A Kinetic Study of Uptake of Some Cationic Entities by The Alga *Chlorella vulgaris*

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ABSTRACT

Biosorption equilibrium and kinetics of proton and copper ions by dead dried *Chlorella vulgaris* were studied in a batch system at constant temperature. The uptake of ions was studied potentiometrically, a quick and simple method not usually used in such studies. Langmuir and Freundlich isotherm models were applied to equilibrium data where it was found that the uptake for both ions fitted better to the Langmuir model. The maximum adsorption Langmuir, (Q values), were 2.01 mmole H^+/g alga and 42.19 mg Cu/g alga. Less than 8% of the total adsorbed protons, while up to 50% of Cu ions, adsorbed after 15 minutes. A second-order rate dependence was found for both the proton and copper ions.

Keywords: Biosorption; *Chlorella vulgaris*; Langmuir; Kinetics; Potentiometry

1. INTRODUCTION

The increasing input of heavy metals into the environment through different industrial processes is a growing problem. Techniques utilized for treatment include precipitation, evaporation, adsorption, ion exchange, membrane processing, solvent extraction etc. These methods have been found to be limited as they involve high capital and operational costs, and may be followed by secondary generation of wastes. Unicellular algae, as well as aquatic microorganisms in general can be used to remove heavy metals and be recycled [1- 5]. Understanding the phenomenon would also be useful in explaining the entry of heavy metals into the food chain [6]. Another advantage of biosorption processes over other techniques is that metal ion concentrations lower than $10mgL^{-1}$ can be removed by microorganisms depending on adsorption equilibrium [7]. *Chlorella vulgaris* (*C. vulgaris*) has been suggested as an alternative method for removing heavy metals from industrial wastewater, especially for metal concentrations in the range mentioned above [8].

There are significant practical limitations for methods using living microorganism systems; perhaps the most significant is that microbial growth is inhibited by too high metal ion concentrations. Dead cells accumulate heavy metals to the same, or to a greater, extent than the living cells [1, 7-12] In addition, the dead biomass cells are readily available, cheap, and transport and storage is always convenient.

Proteins and polysaccharide in the biomembrane of the cells [10, 13-15] are effective types of sites for binding heavy metal ions. These contain diverse anionic functional groups including carboxylate, hydroxyl, amine, phosphate, sulfate etc. [16-20]. The carboxyl and amino groups as well as the nitrogen and oxygen of the peptide bond could be available for characteristic coordination bonding with metallic ions, which could be accompanied by proton displacement. Metal ions could also be electrostatically bonded to unprotonated carboxyl oxygen and sulfide. Polysaccharides often have ion exchange properties [7, 17] while the complex formation ability of their hydroxyl groups has been reported to be negligible [7, 19].

Biosorption typically involves a combination of passive and active transport mechanisms, starting with the diffusion of metal ion to the surface of the microbial cell [21, 22]. Once the metal ion has diffused to the cell surface, it will bind to sites on the cell surface, which exhibits some chemical affinity for the metal. This step contains a number of passive accumulation processes and may include adsorption, ion exchange, coordination, complexation, chelation and microprecipitation. Generally, such metal ion adsorption is fast, reversible and not a limiting factor in bioremoval kinetics when dealing with dispersed cells [21]. Biosorption is followed by a slower binding process in which additional metal ions are bound, often irreversibly. This second step can be due to mechanisms including covalent bonding, surface precipitation, redox reactions, crystallization on the cell surface, or most often, diffusion into the cell interior [21, 23-24].

The mechanism involved in metal binding by algal biomass depends on the species of metal ions, the algal species and state, the chemical composition of the metal ion solution, pH and temperature [14, 20, 21, 24]. Most investigators have been interested in the total exchange capacity and equilibrium of metal uptake by algae and microorganisms, but few workers have, until recently, studied the kinetics of the uptake process.

In ion exchange experiments, algae are usually acid washed in order to saturate all binding sites with protons [17, 20, 25-26]. Hence, understanding the kinetics of proton uptake is of great importance in relation to the kinetics of metal ions uptake. However, very little has been published on proton uptake equilibria, and far less on the kinetics of the process.

In almost all kinetic studies [7, 8, 12, 16, 21], the metal ion uptake by microorganisms has been followed by withdrawing the biomass-ion mixture at given time intervals, separate the biomass and determining the metal ion concentration by atomic absorption spectrometry. Such methods can cause deviation from equilibrium through steps of filtration or centrifugation. In this study, copper ions and protons were determined potentiometrically, which allows continuous monitoring of ions in the presence of the alga in an easy and quick method with very low detection limits.

