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SCIENTIFIC PAPER

UDC 661.183.2:66.081.3:544:547.472.3

## KINETICS AND ADSORPTION ISOTHERM OF LACTIC ACID FROM FERMENTATION BROTH ONTO ACTIVATED CHARCOAL

### Article Highlights

- Activated charcoal was used to remove lactic acid from fermentation broth
- The isotherm was best fitted with the Freundlich isotherm
- The kinetic data were described well by pseudo-second-order model
- Maximum adsorption capacity of 57.06 mg/g was obtained

### Abstract

*Activated charcoal was applied for the recovery of lactic acid in undissociated form from fermentation broth. Lactic acid was obtained from the fermentation of *Lactobacillus casei* TISTR 1340 using acid hydrolyzed Jerusalem artichoke as a carbon source. The equilibrium adsorption isotherm and kinetics for the lactic acid separation were investigated. The experimental data for lactic acid adsorption from fermentation broth were best described by the Freundlich isotherm and the pseudo-second order kinetics with  $R^2$  values of 0.99. The initial adsorption rate was 41.32 mg/g·min at the initial lactic acid concentration of 40 g/L.*

*Keywords: lactic acid, activated charcoal, adsorption kinetics.*

Lactic acid is an important organic acid that is widely used in food, pharmaceutical and cosmetics industries. It can be produced by both chemical synthesis and fermentation. Recently, many researchers have focused on lactic acid recovery from fermentation broth by calcium lactate precipitation. Its economical cost is about 50% of the total capital cost but calcium sulphate was occurred as the waste from this recovery process. So, the separation technologies for lactic acid separations such as solvent extraction, electrodialysis, ion exchange chromatography and membrane separation etc. have been studied. These processes, however, have many disadvantages such as high purification cost, the more complex procedure, the usage of organic solvent, the fouling problem in case of membrane process and the short lifetime of expensive resins. As a result, adsorption technique is of interest for lactic acid separation from fer-

mentation broth. Currently, many different adsorbents such as Amberlite XAD1600, activated carbon, adsorption resin AX-1 and Amberlite IRA-67 have been investigated for lactic acid removal from fermentation broth [1-6]. Therefore, activated charcoal is an interesting resin for lactic acid adsorption due to its good adsorbent characteristics, high porous and low cost. The objectives of this work were to study the adsorption of lactic acid from fermentation broth on activated charcoal and to determine the adsorption isotherm and adsorption kinetics.

## MATERIALS AND METHODS

### Chemicals

Activated charcoal with particle sizes in the range of 20-60 mesh (0.84-2.4 mm) was purchased from Sigma-Aldrich and used in this study.

### Bacterial strains

*Lactobacillus casei* TISTR 1340 was purchased from The Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. Stock cultures were maintained in MRS agar plates at 4 °C and reactivated every month. Cells were cultured in MRS broth

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Paper received: 11 May, 2016

Paper revised: 9 December, 2016

Paper accepted: 1 February, 2017

<https://doi.org/10.2298/CICEQ160511004S>

at 37 °C for 18 h under static and anaerobic conditions.

### Lactic acid fermentation broth for adsorption

To prepare a fermentation broth for adsorption, batch lactic acid fermentation was performed in a 5 L bioreactor (Biostat, B. Braun, Germany) using Jerusalem artichoke hydrolysate as a carbon source. The Jerusalem artichoke hydrolysate was obtained from the hydrolysis of Jerusalem artichoke tubers with 0.1 M H<sub>2</sub>SO<sub>4</sub> at a ratio of tubers to conc. sulfuric acid of 1:2 at 80 °C for 6 h. Then, the mixture was adjusted to pH 6.5 with 4 M NaOH. The hydrolysate was passed through cloth bags to remove solid particles and sterilized at 121 °C for 15 min. The hydrolysate was mixed with MRS medium and the *L. casei* TISTR 1340 inoculum (10 vol.%) was added. Temperature and agitation speed were controlled at 37 °C and 150 rpm, respectively. The pH of the culture was kept at 6.0 by automatic addition of 25% Ca(OH)<sub>2</sub>. At the end of fermentation, cells were removed by centrifugation at 4000 rpm for 10 min. The fermentation broth contained approximately 40.6 g/L lactic acid, 1.05 g/L sucrose and 4.10 g/L acetic acid.

