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1 **Anaerobic Digestion of Corn Stover Pretreated with Sulfuric Acid in Different Soaking**
2 **Durations**

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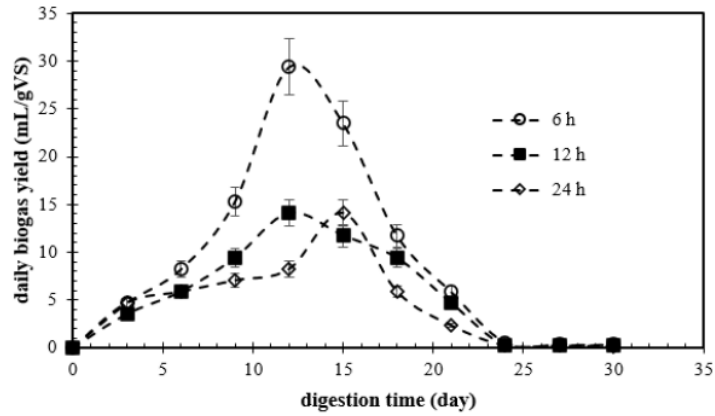
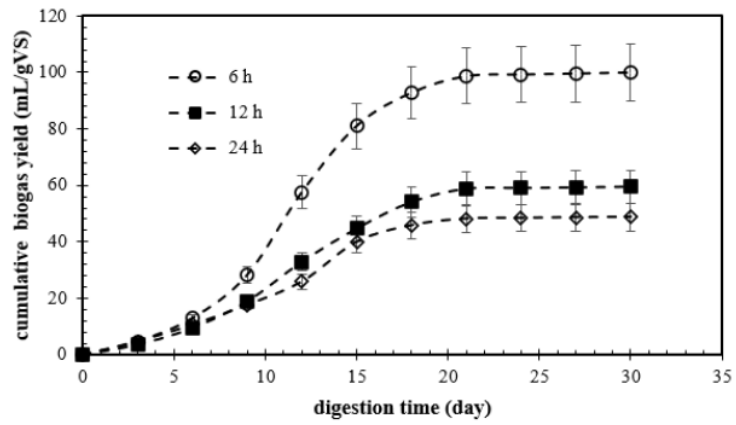
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12 GRAPHICAL ABSTRACT



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23 **ABSTRACT**

24 The biogas production of pretreated corn stover has been determined in different soaking durations.

25 Batch anaerobic digestion applies three different soaking durations in sulfuric acid pretreatment under

26 room temperature. The study aimed to probe the effect of soaking durations during sulfuric acid

27 pretreatment. The observed cumulative biogas yields varied between 48.74 mL/g VS and 99.95 mL/g

28 VS. The highest biogas yield was obtained when corn stover was soaked in sulfuric acid for 6 hours.

29 The 24 h-pretreated corn stover got the lowest biogas yield. The statistical result proved a significant

30 effect of soaking durations on biogas production ($p < 0.05$). The logistic model provided a better fit

31 than the first-order model, with R^2 values ranging from 0.9923 to 0.9987 and divergence between

32 experimental and predicted values varying between 0.12% and 1.48%.

33 **Keywords:** acid pretreatment; biogas; first-order model; kinetic; logistic model

34 **1. Introduction**

35 ¹² Anaerobic digestion is a process that converts biodegradable organic material into biogas through
36 biochemical stages, which are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Hajji &
37 Rhachi, 2022). Its advantages are that it reduces the odour and the size of organic waste, diminishes
38 greenhouse gas emissions, and produces a renewable fuel (Mirtsou-Xanthopoulou et al., 2019).
39 Anaerobic digestion of corn stover has been a broad topic of discussion for renewable fuel production
40 in the form of biogas. However, challenges are faced in treating corn stover as a biogas feedstock
41 because of its characteristic as lignocellulosic biomass. Lignocellulose comprises cellulose bundles
42 scattered with bundles of hemicellulose and lignin. This composition creates complex biodegradation
43 leading to low biogas yield and longer retention times (Fernández-Rodríguez et al., 2022). A
44 Pretreatment stage is needed to change the biomass structures. It also intends to split lignin from
45 cellulose and hemicellulose, decrease cellulose crystallinity and enhance biomass porosity (You et
46 al., 2018).

47 Pretreatment can be run by chemical, physical or biological pretreatment; nevertheless, chemical
48 pretreatment becomes one of the most applied methods due to its high carbohydrate solubility
49 efficiency (Alino et al., 2022). Taherdanak et al. (2016) reported that dilute sulfuric acid pretreatment
50 enhanced biogas production and improved methane yield by 8.9% during the anaerobic digestion of
51 wheat plants. Dahunsi et al. (2019) also stated that sulfuric acid pretreatment significantly reduced
52 hemicellulose and partial cellulose solubilization. Furthermore, Domański et al. (2020) investigated
53 that methane yield increased with increasing sulfuric acid concentrations during methane production
54 of rye straw. Therefore, this study chose sulfuric acid pretreatment as chemical pretreatment for corn
55 stover.

