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Effect of glucose and type of inoculum on biodegradation of phenol

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Adaptation of *Mycoplana dimorpha* on glucose, phenol and phenol/glucose binary substrate significantly affects the values of specific growth rate, lag time etc. The preference of substrate depends upon the type of inoculum. Phenol adapted cultures prefer phenol, glucose adapted cultures prefer glucose whereas, those adapted on the binary substrate prefer phenol and glucose equally. Diauxic growth was not observed in any experiments.

Effects of conventional organic substrates on biodegradation of toxic waste components are of great practical importance[1] due to the fact that biological wastewater treatment facilities deal with multicomponent wastes. In case of microbial degradation of phenol, the specific growth rate of organism increases with the increase in phenol concentration to a maximum growth rate and for any further increase of phenol concentration, there is a sharp decline in specific growth rate. This is indicative of growth inhibited by substrate. This behaviour is in sharp contrast to the non-inhibitory substrate. Specific growth rate increases with substrate concentration and aproaches asymptotically to a maximum specific growth rate. Because of these radically different growth-rate characteristics, it is essential to determine, if some form of substrate interaction could be expected when both inhibitory and non-inhibitory carbon sources are concurrently available to microbial populations.

The interaction of non-inhibitory substrate with inhibitory substrate was investigated by several workers with pure as well as mixed cultures. *Pseudomonas fluorescens*[2,3], *Pseudomonas putida* A3.12[4], *Cellulomonas* species[5], four strains of *Pseudomonas putida* A3.12, IP 6323, ASI and P3[6] used a heterogeneous population[1,7] and *Pseudomonas putida*[8], showed that the oxidation of aromatic substances is accelerated in presence of glucose. This effect is apparently due to the enhanced induction of corresponding catabolic enzymes for synthesis. During the induction phase, glucose was primarily metabolized to ribose-5-phosphate and synthesize RNA[3]. These authors demonstrated that bacterial strain did not incorporate C^{14} from glucose into nucleic acids when grown in presence of protocatechuate and they further suggested that glucose was eventually catabolized via the pentose-phosphate pathway.

Heterogeneous population[1] of microorganisms adapted to two different conditions: one was adapted to phenol as the sole carbon source while another adapted to a mixture of glucose and phenol and reported that the utilization pattern of substrate was significantly influenced by the adaptation characteristics of the culture. The phenol adapted organisms showed an initial preference for phenol but the presence of glucose hindered the phenol utilization rate. The microbes adapted to the mixture of glucose and phenol demonstrated rapid initial glucose utilization with a slower utilization of phenol. This indicated that adaptation condition influences the utilization pattern of substrates. No diauxic growth was also observed.[1,6]

Experimental Procedure

Microorganism—Mycoplana dimorpha NCIM 2383 was used in all studies. The culture was obtained from National Collection of Industrial Microorganism, NCL, Pune, India.

Culture medium—The salt medium was used of following constituents. It consisted of (mgL^{-1}): ammonium sulphate 500, potassium phosphate monobasic 500, potassium phosphate dibasic 1000, magnesium sulphate 100, calcium sulphate 30,

sodium chloride 50, ferric chloride 0.50, supplemented with glucose, phenol or both as needed as carbon source. This medium was used for inoculum preparation and for studies of effect of glucose on phenol degradation.

Adaptation procedure—10 mL of cell suspension grown for 8 h in nutrient broth medium was transferred in 500 mL Erlenmeyer flask containing 80 mL of salt medium. The concentration of glucose, phenol or both in the medium was 100 mgL^{-1}. 10 mL of this broth was used as inoculum for further experiment. Other sets of experiments were carried out in the same fashion by varying the glucose, phenol concentration with gradual increase. This process was repeated upto 500 mgL^{-1} of phenol and glucose concentration in medium. The *p*H of medium was maintained at 7.0±0.50°C and temperature of shaker was maintained at 37 ± 0.5°C during the period of adaptation. The agitation speed of shaker was set at 250 revolution per minute.

Analytical methods—Optical density of cell mass was estimated by Jasco spectrophotometer at 640 nm wave length. Residual reducing sugar in broth was measured by Dinitrosalicylic acid method (DNS)[9]. Residual phenol concentration in broth was estimated directly by Jasco spectrophotometer at 268 nm wave length.