### Adsorption of lactic acid on activated charcoal

*Batch adsorption of lactic acid.* The experiment was carried out in a 250 mL Erlenmeyer flask with either 50 mL of fermentation broth or pure lactic acid solution containing different initial lactic acid concentrations in the range of 10–40 g/L. Then, 5 g of activated charcoal was added and incubated at 100 rpm, 25 °C until equilibrium (~8 h). Samples were taken periodically to determine the amount of adsorbed fermentation broth components at equilibrium. Lactic acid concentrations were determined by HPLC and the amount of adsorbed lactic acid per g of activated charcoal was calculated using Eq. (1):

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where  $q_e$  is the amount of lactic acid adsorbed by the adsorbent (g/L),  $C_0$  is the initial lactic acid concentration (g/L),  $C_e$  is the lactic acid concentration at equilibrium (g/L),  $V$  is the initial solution volume (L) and  $W$  is the weight of activated charcoal.

The adsorption efficiency of lactic acid was determined using Eq. (2):

$$\text{Adsorption efficiency} = 100 \frac{C_0 - C_f}{C_0} \quad (2)$$

where  $C_0$  and  $C_f$  are the initial and final concentration of lactic acid in solution (g/L), respectively.

The equilibrium data obtained from batch adsorption of lactic acid can be applied to predict the adsorption models and related theories, the Langmuir and Freundlich isotherms were compared in this work. The adsorption isotherms of lactic acid in the fermentation broth and in the pure lactic acid solution at the concentration of 10 g/L were also evaluated because of the high adsorption efficiency of lactic acid at this concentration. The pseudo-first order and pseudo-second order equations were applied to model the kinetics of lactic acid adsorption onto activated charcoal.

### Analytical methods

Samples were taken periodically and centrifuged at 7000 rpm for 10 min to remove any particulate solids. The supernatants were used for the analysis of lactic acid and other components in the fermentation broth. The lactic acid, organic acid and sugar concentrations were measured with an HPLC with a RI detector (Shimadzu, Japan). The separation was performed on an Aminex HPX-87H column (Bio-Rad, CA) at 55 °C and the flow rate of 0.005 M sulfuric acid as a mobile phase was set at 0.5 mL/min.

## RESULTS AND DISCUSSION

### Adsorption efficiency

Figure 1 shows the adsorption efficiency of activated charcoal for the removal of lactic acid from fermentation broth at 25 °C and pH 2. As can be seen, the maximum acid removal (~45%) was obtained at an initial concentration of 10 g/L, the increase of the lactic acid concentration from 10 to 40 g/L decreased the adsorption efficiency from 45 to 20%. Therefore, a comparison between pure lactic acid and lactic acid from fermentation broth at 10 g/L was chosen and selected for further study on the adsorption isotherms and kinetics. The similar results were also presented

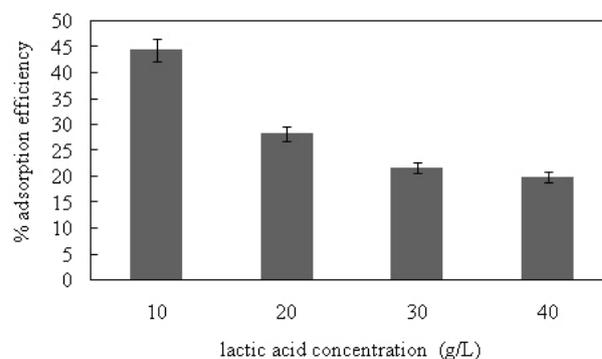


Figure 1. Adsorption efficiency of activated charcoal on the removal of lactic acid from fermentation broth under different initial lactic acid concentrations.

by Yousuf *et al.* [7] that the percentage of acid removal using activated carbon as an adsorbent was approximately 37% at pH 3.