The goal of this work was to study the kinetics of H^+ and Cu(II) binding to dead *C. vulgaris* cells during a short time (15 minutes), since kinetic studies with organisms during long periods are much more difficult to interpret [16]. Experiments were carried out at different ionic strengths.

2. EXPERIMENTAL

The alga *C. vulgaris* was generously provided as a dry green powder by Dr. F. Al-Baz from the Botany Department at the National Research Center, Cairo, where the alga was prepared using the single cell technique in indoor cultures, then transferred to outdoor cultures. It was provided with air, CO_2 , and a nutrient solution containing urea and trace elements. Harvesting took place seven days after inoculation

and at the day of maximum growth, using a continuous centrifuge, then passed through a homogenizer and pumped on a roller drier at 120ºC for a few seconds. The green powder yeild contained 4-6% moisture. In our laboratory the algal powder was then washed several times with distilled deionized water, filtered and dried at ~80°C overnight. The dry biomass was sieved to a particle size of ≥355μm and stored in closed bottles in a descicator.
A WTW pMx 3000/ion ionometer was used to determine the cation concentration using solid state electrodes for copper ions and the glass electrode for protons. HCl (BDH), $CuSO_4$ (WTW prepared standard solution), and $NaNO_3$ (WTW prepared 5M solution) were used as obtained. Deionized water was used in preparing all solutions. Cation concentrations varied in the range of 1-100 ppm.

To 50 ml of the continuous stirred acid or copper solution, 0.1 g *C. vulgaris* was added and the concentration of the studied cation was recorded every minute for 15 minutes (1 ml of 5M $NaNO_3$ added to maintain ionic strength in copper experiments). After 24 hours, concentrations were recorded and taken as equilibrium concentrations. All experiments were carried out in duplicates at room temperature, and average values used in analyses. No special care was taken to maintain the pH, (which will be the case in environmental and industrial applications).

3. RESULTS AND DISCUSSION

3.1. Equilibrium Parameters

Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. The classical adsorption models (Langmuir and Freundlich) were used [7, 12, 21, 27-29] to describe the equilibrium between the adsorbed cationic species on the algal cell (q_e) and the cationic species concentration in solution (C_e) at a constant temperature. The Langmuir equation is valid for monolayer sorption onto a surface with a finite number of identical sites;

$$q_e = Q\, b\, C_e / \; 1 + b\, C_e \qquad (1)$$

Where Q is the maximum amount of the cationic species per unit weight of the alga to form a complete monolayer on the surface formed at high C_e, and b is a constant related to the affinity of the binding site. Linearizing equation (1) and plotting C_e/ q_e versus C_e resulted in straight lines for proton uptake (Figure 1) and copper uptake (Figure 2). The values of Q and b are listed in Table 1.

The empirical Freundlich equation based on heterogeneous biosorption is linearized as:

$$\ln q_e = \ln K + n \ln C_e \qquad (2)$$

where k and n are the Freundlich constants, and are indicators of adsorption capacities and intensities respectively. The values of K and n determined from plots of $\ln q_e$ versus $\ln C_e$ for all species (figures 3 and 4) are listed in table (1)

Regression values (R^2) (Table 1) suggest that the biosorption of protons as well as copper ions fitted better to the Langmuir model, which is in accord with results obtained using other methods for *C. vulgaris* [8-21] and for other microorganisms [7, 27-29]. The value of Q for copper ions (42.19 mg Cu/g alga) is in good agreement with findings [21] for nickel ions adsorbed amounts by *C. vulgaris* at similar conditions, but are almost five times the value reported [12] for copper and nickel ions for the same alga. It is difficult to compare equilibrium constants reported from different studies, even for the

same species of algae as different conditions of growing the alga may affect the type of binding sites. The very small value (1/b) that approaches zero means that the algal surface has a very strong affinity towards protons, which is expected for such a small ion.

3.2. Kinetics Parameters

Generally speaking, the uptake of heavy metal ions by microorganisms has been observed to occur in two stages, an initial rapid uptake due to surface adsorption on cell walls and a subsequent slow uptake due to membrane transport [6, 11, 12, 17, 19, 22]. In this study, it is assumed that the algal particles are spherical [21-22] and only surface adsorption occurs, and that biomass is employed as free cell suspension in well-stirred batch systems.

Since a fixed cell biomass offers a finite number of surface binding sites, the initial uptake (being surface adsorption) would be expected to show saturation kinetics with increasing ion concentration. This trend was observed for protons and copper ions under study (see Figures 5 and 6). Two types of proton uptake were known [19] to take place, a rapid one due to neutralization of the carboxylate sites and a slow process that carries on for several hours. Table 2 lists the percent proton biosorbed after time intervals of 1, 5, 10, 15 minutes and after 24 hours (at equilibrium) for different acid concentrations. From the values in the table it is clear that less than 8% of the total adsorbed amount was taken up after 15 minutes.

