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Adsorption of a mutant strain of *Pseudomonas pictorum* on rice bran based activated carbon

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Abstract

Microbial cultures immobilised on various matrices are used to protect the microbes from confronting shock loads of concentrated organic pollutants during wastewater treatment. Activated carbon is generally used as carrier material in comparison to other matrices. In this study a mutant strain of *Pseudomonas pictorum* (MU 174) was immobilised on rice bran based activated carbon. The effect of contact time, pH, mass of activated carbon, temperature and ionic strenth on adsorption of MU 174 on to activated carbon has been investigated. The optimum temperature and pH for adsorption of MU 174 on to activated carbon were 31 °C and 7, respectively. The adsorption kinetic parameters K_p, K_{ad} and ΔH have been determined. The rate constant values and the intraparticle diffusion of MU 174 on to activated carbon obtained were 5×10^{-3}–17.5×10^{-3} min^{-1} and 0.75–2.9 $min^{0.5}$, respectively, and the enthalpy of adsorption was observed to be −47.9 kJ/Avogadro number of cells.

Keywords: Rice bran based activated carbon; Mutant strain of *P. pictorum*; Adsorption isotherm; Intraparticle diffusion

1. Introduction

In secondary biological wastewater treatment the on-site application of activated carbon is a known process [1, 2] for improving the removal efficiency of dissolved organics. The dissolved pollutants in wastewater adsorb on the surface of activated carbon and subsequently undergo oxidation under aerobic conditions [3]. In certain instances the metabolites resulting during biological processes also adsorb on carbon and they are removed from the wastewater during secondary settling [4]. However, shock load application of pollutants upsets the performance of suspended microbial culture in the wastewater treatment unit [5]. Thus, wastewater having

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inconsistent pollution load has to be treated by protecting microbes from exposure to shock load [6]. Immobilisation of microbial culture in certain porous supports reduces the possibility of the microbes to confront the shock load [7]. The dissolved organics in wastewater first adsorb on the surface and then gradually penetrate through the immobilising matrix [8]. Certain matrices like sodium alginate [9], polyacryl amide hydrazide gel [10], chitosan [11], activated carbon [12, 13], sintered glass [14] have been reported in the literature for immobilisation of microbes. In this study activated carbon obtained from rice bran [15] was used for the immobilisation of the mutant strain of *Pseudomonas pictorum* (MU 174) for its high bulk density and quick settling that are considered to be effective characteristics of carbon used in wastewater treatment.

2. Experimental procedure

The characteristics of activated carbon obtained from rice bran are given in Table 1. Adsorption of MU 174 on to activated carbon was studied in the bulk concentration range 5.3×10^4–17.5×10^4 cells/ml of nutrient medium. The composition of the nutrient medium selected was according to that of Chitra et al. [16]. The pH values of the nutrient medium used in this study were in the range 6–9.

In each of the two dry, well-stoppered bottles, 50 ml of this cell suspension was taken. About 0.1 g of accurately weighed rice bran based activated carbon (dried over H_2SO_4) was added in the bottle (sample) while the other bottle (reference) containing cell suspension without activated carbon served as control. A magnetic stirrer was placed inside the bottles under study and it was gently stirred. In batch adsorption studies the temperature of the incubator was set at 28–34 °C. The stirrer was then removed and the solution was filtered through a stainless steel wire gauze. The optical density of the filtrate was determined spectrophotometrically.

From the known concentrations of cells before and after adsorption by 1 g of activated carbon, the number of MU 174 cells adsorbed per square metre of the solid surface was calculated.