### Adsorption isotherms

The adsorption of lactic acid was performed at pH 2 due to its undissociated form at the pH lower than the  $pK_a$  value ( $pK_a$  of lactic acid = 3.78). Even though the physical properties of the used activated charcoal were not estimated, these physical properties can be obtained from the work of Pyrzyńska and Bystrzejewski [8] which used the same type of commercial activated charcoal. As reported in their work, the pH of the point of zero charge of this commercial adsorbent was 4.44 with the methylene blue-relative surface area of 118.7 m<sup>2</sup>/g. Therefore, the possibility of using activated carbon for the adsorption of lactic acid from fermentation broth in a low pH region was studied and presented in Figure 2. The adsorption capacity of lactic acid from the fermentation broth by activated charcoal was 57 mg/g adsorbent at an initial lactic acid concentration of 40 g/L. As can be seen, lactic acid is adsorbed on activated carbon whereas other components such as sugars still remained mainly in the solution which agrees with the previous work of Thang and Novalin [1].

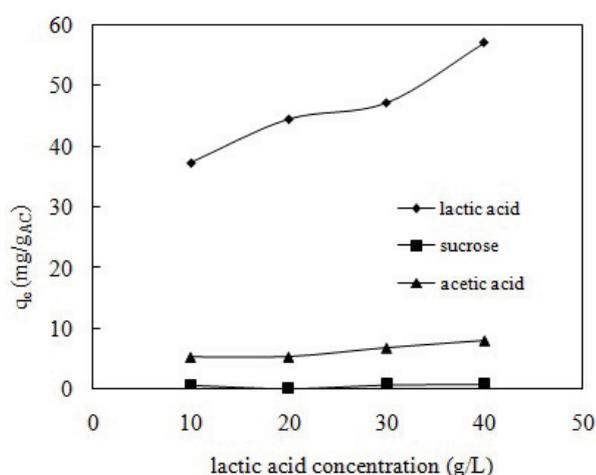


Figure 2. Adsorption capacity of lactic acid, acetic acid and sucrose at equilibrium at different lactic acid concentrations.

The Langmuir and Freundlich isotherms were studied to find the equilibrium characteristics of adsorption. The Langmuir adsorption model is based on the assumption that the solute molecules adsorbed on the adsorbent surface in a monolayer without any interactions between the adsorbed molecules. The linear form of Langmuir equation can be expressed by Eq. (3):

$$\frac{1}{q_e} = \frac{1}{K_L q_m} \frac{1}{C_e} + \frac{1}{q_m} \quad (3)$$

where  $q_e$  is the amount of lactic acid adsorbed at equilibrium (g/g<sub>AC</sub>),  $q_m$  is the maximum adsorption capacity (g/g<sub>AC</sub>),  $C_e$  is lactic acid concentration at equilibrium (g/L) and  $K_L$  is equilibrium constant of the Langmuir constant (L/g). The values of  $q_m$  and  $K_L$  were determined from the intercept and slope, respectively, and are presented in Table 1.

To determine whether the adsorption is favorable or unfavorable, a dimensionless constant separation factor ( $R_L$ ) is presented and can be expressed by Eq. (4):

$$R_L = \frac{1}{1 + K_L C_0} \quad (4)$$

where  $K_L$  is the Langmuir constant and  $C_0$  is the initial concentration of adsorbate. The  $R_L$  value presents the nature of adsorption as irreversible ( $R_L = 0$ ), favorable ( $0 < R_L < 1$ ), linear ( $R_L = 1$ ) or unfavorable ( $R_L > 1$ ). In this study, the calculated  $R_L$  values were between 0 and 1 as listed in Table 1, indicating that the adsorption characteristic of lactic acid in fermentation broth on activated charcoal is favorable.

The Freundlich isotherm is an empirical equation for heterogeneous systems and was used in this study as a second isotherm and the linear form of this model is expressed by Eq. (5):

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (5)$$

where  $K_f$  is a Freundlich constant,  $n$  indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. The values of  $K_f$  and

Table 1. The parameters of Langmuir and Freundlich isotherm of lactic acid adsorption

Lactic acid concentration, g/L	Langmuir isotherm				Freundlich isotherm		
	$q_m$ / g g <sub>AC</sub> <sup>-1</sup>	$K_L$ / L g <sup>-1</sup>	$R_L$	$R^2$	$K_f$ / L g <sup>-1</sup>	$n$	$R^2$
10	0.018	0.329	0.239	0.905	0.37	0.770	0.975
20	0.021	0.116	0.302	0.863	12.04	0.491	0.965
30	0.014	0.042	0.446	0.971	31282	0.241	0.993
40	0.011	0.008	0.766	0.962	331589	0.229	0.991
10 (pure)	0.008	0.250	0.320	0.905	1.28	0.455	0.975

$n$  were determined from the intercept and slope, respectively, as shown in Table 1.