For copper ions the view is different, and values in Table 3, suggest that initial adsorption is very much affected by the ion concentration. Different binding sites with varied affinities towards copper ions may act in a way that seems anomalous at different ionic strengths. But after 24 hours (equilibrium) the percent of adsorbed copper ions showed a systematic decrease with increase in ions available in solution. It has been reported in an earlier study [30] that electrostatic binding of Cu predominates at higher concentrations while ion exchange mechanism predominates at lower concentrations.

First and second order rate equations [12] are used to describe the kinetics of uptake of cationic entities by *C. vulgaris*. From plotting log(q_e-q) versus t for protons and copper ions, straight lines were obtained showing a degree of fitness to first order rate kinetics for both (Figures 7 and 8). However, plotting t/q versus t exhibited better fitting to second order kinetics models (Figures 9 and 10), which can be seen from comparing R^2 values (see Table 4).

4. CONCLUSION

This study offers a precise, simple, and economical technique to determine heavy metal ion uptake by algae and other microorganisms. Both proton and copper ions biosorption fitted the Langmuir model better than the Freundlich, indicating similar equilibrium for the two species at the algal surface after 24 hours.

Proton percent uptake with time exhibited a uniform decrease with increase in initial concentration, where only a maximum of 8% was adsorbed after 15 minutes. For copper, up to 50% of the total adsorbed amount was adsorbed after 15 minutes. After 24 hours, copper achieved a uniform trend in decrease in percent uptake with increase in initial concentration. It is important to study the differences in binding in depth to understand the variations in affinities in relation with time and concentration.

Proton uptake by untreated dead dried *C. vulgaris* followed second order kinetics, as did copper uptake, with respect to cation concentration.

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Table 1: Langmuir and Freundlich values

	Langmuir			Freundlich		
	R^2	Q	1/b	R^2	lnK	n
protons	0.998	2.09	- 0.004	0.841	-5.530	0.124
copper	0.961	42.19	1.33	0.913	0.212	3.05

Table 2: Proton % uptake

	Proton % Adsorbed				
Conc. (mmole)	**1 min**	**5 min**	**10 min**	**15 min**	**24h**
50	32.23	35.13	35.87	36.46	34.08
30	32.57	36.63	37.07	37.21	37.93
20	36.18	43.90	45.80	46.42	46.42
10	40.71	56.95	61.10	62.68	65.65
5	53.00	79.82	87.67	90.86	98.69
1	85.61	99.67	99.84	99.91	99.99

Table 3: Cu % uptake

	Cu % adsorbed				
Conc. (ppm)	**1 min**	**5 min**	**10 min**	**15 min**	**24h**
100	39.50	62.28	64.06	64.25	81.41
80	8.36	23.40	34.89	40.44	84.09
60	1.42	31.98	44.74	51.72	97.77
40	20.00	50.44	62.21	68.53	98.53

Table 4: Comparison of regression coefficients for first and second order kinetics at different concentrations

	First Order		Second Order	
mmole protons	**1**	**30**	**1**	**30**
R^2	0.6828	0.7526	0.9999	0.9993
ppm copper	**40**	**100**	**40**	**100**
R^2	0.9117	0.5339	0.9992	0.9809