56 Based on past literature, the previous authors still need to study the biogas production from corn
57 stover using sulfuric acid pretreatment. Olugbemide et al. (2020) produced biogas from corn stover
58 without chemical pretreatment. Jie et al. (2020) compared mass ratio during anaerobic ²¹ co-digestion
59 of corn stover and cattle manure. Ajayi-Banji et al. (2020) investigated biogas production from corn

60 stover with daily manure in different particle sizes of corn stover; thus, this study was original and
61 novel. ⁶ This study aims to investigate the effect of soaking durations in sulfuric acid pretreatment on
62 the biogas production of corn stover. The kinetic model was also evaluated in predicting biogas
63 production and determining the equivalent kinetic parameters.

64 ¹³ **2. Materials and methods**

66 *2.1. Substrate and inoculum preparation*

67 Corn stover was collected from Yogyakarta, Indonesia. Corn stover was dried, milled into 2-3 mm
68 mesh sieve lengths by a hammer mill, and then stored at room temperature. The bovine rumen fluid
69 was used as inoculum.

70 *2.2. Sulfuric acid pretreatment*

71 Dried corn stover was pretreated with sulfuric acid (10% w/v) at 121°C at three different soaking
72 times of 6, 12, and ¹⁶ 24 h. Then, the pretreated corn stover was cooled and kept at room temperature
73 until use.

74 ⁶ *2.3. Anaerobic digestion process*

75 The pretreated corn stover was mixed with inoculum, and then the mixture was fed into a batch
76 digester. The total working volume of each digester was 600 mL with the addition of water. The batch
77 test ¹⁵ was conducted at room temperature. The daily biogas volume was measured using the water
78 displacement method every three days.

79 **2.4. Kinetic model**

80 *2.4.1. First-order kinetic model*

81 Anaerobic digestion assumes hydrolysis as a rate-limiting reaction, mainly when breaking down
82 solid matter, and the degradation of the substrate may follow a first-order rate (Marañón et al.,
83 2021). The production of biogas is written below:

$$84 \quad B = B_0(1 - e^{-kt}) \quad (1)$$

85 Where, B is the cumulative biogas yield at time t (mL/gVS), B₀ is the biogas potential of the
86 substrate (mL/gVS), k is the first-order biogas production rate constant (1/day), t is digestion time
87 (days)

88 2.4.2. Logistic model

89 The logistic model represents a linear correlation between specific growth rate and biomass
90 concentration. This model was used to express cell growth kinetics by way of the deviation of
91 growth from the exponential ratio (Habchi et al., 2022).

$$92 \quad B = \frac{B_0}{\left[1 + \exp\left\{\frac{4R_m(\lambda - t)}{B_0} + 2\right\}\right]} \quad (2)$$

93

94 ⁸ R_m is the maximum biogas production rate (mL/gVS/d), λ is the lag phase time (days).

95 ² 2.5. Statistical analysis

96 The significant deviation was determined using analysis of variance (ANOVA) with a p-value less
97 than 0.05. Non-linear regression analysis was operated using solver Microsoft Excel to determine
98 R_m, k, λ, and the predicted biogas potential. Microsoft Excel also implemented the coefficients of
99 determination (R²) and root means square error (RMSE).

$$100 \quad RMSE = \sqrt{\frac{\sum_{i=1}^n (Y_{exp,i} - Y_{mod,i})^2}{n}} \quad (3)$$

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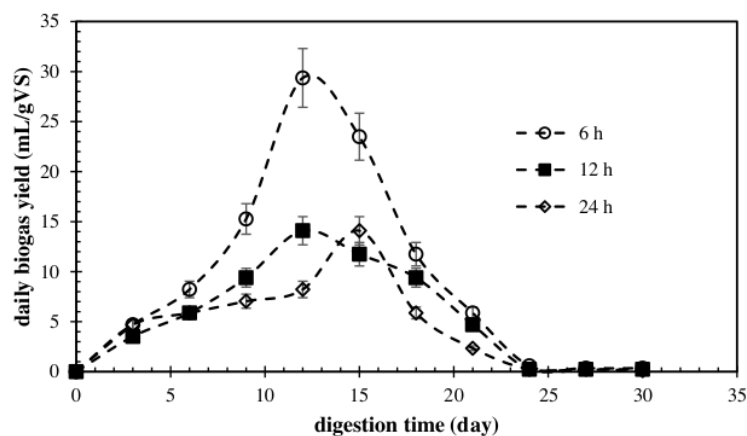
102 Y_{exp,i} is the biogas yield obtained from the experimental results, Y_{mod,i} is the biogas yield obtained
103 from the model, and n is the number of observations