3. Results and discussion

3.1. *Effect of contact time*

The effect of contact time on immobilisation of MU 174 is shown in Fig. 1. The results plotted in the figure show that the adsorption increased with contact time and it attained equilibrium after 4 h irrespective of the bulk concentration of the cell suspension. The number of cells remaining under equilibrium increases with initial microbial concentration, temperature and ionic strength. The curve shown in Fig. 1 presents a double nature. The initial portion of the curve rises linearly and is changed into a curve and levels off after 4 h of contact time. The plateau portion of the curve corresponds to pore diffusion and the linear portion of the curve reflects

Table 1
Characteristics of activated carbon

Elemental analysis	C	48.45%
	H	0.70%
	N	0.10%
	Ash	50.75%
Bulk density		0.69 g/m^3
Specific surface area		218 m^2/g

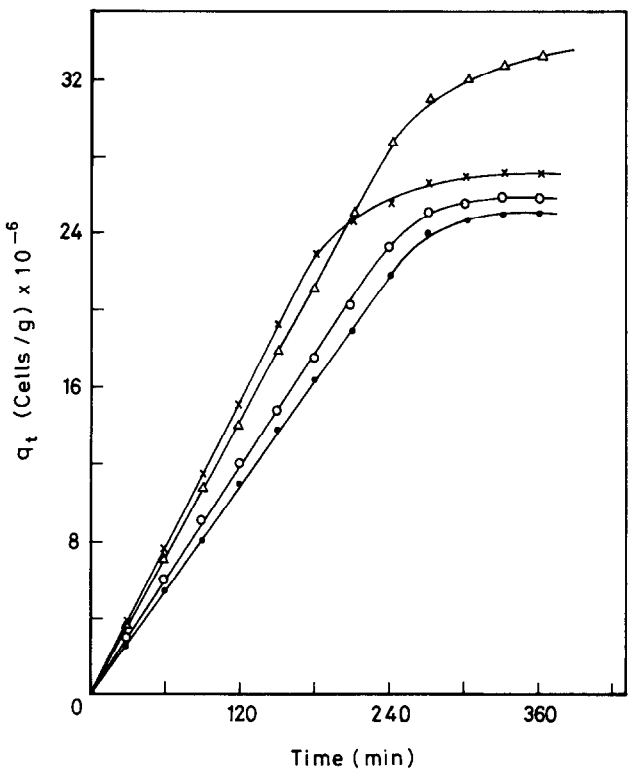

Fig. 1. Effect of contact time on adsorption of MU 174 on to rice bran based activated carbon at different concentrations. pH = 7, temperature = 31 °C, (O) 5.3×10^4, (×) 10.3×10^4, (△) 12.8×10^4, (1) 18.3×10^4 (cells/ml).

surface layer diffusion [17]. The amount of cell removal from the bulk solution increased with concentration in a given time. The concentration of cell suspension is a driving force [18] for cell removal from bulk solution up to a certain critical concentration and beyond this level cell removal tendency decreased. At high concentrations, i.e. 17.5×10^4 cells/ml, the microbial cells may cluster together to form floc like masses which could slide off under the influence of gravity leading to poor adsorption of cells.

The diameter of *Pseudomonas* cell is 5×10^{-7} m. The area covered by individual cell is (1/2 of the spherical surface area) 3.1×10^{-13} m^2. Calculations showed that surface area per unit g of the activated carbon can be completely covered by 10^6 cells. However, it has been observed that only 10^{14} cells were removed under existing experimental conditions. It is reasonable to assume that a certain proportion of the surface area is covered by the adsorbed cells leading to a net decrease in the net effective area available for the microbial cells to adsorb. It can be shown that each microbial cell covers an area of 6×10^{-13} m^2.

3.2. Rate constant study

The rate constant for the adsorption of microbial cells on to activated carbon has been calculated using lagergran rate equation [19].