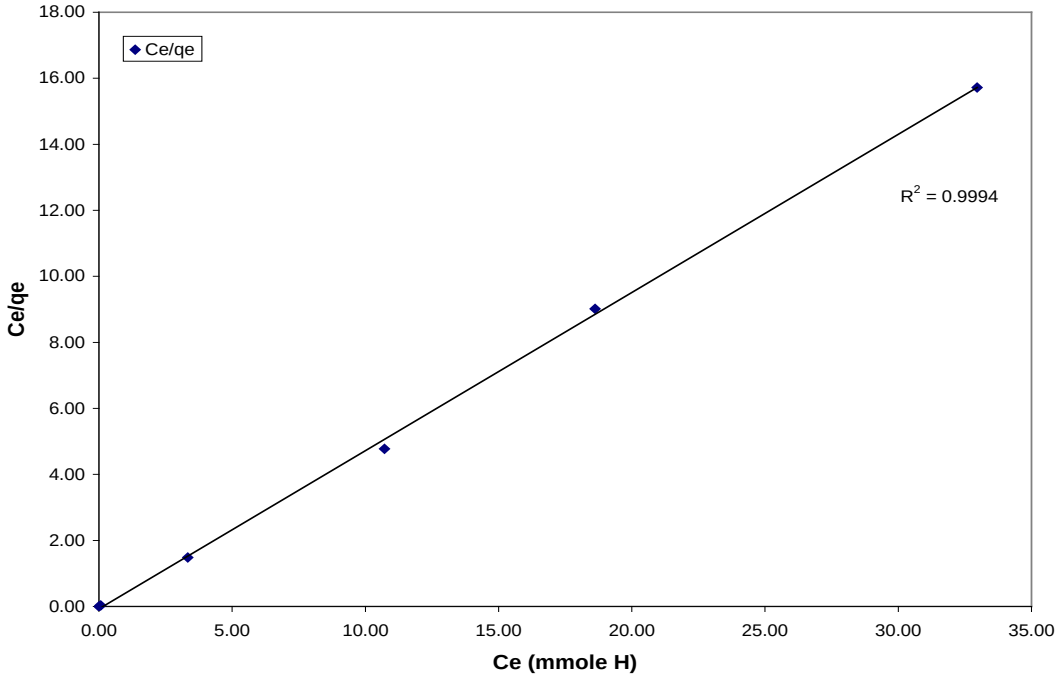

Fig.1 Langmuir isotherm of protons uptake

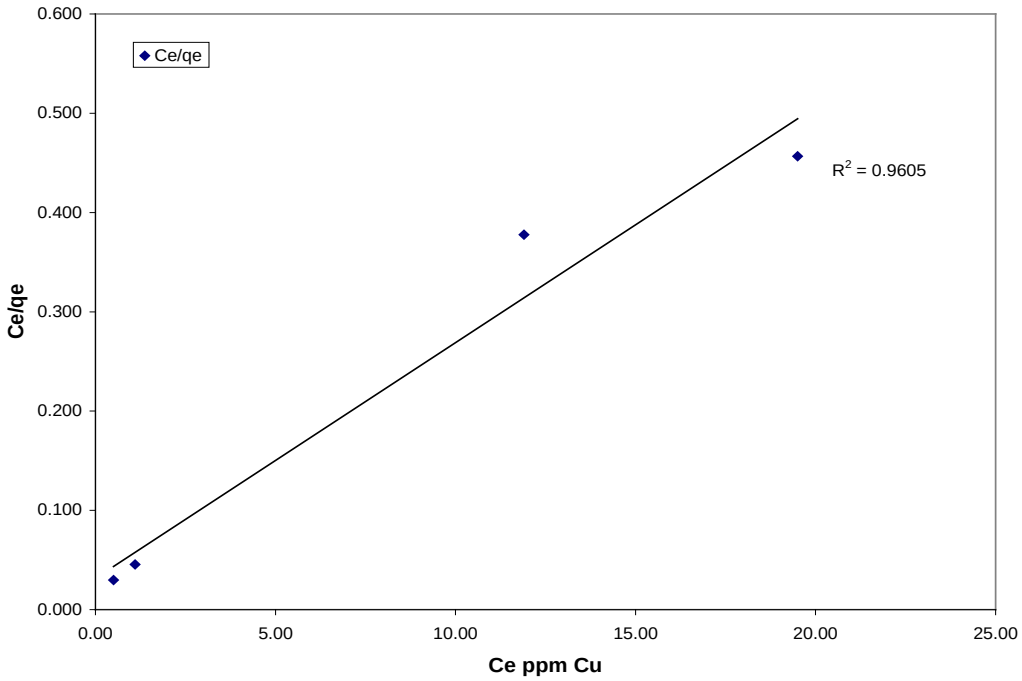

Fig. 2 Langmuir isotherm for Cu uptake