Experiments were carried out to study various substrate interactions that could occur when glucose is available as a more preferential carbon source to three differently adapted population of *Mycoplana dimorpha*. In Type 1, cells were adapted to phenol as the sole source of carbon and energy; in Type 2, cells were adapted to glucose as sole carbon and energy source and in Type 3, they were adapted to a binary mixture of phenol/glucose substrate in salt medium. These three types of inoculum were used to study specific growth rate, phenol consumption and lag time, etc. Constant environmental conditions of temperature (40±1°C) and *p*H (6.8±0.2) were maintained in all the experiments. Substrate used was either phenol, glucose or a mixture of phenol/glucose. The samples were withdrawn at regular intervals of time and cell mass, residual phenol and glucose concentrations were determined. The data thus obtained are plotted in Figs. 1-5. From these figures, specific growth rate, length of lag phase,

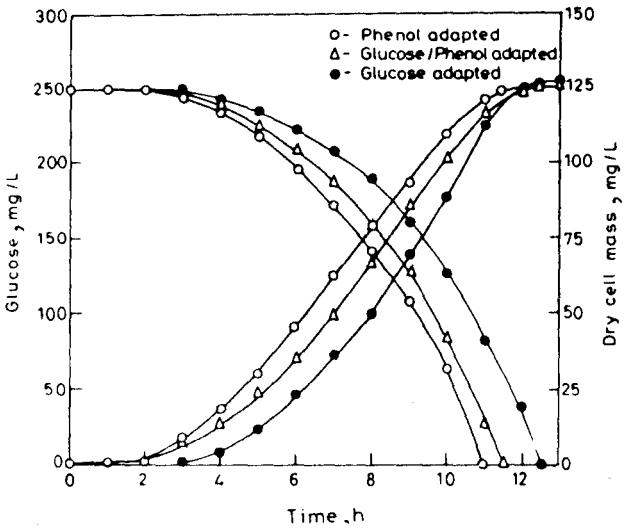

Fig. 1—Effect of adaptation on phenol consumption and the growth of *Mycoplana dimorpha*

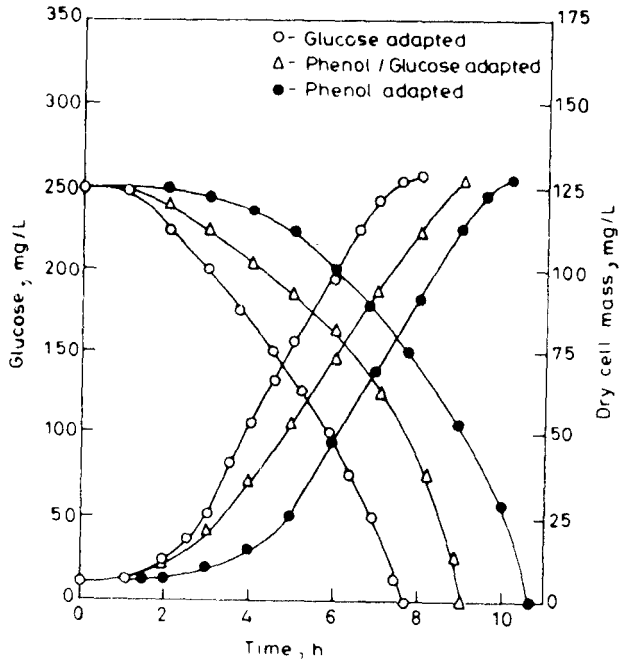

Fig. 2—Effect of adaptation on glucose consumption and the growth of *Mycoplana dimorpha*

overall consumption rates of phenol and glucose have been calculated and are shown in Table 1.

Results and Discussion

Phenol as the sole carbon source—Three sets of experiments with variation of the type of inoculum were carried out in shake flasks. In each set of experiments, inoculum level and phenol concentration were 10 mL and 250 mg/L, respectively. The results are plotted in Fig. 1. It is clear from the figure that the phenol adapted