$$\log (q_e - q_t) = \log q_e - K_{ad} t / 2.303. \quad (1)$$

The straight line plots of the $\log(q_e - q_t)$ versus time (Fig. 2) at different temperatures show the applicability of the equation. The rate constants for the surface adsorption of cells at different temperatures have been calculated from the slope of the plot $\log(q_e - q_t)$ versus time. The plot of k_{ad} versus the initial concentration of cell suspension gave a straight line form on which the following relationship can be derived:

$$K_{ad} = x C_0^y. \quad (2)$$

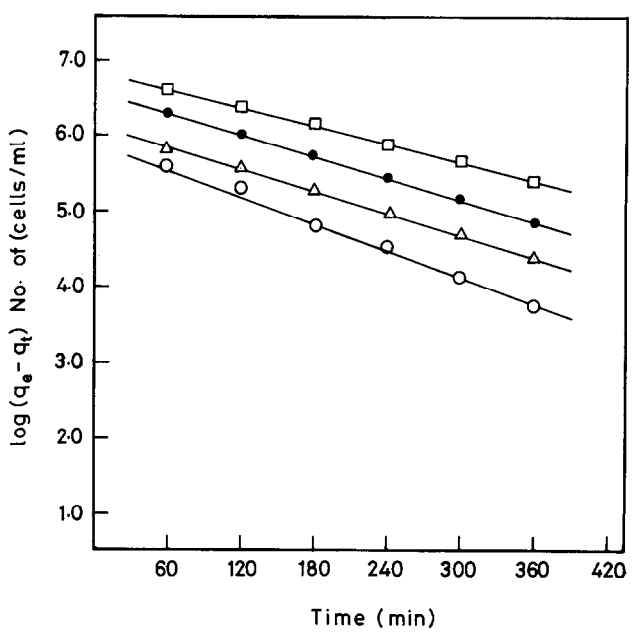

Fig. 2. $\log(q_e - q_t)$ versus time for the adsorption of MU 174 on to rice bran based activated carbon at different temperatures. pH = 7, cell concentration = 17.5×10^4. (O) 28 °C, (△) 31 °C, (1) 34 °C, (□) 37 °C.

The data listed in Table 2 demonstrated that the values obtained from the graphical method and the computational method are in close agreement.

3.3. Intraparticle diffusion

In addition to the adsorption of micro-organisms on the surface of the carbon they diffuse into the pores of the activated carbon and attach on the walls of the pores. This intraparticle diffusion is influenced by particle layer thickness [20], which, in turn, is controlled by concentration of the bulk solution. The particle layer thickness and intraparticle diffusion rate constants have been determined from the plot of amount of cell adsorbed (q_t) versus $t^{0.5}$. The particle diffusion rate constants at different temperatures are given in Table 3. It can be determined from the figure that the surface adsorption is 22.5×10^5 cells/g and the adsorption due to pore diffusion is 1.11×10^5 cells/g, i.e the ratio of adsorption due to pore diffusion and surface adsorption is 0.049. This is in close agreement with the proportion of pore volume to bulk volume of the activated carbon (0.0431). The pores in the activated carbon are classified into macropore, micropore and transitional pore in the decreased order of pore diameter [21]. The small proportion of pore volume indicates the presence of only micropores or transitional pores in the activated carbon under this study. These micropores can contain the microbes more strongly than that can be held in macropores. In micropores the microbes develop cohesion between

Table 2
Rate constant parameters at different concentrations for the adsorption of MU 174 on to activated carbon

Sl. no.	Temperature (°C)	Concentration (cells/ml)	K_{ads} (min^{-1})	
			Graphical method	Computational method
1.	28	4.9×10^4	0.0114	0.0118
2.		10.4×10^4	0.0123	0.0122
3.		13.6×10^4	0.0168	0.0166
4.		19.8×10^4	0.0173	0.0170
5.	31	5.3×10^4	0.0069	0.0068
6.		10.3×10^4	0.0073	0.0071
7.		12.8×10^4	0.0076	0.0073
8.		18.3×10^4	0.0083	0.0085
9.	34	5.1×10^4	0.0055	0.0054
10.		10.0×10^4	0.0059	0.0058
11.		12.6×10^4	0.0070	0.0071
12.		17.7×10^4	0.0077	0.0078
13.	37	5.5×10^4	0.0053	0.0051
14.		10.2×10^4	0.0053	0.0053
15.		12.6×10^4	0.0069	0.0068
16.		17.6×10^4	0.0076	0.0078

pH = 7.0, glucose = 0.05%, sodium nitrate = 0.1%.