From Table 1, in case of Langmuir isotherm, the value of  $q_m$  and  $K_L$  decreased with increasing initial lactic acid concentration indicates that the lactic acid was adsorbed on the activated charcoal at lower concentration. The Freundlich isotherm, the  $K_f$  value indicates the adsorption capacity of the adsorbent or the bonding energy which increased when increasing initial lactic acid concentration. Whereas  $n$  is the adsorption intensity, smaller  $n$  values at high lactic acid concentration implies that the adsorption is better with increasing lactic acid concentration [9]. The comparison between  $n$  value of pure lactic acid solution and the fermentation broth indicates that the lactic acid is adsorbed at higher intensity than that from fermentation broth. However, as seen in Table 1,  $n$  value was not high enough for separation ( $n < 1$ ). The coefficient determination ( $R^2$ ) values in the Freundlich isotherm of lactic acid adsorption were higher than those in the Langmuir isotherm. As a result, the Freundlich isotherm is more suitable to describe the adsorption of lactic acid adsorption.

### Adsorption kinetics

In this study, adsorption data are applied to the pseudo-first order, pseudo second-order kinetic models to find the rate constant of adsorption.

#### Pseudo-first-order

A linear form of pseudo-first order model was described by Lagergren as shown in Eq. (6):

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (6)$$

where  $q_e$  and  $q_t$  are the amount of lactic acid adsorbed at equilibrium and time  $t$  (mg/g), respectively, and  $k_1$  is the rate constant of the first-order adsorption ( $\text{min}^{-1}$ ).

The plots of  $\log(q_e - q_t)$  versus  $t$  were used to determine the rate constant ( $k_1$ ),  $q_e$  and correlation coefficients ( $R^2$ ) for different lactic acid concentrations as shown in Fig. 3.

#### Pseudo-second order

The pseudo-second order kinetic may be presented in a linear form as in Eq. (7):

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (7)$$

where  $k_2$  is the rate constant of second-order adsorption ( $\text{g}/\text{mg}\cdot\text{min}$ ) and  $q_e$  and  $q_t$  is amount of lactic acid adsorbed at equilibrium and time  $t$  (mg/g).

The plot of  $t/q_t$  versus  $t$  should give a straight line, and  $q_e$  and  $k_2$  can be determined from the slope and intercept, respectively, as shown in Fig. 4.

The pseudo-second order kinetic analysis presented that the values of the initial adsorption rates ( $h$  in the unit of  $\text{mg}/(\text{g}\cdot\text{min})$ ) increased with increasing initial lactic acid concentrations as shown in Table 2. It could be explained by the fact of the increase in driving force at high lactic acid concentrations which is also in agreement with the previous literature work [10-13]. The equilibrium adsorption capacity ( $q_e$ ) also