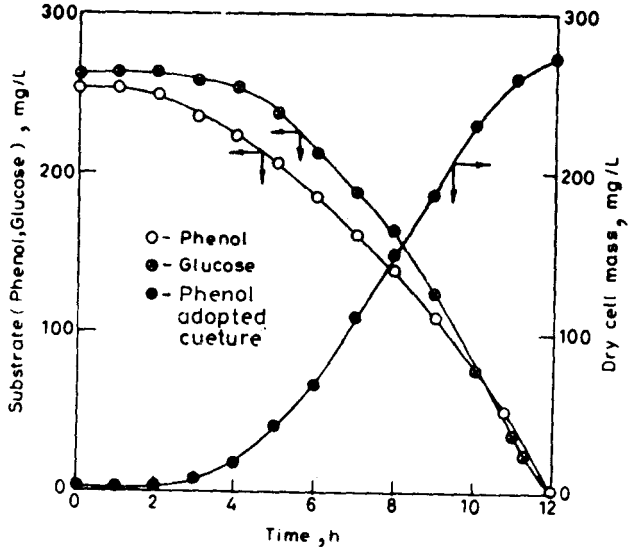

Fig. 3—Effect of phenol adaptation on the utilization of glucose and phenol

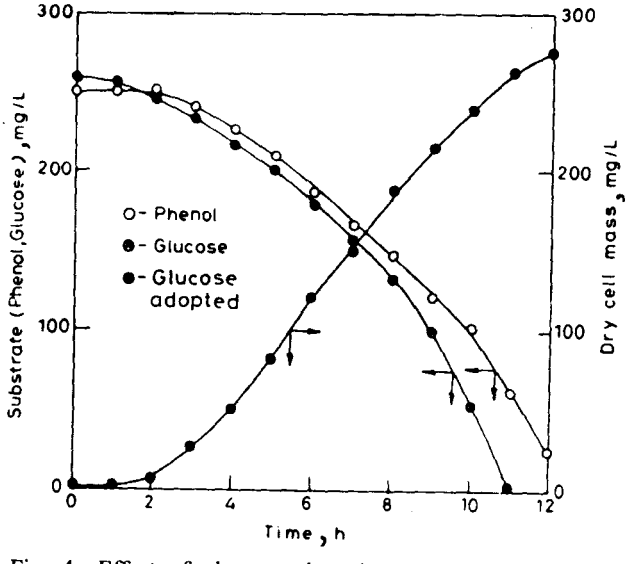

Fig. 4—Effect of glucose adaptation on the utilization of phenol and glucose

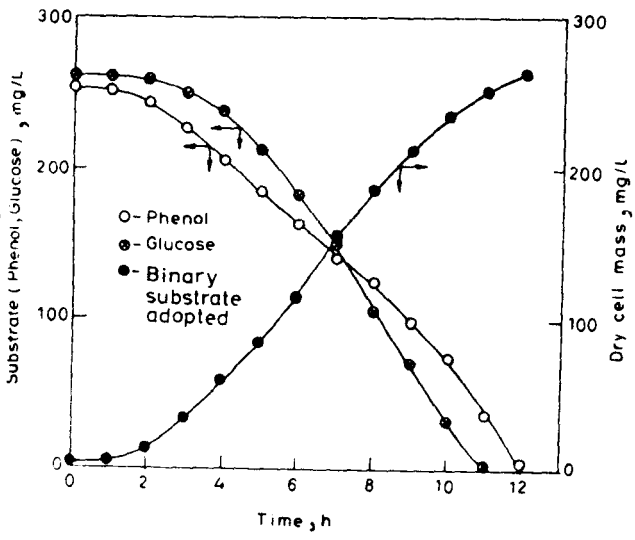

Fig. 5—Effect of binary substrate adaptation on the utilization of phenol and glucose

culture grows at a specific growth rate of 0.232 h^{-1} with lag time of about 2 h. Complete consumption of phenol is possible after 11 h of growth. The overall phenol consumption rate is approximately 22.73 mg/L h. The glucose adapted culture takes more time to grow in exponential phase and reaches its maximum cell mass concentration in about 12 h. The overall phenol consumption rate is 20 mg/L h. In the case of binary substrate adapted culture, the pattern of growth of microorganism remains same as in other cases, however, the lag phase lies in between those observed for the other two cases. Its specific growth rate is 0.229 h^{-1} and reaches to maximum cell concentration at 12 h of growth. This indicates that the cells adapted on either phenol or binary substrate are more active for phenol utilization than glucose adapted cells.