Table 3
Intraparticle diffusion rate constant parameter at different concentrations for the adsorption of MU 174 on to activated carbon

Sl. no.	Temperature (°C)	Concentration (cells/ml)	K_p ($min^{0.5}$)
1.	28	4.9×10^4	1.00
2.		10.4×10^4	2.00
3.		13.6×10^4	2.50
4.		19.8×10^4	2.86
5.	31	5.3×10^4	0.865
6.		10.3×10^4	1.750
7.		12.8×10^4	2.080
8.		18.3×10^4	2.38
9.	34	5.1×10^4	0.735
10.		10.0×10^4	1.458
11.		12.6×10^4	1.667
12.		17.7×10^4	2.190
13.	37	5.5×10^4	0.647
14.		10.2×10^4	1.250
15.		12.6×10^4	1.364
16.		17.6×10^4	1.810

pH = 7.0, glucose = 0.05%, sodium nitrate = 0.1%.

each other so that they are difficult to be dislodged from the pores. The microbes held in micropores resist mechanical attrition force of water [5]. This fact was confirmed by washing the carbon loaded with cells of MU 174 by water at a high flow rate of 5×10^{-2} l/min for 6 h. The wash water contained negligible concentration of 5×10^2 cells.

3.4. Adsorption isotherms

The study of adsorption isotherms is helpful in determining the maximum adsorption capacity of adsorbate for the given adsorbent [22]. The equilibrium data for the adsorption of microbes at different temperatures have been found to obey the rearranged Langmuir isotherm, i.e.

$$C_e/q_e = 1/Q_0 b + C_e/Q_0. \tag{3}$$

The applicability of the Langmuir isotherm for the present system, which indicates the formation of monolayer coverage of the adsorbate on the surface of the adsorbent have been verified from the plot of C_e/q_e versus C_e. Values of Q_0 and b have been calculated using graphical method at various temperatures and are presented in Table 4.

3.5. Effect of temperature

The effect of temperature on the adsorption of microbes on to activated carbon can be explained in accordance with adsorption isotherm findings. In Fig. 3 the

Table 4
Langmuir isotherm parameters for the adsorption of MU 174 on to activated carbon

Sl. no.	Q_0 (g/ml)	b (ml/cells)	Temperature (°C)
1.	80.00	0.833	28
2.	27.27	0.982	31
3.	28.75	1.265	34
4.	20.00	1.429	37

pH = 7, glucose = 0.05%, sodium nitrate = 0.1%

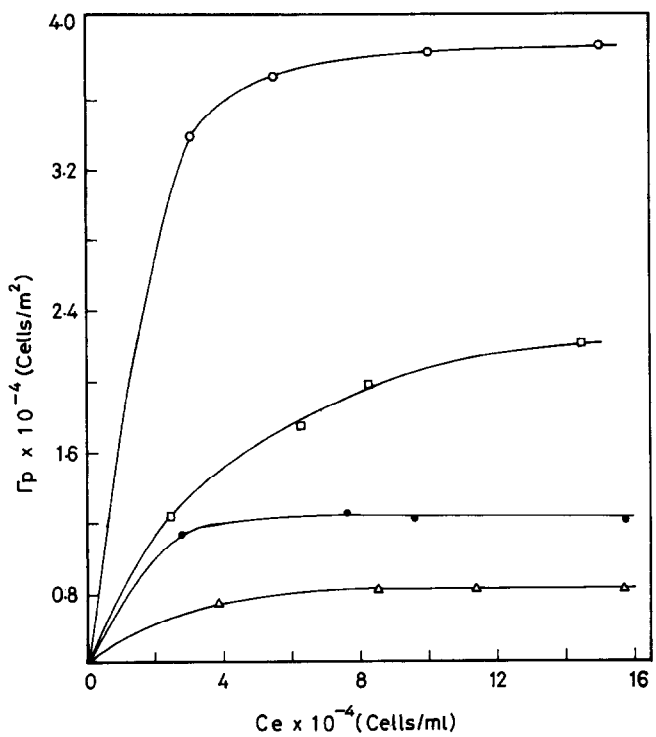

Fig. 3. Adsorption isotherm of MU 174 on to activated carbon at different temperatures. pH = 7, cell concentration = 17.5×10^4. (O) 28 °C, (1) 31 °C, (□) 34 °C, (△) 37 °C.