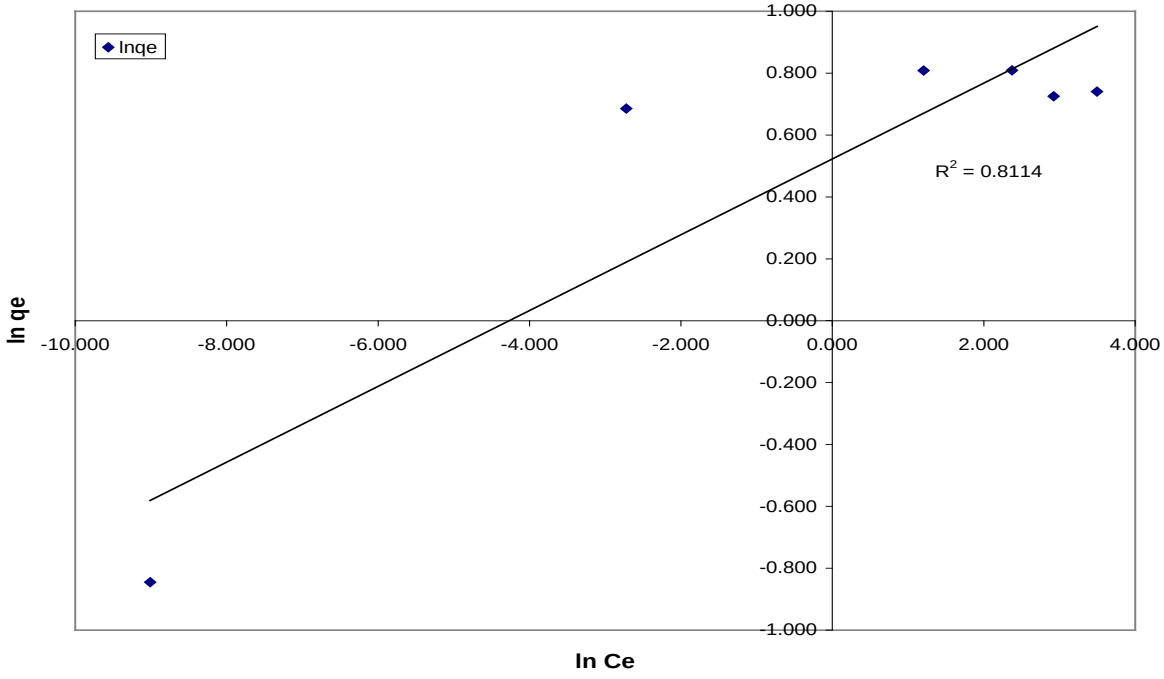

Fig. 3 Freundlich isotherm for proton uptake

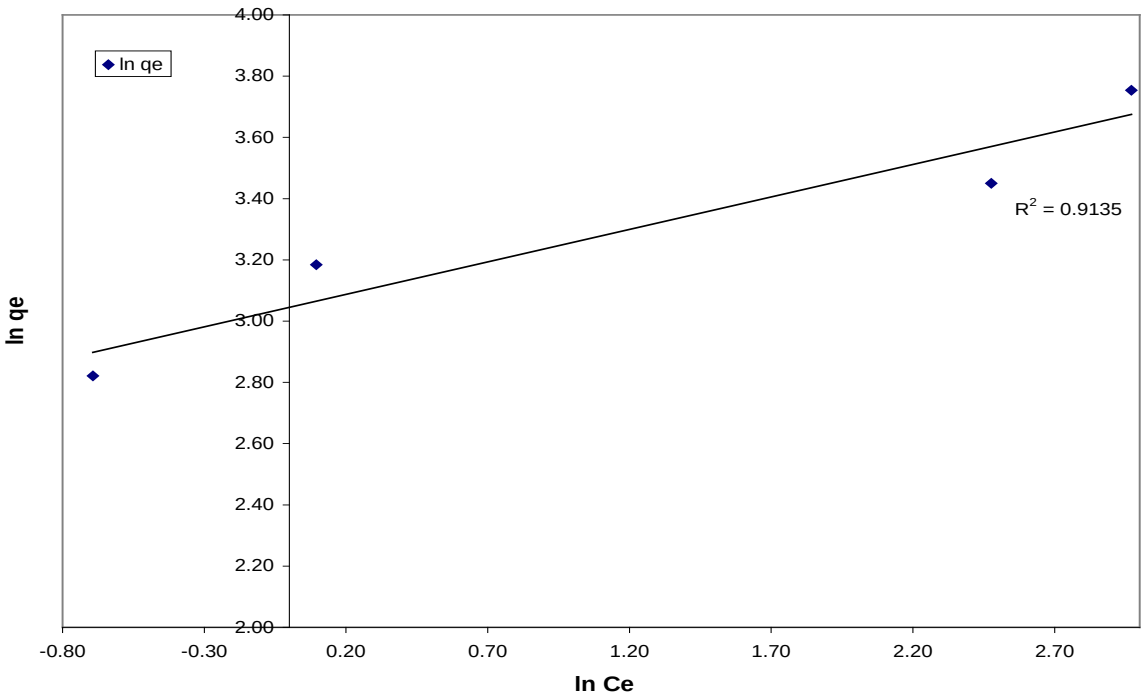

Fig.4 Freundlich isotherm for Cu uptake

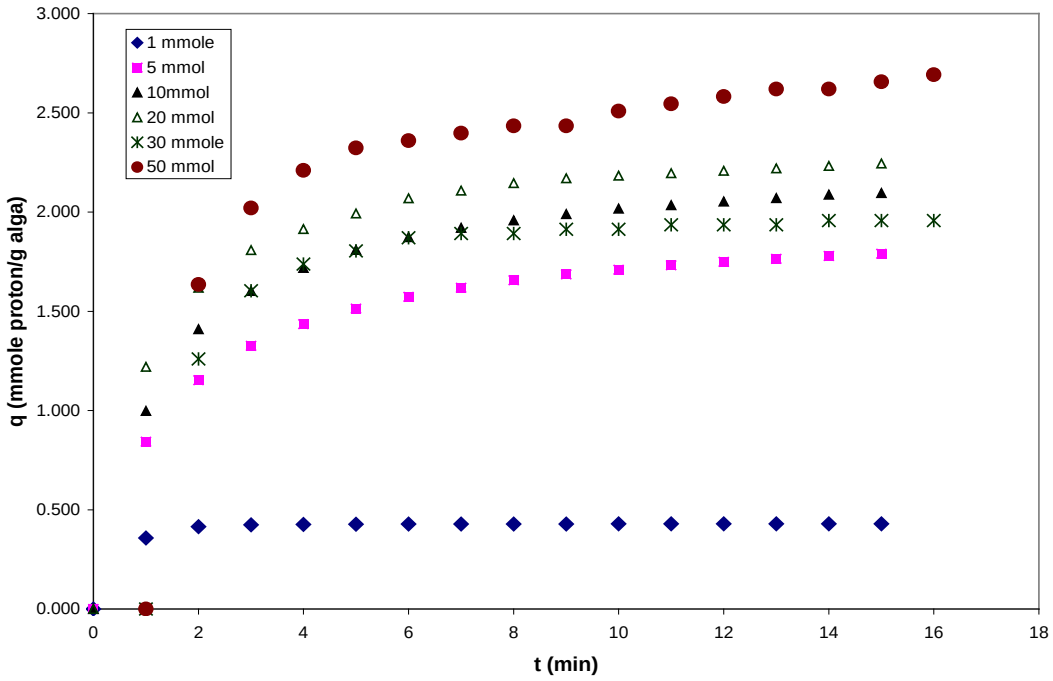

