D-xylose in the mixture of xylose and xylulose,which proves the easier uptake of xylose sugars by this microbes. There was no significant increment in cell biomass have been observed after 42 hr, in xylose and sucrose based culture media, probably due to the depletion of nutrients and carbon source in the media therefore booming growth stop whereas in case of lactose, maltose and glucose based culture media this phase has been started 48,54 and 72 hr respectively, as has been observed by Table 1. After that cell growth was moves towards stationary and death phase .As in death phase toxic waste products build up, food is depleted and the microbes begin to die. When compared the growth of *Aspergillus* under various sugars containing growth media than we observed that in xylose, maltose and sucrose based media growth rate was faster and closely resemble to growth in glucose containing media which indicates that this strain of *Aspergillus* having much faster xylose, maltose and sucrose exhausting capacity, as almost similar to glucose whereas in case of *Neurospora* distinctive situation has been observed, as shown by Fig 2. The stable lag phase has been observed in *Neurospora crassa* which indicates that this microbes takes much more time for reaching their active state .Afterward a sharp inclination has been seen in sucrose, glucose and maltose based M_2 media whereas much lesser growth observed in xylose based culture media. Exponential phase was observed upto 36 hr in maltose and sucrose based

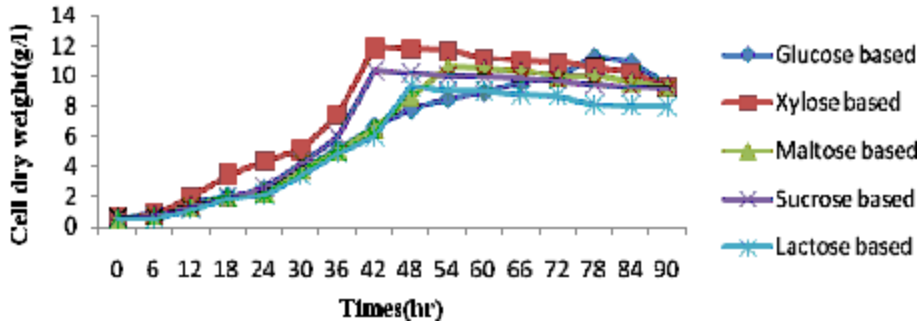

Fig. 1. Batch growth curve of *Aspergillus niger* in glucose, xylose, maltose, sucrose and lactose containing potato broth medium at 180 rpm and 30°C.

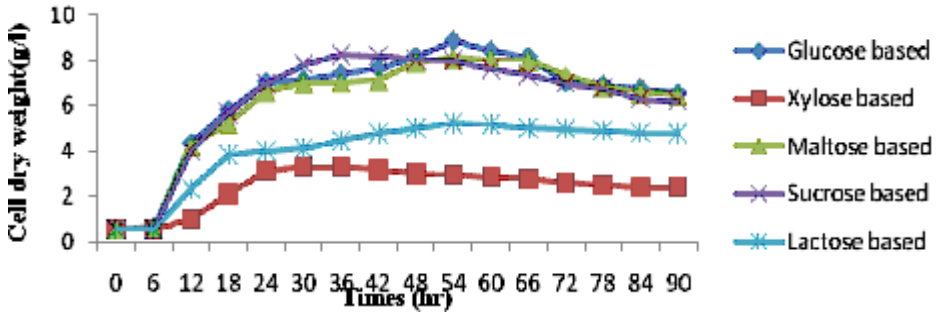

Fig. 2. Batch growth curve of *Neurospora crassa* in glucose, xylose, maltose, sucrose and lactose containing M_2 broth medium at 180 rpm and 30°C

culture media whereas in glucose based media this was observed till 54 hr. On the other hand in lactose and xylose based culture media exponential phase was much smaller with lesser growth. The maximum cell dry weight(g/l) attained by *Neurospora crassa* was 8.86,3.28,8.11,8.24 and 5.25 at 54,36,54,36 and 54 hr in glucose, xylose, maltose, sucrose and lactose based culture media respectively, which indicates that growth rate was much faster in glucose, maltose, sucrose based culture media with respect to xylose and lactose based culture media as shown by Fig 2. After 36 hr in xylose and sucrose based culture media no significant increment in the growth has been observed probably due to the depletion of nutrients and carbon source in the media whereas such condition has been observed, after 54 hr in glucose, maltose and lactose based culture media. After that cell growth has been shifted towards stationary and death phase. It has been observed from Fig 2. that growth rate of *Neurospora crassa* was much faster in maltose and sucrose based culture media which closely resemble to growth in glucose based culture media hence we can say that sugar uptake capacity was much faster in *Neurospora crassa* for maltose, sucrose and glucose in comparison to lactose and xylose.

CONCLUSION

The morphological pattern and growth kinetic studies of *Aspergillus* and *Neurospora* in their respective cultures medium, might be helpful in knowing the growth patterns of microbes which ultimately beneficial for their mass scale production. The information about the microbial growth rate and maximum growth in terms of cell dry weight, gives the detailed knowledge about the nature of microbial growth which ultimately beneficial for the industrial and environmental application of these industrial strains. It can also be concluded that microbes have distinctive sugar uptake capacity for various sugars used as a carbon source for microbial growth.

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