adsorption isotherms of MU 174 on to carbon at different temperatures have been compared with each other. The ionic strength and pH in all the experiments were fixed at pH 7.0. At a given value of concentration the intensity of adsorption was observed to decrease significantly with a 3–9 °C rise in temperature. This sort of result would be exhibited when the adsorption process is usually physical in nature. It is also apparent that Γ_p^m also decreases with an increase in temperature.

This indicates that biopolymers may orient, expand or contract laterally with an alteration in temperature [23]. It is clear that the adsorption capacity, Q_0, for uptake

of the microbes by carbon decreases on increasing the temperature. The net enthalpy of adsorption ΔH is related to the Langmuir constant b using the following equation:

$$b = b' e^{-\Delta H/RT}. \quad (4)$$

The value of ΔH has been calculated from the plot of $\log b$ versus $1/T$. The value of ΔH was found to be -47.91 kJ/N cells (Avogadro number = N), suggesting that the adsorption process is physical in nature [24].

3.6. Effect of pH

Fig. 4 shows the effect of pH values on the adsorption on to activated carbon. The results from the study of pH on adsorption indicated that maximum adsorption capacity occurred at pH 7.0 and the same was decreased on either side of pH 7.0. The adsorption of cells on the surface of the activated carbon is through

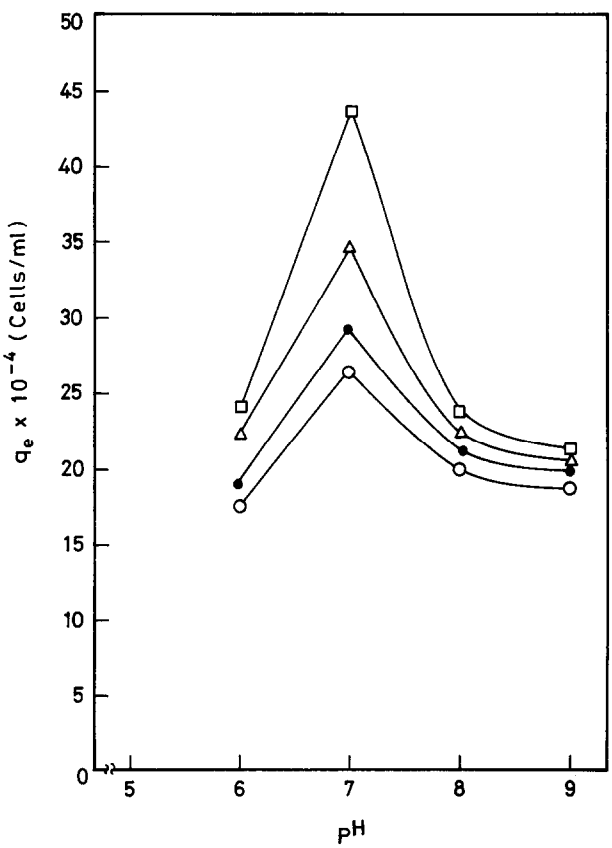

Fig. 4. q_e versus pH for the adsorption of MU 174 at different concentrations. Temperature = 31 °C. (O) 5.3×10^4, (1) 10.3×10^4, (□) 12.8×10^4, (△) 18.3×10^4 (cells/ml).

extracellular polymers which are monopolysaccharides in nature. The extracellular polymers exhibit Zwitter ionic characters that is highly influenced by the pH of the medium. At low and high pH values the polymers are positively charged. The adsorption was found to be very high at pH = 7.0 compared to other ranges of pH values because of the fact that the adsorbate molecules would undergo lateral expansion or lateral repulsion at these pH values accounting for a low degree of adsorption [25]. At pH 7.0 the biopolymers have zero net charge with an additional negative free energy term for adsorption process such that two-dimensional phase transition to a dense surface layer occurs [26]. The biopolymers lie either flat or lateral to the surface of the activated carbon. The monolayer coverage of biopolymer on the carbon surface is controlled by available free vacant sites. This has been verified in the experiment relating magnitude of adsorption and amount of activated carbon.