Glucose as the sole carbon source—Similar experiments were carried out with glucose in salt

Table 1—Effect of Adaptation on Biodegradation of Phenol, Glucose and Binary Substrate (Phenol/Glucose)

Substrate	Inoculum (Adapted)	Specific growth rate, μ, h^{-1}	Length of lag phase, h	Overall phenol consumption rate mg/L h	Overall glucose consumption rate mg/L h
Phenol	Phenol	0.232	2.0	22.73	—
	Glucose	0.224	4.5	20.00	—
	Phenol^{+} glucose	0.229	3.0	21.74	—
Glucose	Phenol	0.354	2.5	—	23.26
	Glucose	0.379	1.5	—	32.26
	Phenol^{+} glucose	0.359	1.5	—	27.18
Phenol/ glucose	Phenol	0.361	2.5	20.83	22.50
	Glucose	0.325	1.5	18.75	24.55
	Phenol/glucose	0.363	1.5	20.83	24.09

medium as the carbon source. The results obtained are shown in Fig. 2. It is clear from this figure, that length of lag phase remains almost same for all types of inoculum. The glucose adapted cells grow at a higher specific growth rate of 0.379 h^{-1} compared to other two types of inoculum (μ=0.354 and 0.359 h^{-1}). This indicates that the phenol adapted culture has a lower specific growth rate (0.354 h^{-1}) than the glucose adapted one. Therefore, it can be stated that phenol adaptation reduces the growth of organism. Results presented in Table 1 indicate that glucose is a more degradable substrate than phenol.

Phenol/Glucose Mixture as Carbon Source—In order to study the mixed substrate metabolism with three different types of inoculum, as described earlier, a mixture of phenol (250 mg/L) and glucose (270 mg/L) with double concentration of salts in salt medium were taken. The data obtained are shown in Figs. 3 to 4. From these figures it is clear that cells do not show diauxic behaviour of growth. Diauxic behaviour, wherein presence of two carbon source, microorganism utilize preferential substrate up to exhausted, then utilize second carbon source. They are not utilize both substrate at a time. The phenol adapted culture showed a preference for phenol and utilized glucose only after 2 h of growth. The specific growth rate of cells is estimated to be 0.361 h^{-1} and the consumption rates of phenol and glucose are almost the same. Results also indicate that the presence of glucose decelerates phenol consumption rate from 22.73 to 20.83 mg L^{-1} h^{-1}.

The results of studies on glucose adapted culture are shown in Fig. 5. It is clear from the figure that the cells adapted on glucose do not consume phenol at all during the first two hours of growth. However, after 2 h of growth, cells start utilizing phenol also. It is further observed that the phenol consumption rate is adversely affected and decreases to 18.75 mg L^{-1} h^{-1} from 22.73 mg L^{-1} h^{-1} when cells are adapted on phenol.

The data for binary substrate adapted cells are shown in Fig. 4. It is clear from this figure that cells do not show any preference towards glucose or phenol. The growth pattern remains the same as in both the earlier cases. However, overall phenol consumption rate is slightly more compared to that of glucose adapted cells but lower than phenol adapted cells.Diauxic growth was not observed in any of the experiments, where binary substrate (phenol/glucose) was used. This observation is similar to that of Reber and Kaiser[6] and Rozich and Colvin[1]. This behaviour of growth may be due to different pathways of degradation of phenol and glucose utilized by the microorganism. Kirkland and Durham[3] have suggested that glucose is catabolized via the pentose-phosphate pathway and also that carbon of nucleic acid is incorporated from aromatic substrate rather than from glucose.

Rozich and Colvin[1] had observed that the presence of glucose enhanced phenol consumption rate which is not in conformity with the results of the present study which indicate that the presence of glucose slightly reduced the phenol consumption rate. The difference in observations may be because of the fact that Rozich and Calvin[1] used a heterogeneous population, which could not clearly explain the cause of enhancement in phenol consumption. Whereas in the present study, the specific growth rate of a pure culture *Mycoplana dimorpha* was observed. The observation of Rozic and Colvin[1] is also limited by the type of inoculum. They used only phenol and phenol/glucose adapted cultures.

Above results clearly indicate that adaptation significantly affects the specific growth rate, length of lag phase, overall consumption rates of phenol and glucose. The preference of substrate depends upon the type of inoculum. This observation is similar to that of Rozich and Colvin[1]. Phenol adapted cultures prefer phenol, glucose adapted cultures prefer glucose whereas, those adapted on the binary substrate prefer phenol and glucose equally. Longest duration of lag phase was observed with glucose adapted culture inoculated in a phenol substrate medium. It is primarily due to the previous history of the cells.

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