Fig. 5 Kinetics of proton sorption at different concentrations

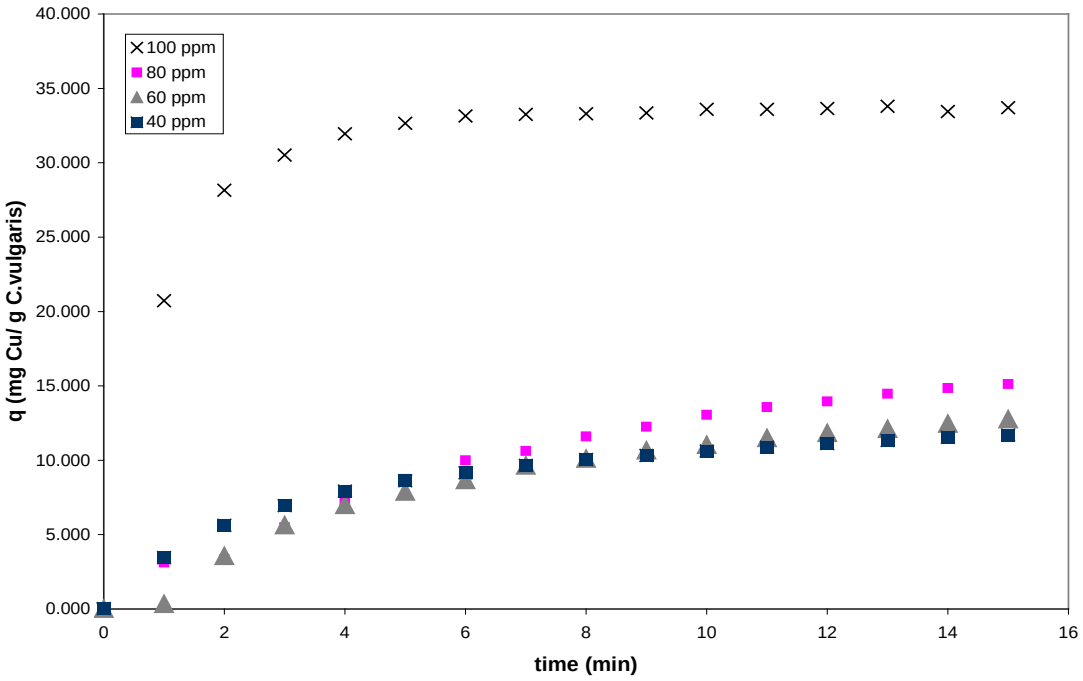

Fig.6 Kinetics of Cu sorption at different concentrations