3.7. *Effect of mass of adsorbent*

Fig. 5 shows the effect of the mass of activated carbon on the adsorption of microbial cells from bulk solution. The removal of cells was found to increase linearly with the increase in mass of carbon equilibrated with the cell suspension. The probable explanation is that as the carbon mass is increased both the available adsorbent surface area and pore surface area are increased.

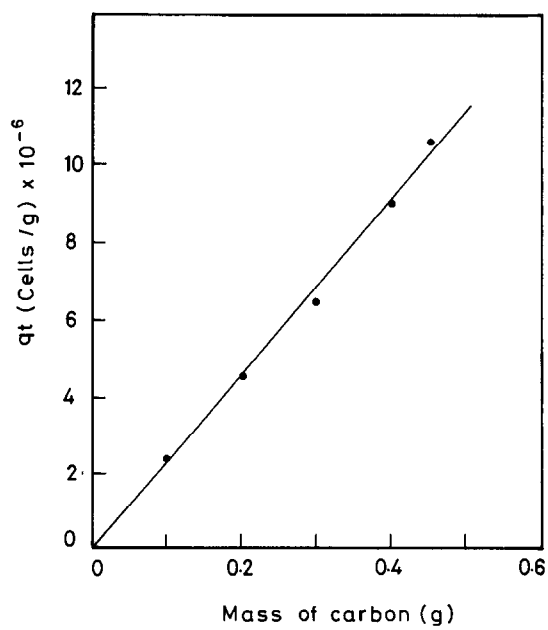

Fig. 5. Effect of mass of activated carbon on adsorption of cells in suspension. Cell concentration $= 17.5 \times 10^4$, pH = 7, temperature = 31 °C.

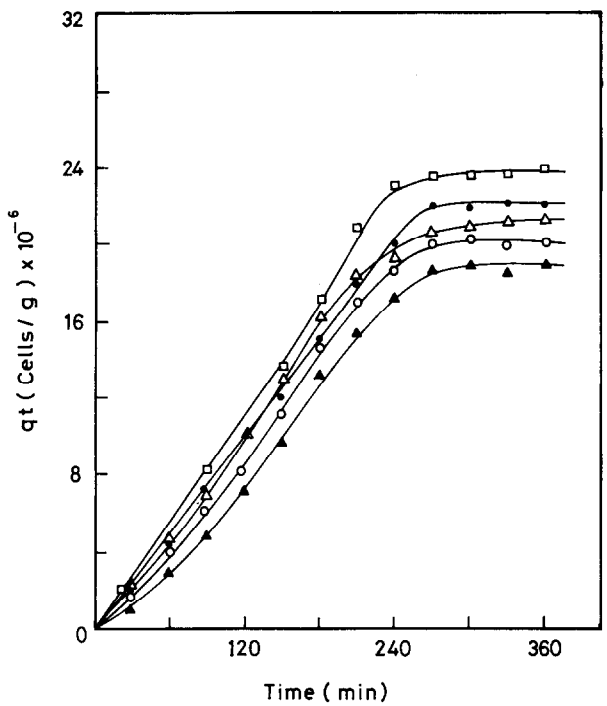

Fig. 6. Effect of sodium nitrate on adsorption of cells in suspension on to activated carbon. pH = 7.0, temperature = 31 °C. (□) 0.1%, (1) 0.2%, (△) 0.3%, (○) 0.4%, (▲) 0.5%.