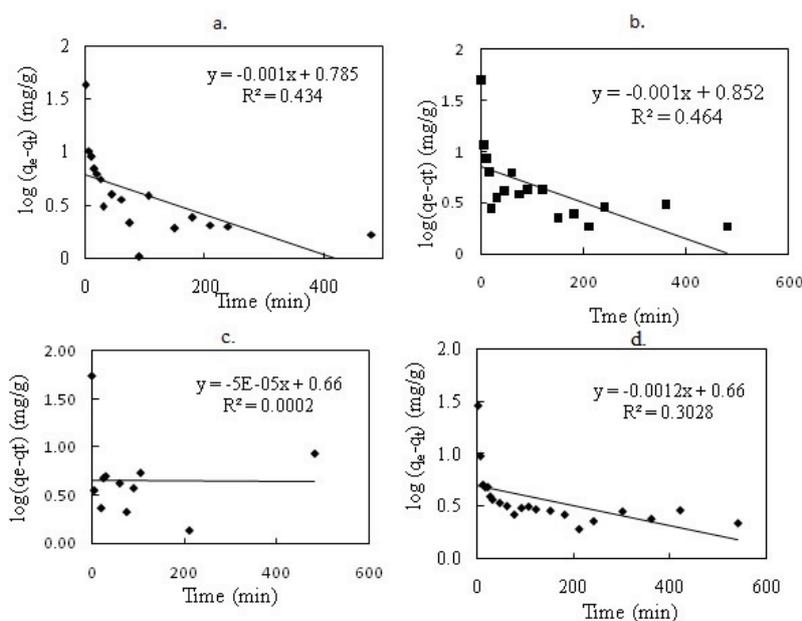


Figure 3. The pseudo-first order kinetic plots for the adsorption of lactic acid on activated charcoal. At conditions: 100 rpm, pH 2 and temperature 25 °C. Different initial lactic acid concentrations: a) 10, b) 20, c) 30 and d) 10 g/L lactic acid solution.

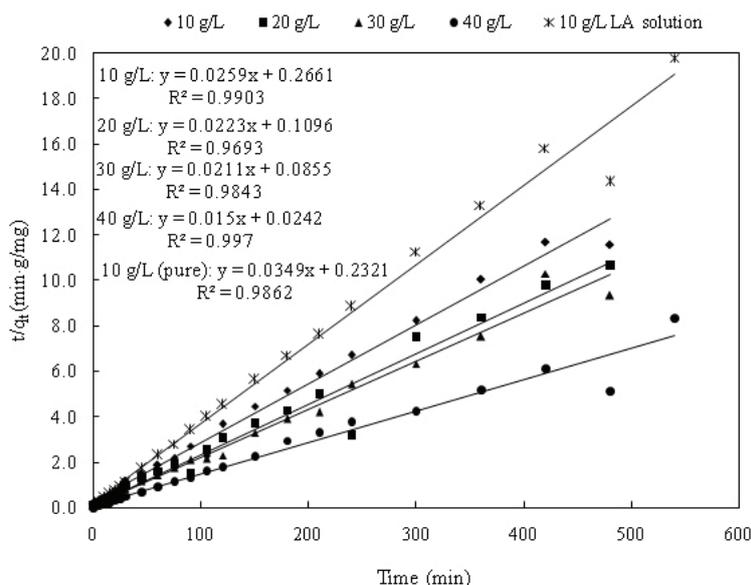


Figure 4. The pseudo-second order kinetics plots for the adsorption of lactic acid on activated charcoal. Different initial lactic acid concentrations at conditions: 100 rpm, pH 2 and temperature 25 °C.

Table 2. Comparison of pseudo-first order and pseudo-second order rate constants, normalized standard deviation ( $\Delta q$ ) and correlation coefficients ( $R^2$ ) at different initial lactic acid concentrations

Lactic acid concentration, g/L	$q_{e,exp}$ mg g <sup>-1</sup>	Pseudo-first order			$\Delta q$ %	$h$ mg g <sup>-1</sup> min <sup>-1</sup>	Pseudo-second order			$\Delta q$ %
		$k_1$ min <sup>-1</sup>	$q_{e,cal}$ mg g <sup>-1</sup>	$R^2$			$k_2$ g mg <sup>-1</sup> ·min <sup>-1</sup>	$q_{e,cal}$ mg g <sup>-1</sup>	$R^2$	
10	37.38	0.0044	6.104	0.434	83.67	3.758	0.0025	38.61	0.990	3.29
20	44.52	0.0042	7.12	0.464	84.01	9.124	0.0045	44.84	0.969	0.72
30	47.22	0.0001	4.571	0.0002	90.32	11.69	0.0052	47.39	0.984	0.36
40	57.06	-	-	-	-	41.32	0.0093	66.67	0.997	16.84
10(pure)	22.99	0.0021	4.939	0.303	78.52	4.308	0.0052	28.65	0.986	24.62

increased with increasing initial lactic acid concentrations. This may be due to the large number of adsorbed lactic acid at the available adsorption sites.