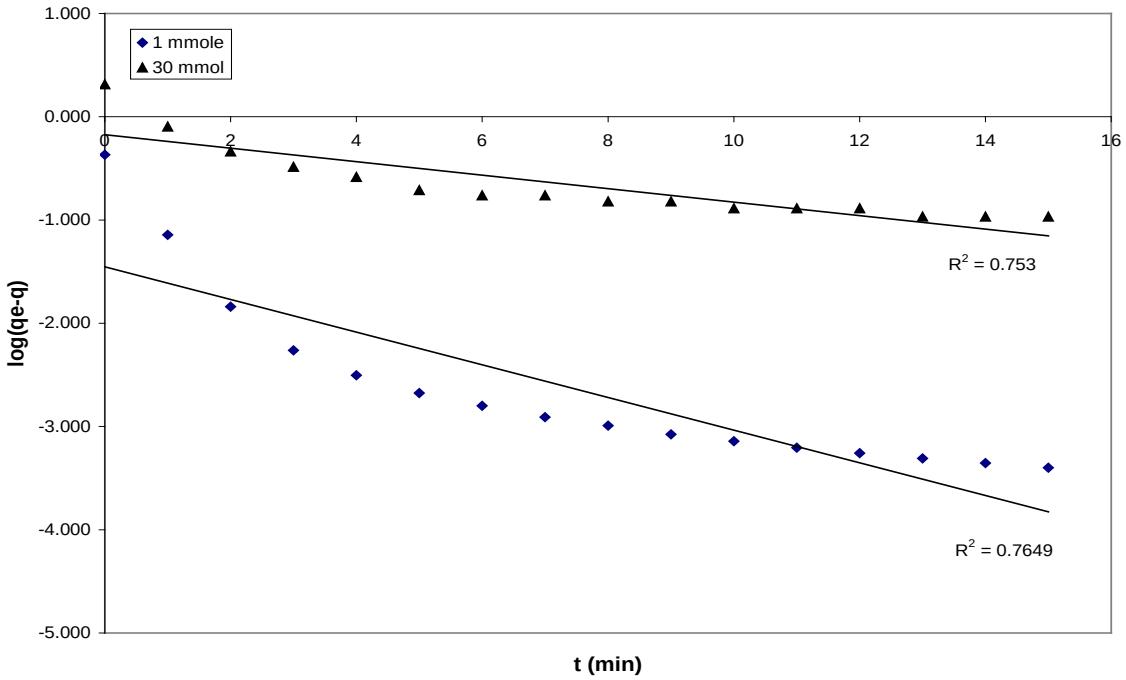

Fig. 7 First-order rate plots for proton uptake

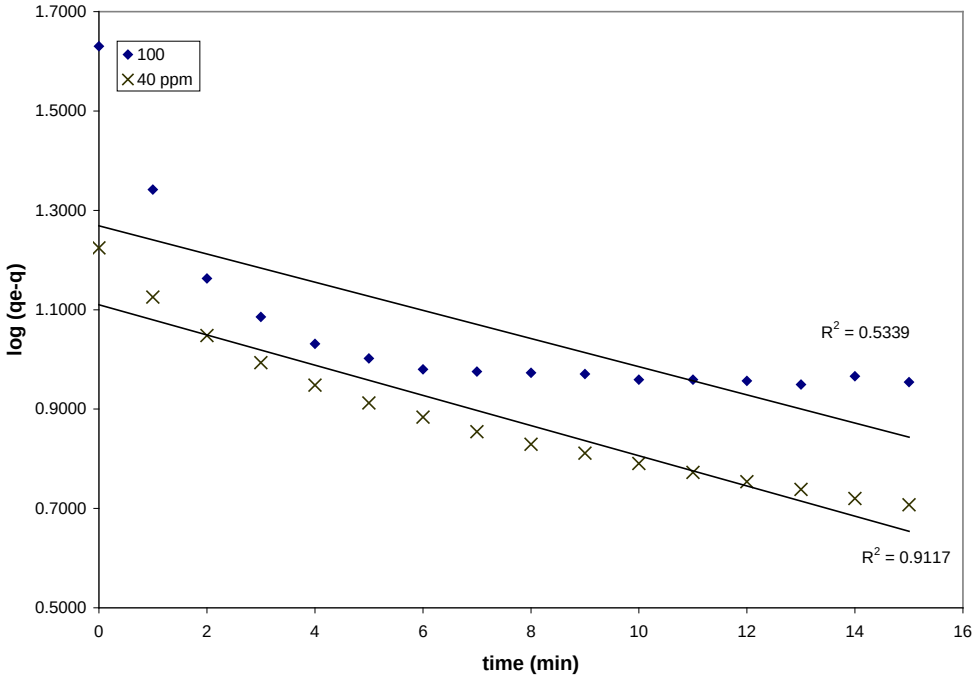

Fig. 8 first-order rate plots for Cu uptake