3.8. *Effect of ionic strength*

The amount of cell removal from cell suspension is influenced by the presence of nutrients like sodium nitrate (Fig. 6) and glucose (Fig. 7). It was found that adsorption of cells from bulk cell suspension decreased with increase in concentration of sodium nitrate and glucose. In view of the charge shield action of electrolytes [27], it might be expected that the overall electrostatic interaction determines the amount of adsorption. Increasing the concentration of sodium nitrate or glucose would lead to a lower affinity of the biopolymers on the carbon surface. The electrolyte primarily exerts its influence on (MU 174) cell adsorption by affecting conformational stability of the cells and/or by being adsorbed simultaneously on the carbon surface. Consequently adsorption of cells on the carbon surface in the presence of an electrolyte was considerably reduced.

4. Conclusion

Rice bran based activated carbon used in this study has a large surface area and desirable micropores. MU 174 appears to have been immobilised strongly inside the

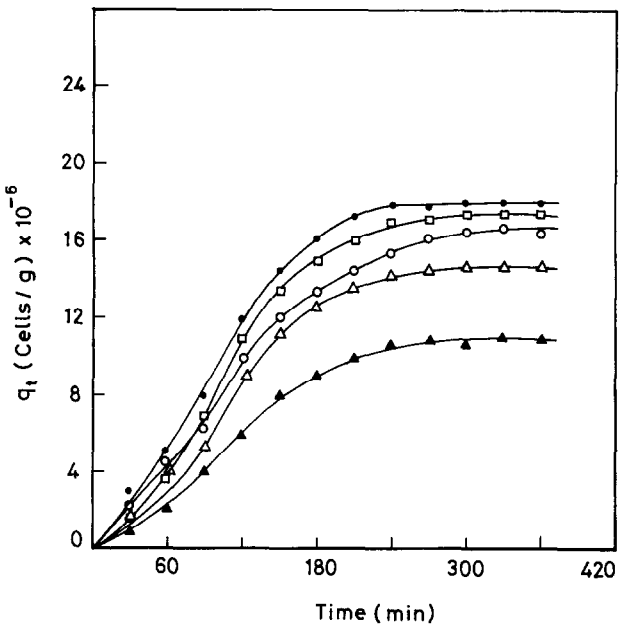

Fig. 7. Effect of glucose on adsorption of cells in suspension on to activated carbon. pH = 7.0, temperature = 31 °C. (□) 0.01%, (1) 0.02%, (△) 0.03%, (○) 0.04%, (▲) 0.05%.

pores, thereby avoiding it from attrition. The optimum pH and temperature for the adsorption of MU 174 on activated carbon were found to be 7.0 and 31 °C, respectively. The values of rate constant for the adsorption of MU 174 (K_{ad}) and intraparticle diffusion rate constant (K_p) varied with temperature. The value of K_{ad} was in the range of 5×10^{-3}–17.5×10^{-3} min^{-1} and that of intraparticle diffusion rate constant was of the order of 0.75–2.9 $min^{0.5}$. The enthalpy of the adsorption of MU 174 on to activated carbon was found to be −47.9 kJ/Avogadro number of cells, indicating that the adsorption is physical in nature.

5. Nomenclature

q_e	MU 174 cells adsorbed on to activated carbon at equilibrium (cells/ml)
q_t	MU 174 cells adsorbed on to activated carbon at time t (cells/g)
b	Langmuir constant (ml/cells)
Q_0	Langmuir number of monolayer capacity (g/ml)
K_{ad}	rate constant of adsorption (min^{-1})
K_p	rate constant of pore diffusion ($min^{0.5}$)
C_e	equilibrium concentration of cells (cells/ml)
C_0	initial concentration of cells (cells/ml)
T	temperature (°C)
R	gas constant (J/deg/mol)

x constant (0.7943)
y constant (-0.36)
Γ_P^m adsorption density of MU 174 cells (cells/m^2 of activated carbon).

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