The pseudo-first order and pseudo-second order rate constants are presented in Table 2 along with the corresponding correlation coefficients ( $R^2$ ) and the normalized standard deviation ( $\Delta q$ ), which is calculated by the following equation:

$$\Delta q = \sqrt{\frac{(q_{exp} - q_{cal}) / q_{exp}^2}{N - 1}} \quad (8)$$

where the subscripts exp and cal are the experimental and calculated data, respectively and  $N$  is the number of data points [3]. From Table 2, it was observed that the pseudo first-order model did not fit well because the calculated  $q_e$  values did not agree with the experimental  $q_e$  values and the  $\Delta q$  was quite high. This suggests that the adsorption of lactic acid did not follow the first-order kinetics. The pseudo-second order model (Fig. 4) gave good straight lines as com-

pared to the pseudo-first order (Fig. 3). The pseudo-second order rate constants were in the range of 0.0025 to 0.0093 g/(mg min). The theoretical values of  $q_e$  also agree very well with the experimental ones. These suggest that the sorption process could be described using the pseudo second-order kinetic model with higher correlation coefficients and lower normalized standard deviation values than the first-order kinetic model. The parameters  $k_1$ ,  $k_2$  and  $h$  from both models indicate the adsorption rate of activated charcoal that can be applied for further study. Interestingly, the  $q_e$  value of the pure lactic acid solution was around 50% less than that of the fermentation broth. This could be explained by the differences in the chemical potentials and the activity coefficients of the fermentation broth which may be higher than those in the pure solution due to the amount of other components in the solution. That would lead to a higher chemical potential and the adsorption capacity.

In this present case, activated charcoal was used as an adsorbent for the adsorption of low concentration of lactic acid from fermentation broth. The adsorption capacity of 37.4 mg/g activated charcoal was obtained at pH 2, with an initial lactic acid concentration of 10 g/L in the fermentation broth. This result was better than that reported by Yousuf *et al.* [7] which obtained the maximum adsorption capacity of 18.63 mg lactic acid/g activated carbon from the mixture of acid system with an initial lactic acid concentration of 11.66 g/L, at pH 2, temperature 298 K and 5 g of adsorbent. This work presents a simple process to recover lactic acid from the fermentation broth at lower concentration and low pH value. The efficiency of desorption of lactic acid is also another important factor that needs to be considered. However, this is outside of the scope of this study. It has been reported that acetone is an efficient solvent for desorption of lactic acid from activated charcoal [14]. With the ratio of acetone to activated charcoal at 4, the recovery efficiency of lactic acid at around 80% could be achieved. The acetone in lactic acid solution can be subsequently removed under vacuum. In addition, a long-term performance of the activated charcoal is another important factor that needs to be evaluated in the further study.

## CONCLUSIONS

In this work, a possible approach to easily separate lactic acid from the fermentation broth in the form of undissociated acids was presented. Lactic acid was much more adsorbed on to the adsorbent than other components such as acetic acid and sucrose in the fermentation broth. Adsorption isotherm data of lactic acid could be well explained by the Freundlich model. The kinetic experimental data correlated well with the pseudo-second order kinetic

more than the pseudo-first order kinetic model. The maximum adsorption capacity of 57 mg/g adsorbent was achieved at pH 2.

## Acknowledgement

This work was financially supported by The Research and Creativity Fund, Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University.

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NAUČNI RAD

## KINETIKA I RAVNOTEŽA ADSORPCIJE MLEČNE KISELINE IZ FERMENTACIONE TEČNOSTI NA AKTIVNOM UGLJU

*Aktivni ugalj je primenjen za izdvajanje mlečne kiseline u nedisociranom obliku iz fermentacione tečnosti. Mlečna kiselina je dobijena fermentacijom kiselog hidrolizata Jerusalim artičoke, koji je izvor ugljenika, korišćenjem Lactobacillus casei TISTR1340. Analizirana je ravnotežna adsorpciona izoterma i kinetika izdvajanja mlečne kiseline. Eksperimentalni podaci za adsorpciju mlečne kiseline iz fermentacione tečnosti najbolje su opisani Frojndlihovom izotermom, a kinetika modelom pseudo-drugog reda sa vrednostima  $R^2$  od 0,99. Početna brzina adsorpcije je bila 41,32 mg/(g·min) pri početnoj koncentraciji mlečne kiseline od 40 g/L.*

*Ključne reči: mlečna kiselina, aktivni ugalj, kinetika adsorpcije.*