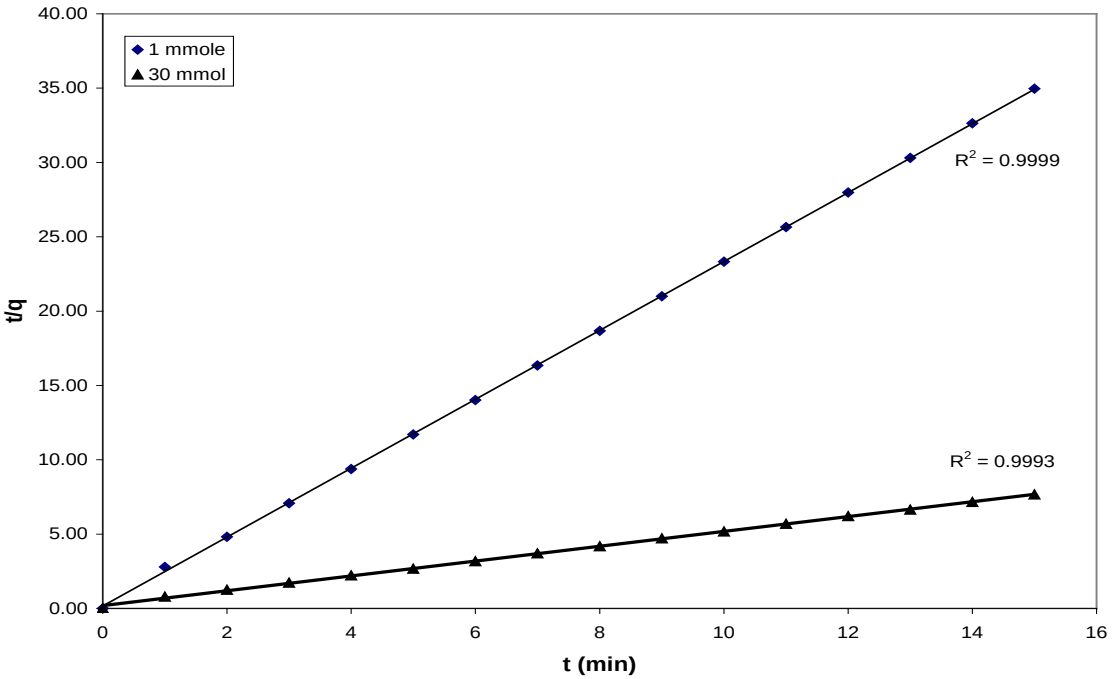

Fig.9 Second- order rate plots for proton uptake

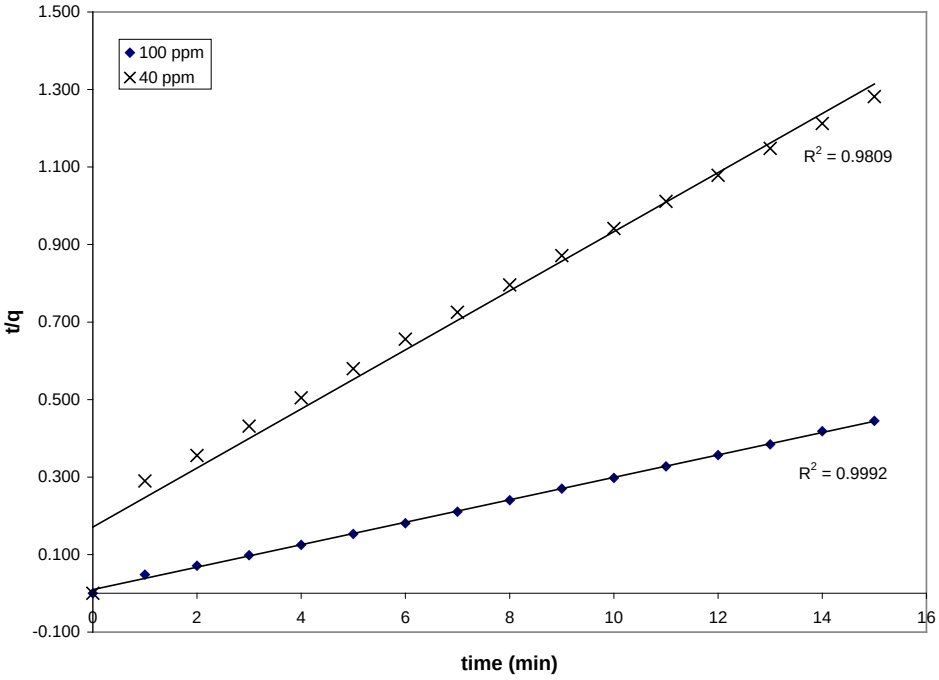

Fig. 10 Second-order rate plots for Cu uptake