104 3. Results and Discussion

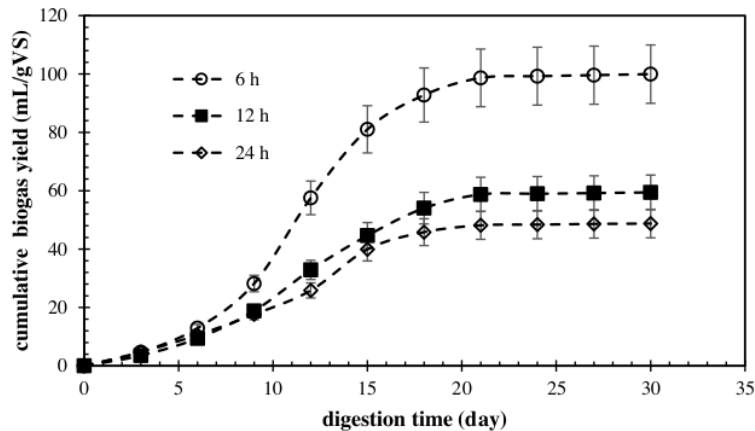
105 3.1. Effect of pretreatment soaking on biogas production

106 The pretreated corn stover samples were subjected to batch anaerobic digestion, and the results of
107 daily biogas production are illustrated in Figure 1. The initial biogas production from the 6 hours and
108 24 pretreated corn stover was the same in this period. (4.70 mL/g VS). The 12 h- pretreated corn

109 stover gained the lowest initial biogas yield of 3.52 mL/g VS Biogas production increased gently
110 from day 3 to day 12. During the 12 days of anaerobic digestion, 29.36 mL/g VS, 14.09 mL/g VS,
111 and 8.22 mL/g VS of peak biogas yields were obtained from the 6 h, 12 h, and 24 h-pretreated corn
112 stover samples, respectively. Afterwards, biogas production dropped gradually from day 15 to day
113 30.



114 **Figure 1.** Daily biogas yield of pretreated corn stover at the soaking durations of 6 h, 12 h, and 24
115 h, respectively
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117
118 The cumulative biogas yield is presented in Figure 2. The highest biogas yield of 99.95 mL/g VS was
119 obtained from the 6 hours-pretreated corn stover, which was 68% higher than the 12-hour-pretreated
120 corn stover (59.43 mL/g VS). Meanwhile, the cumulative biogas yield of 12 hours of corn stover was
121 22% higher than the 24 hours of corn stover. Pretreatment for 6 hours led to the highest cumulative
122 biogas yield, indicating that the decomposition of corn stover was easily degradable in soaking
123 pretreatment of 6 hours.



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125 **Figure 2.** Cumulative biogas yield of pretreated corn stover at the soaking durations of 6 h, 12 h,
 126 and 24 h, respectively
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129 As seen in Figure 2, biogas production decreased when the pretreatment time increased. This
 130 phenomenon might attribute to the loss of dry material during the pretreatment, which led to the
 131 reduction of feed for microbes, as a result, the biogas production declined when extending the
 132 pretreatment duration (Zheng et al., 2010). The statistical result showed that soaking durations in
 133 sulfuric acid pretreatment affected biogas production significantly ($p < 0.05$).

134 3.2. Effect of pretreatment soaking on pH stability

135 The steadiness of the biodegradation process can be checked from the pH value. A pH value is one
 136 of the important parameters affecting the performance of biogas production. The pH value
 137 generated by pretreated corn stover is depicted in Figure 3.

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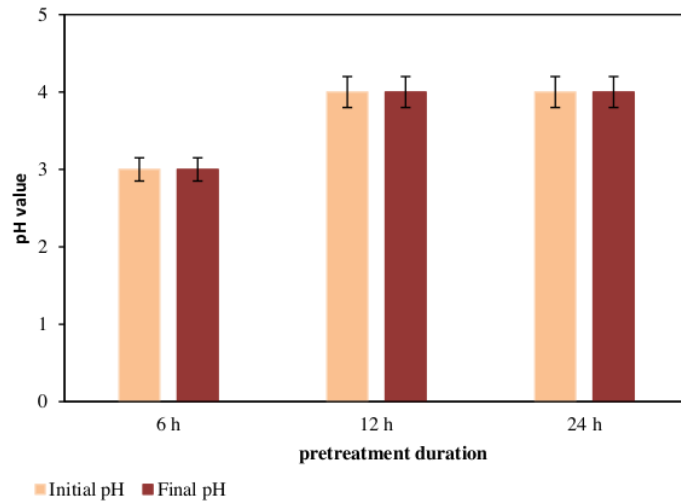


Figure 3. The initial and final pH of pretreated corn stover

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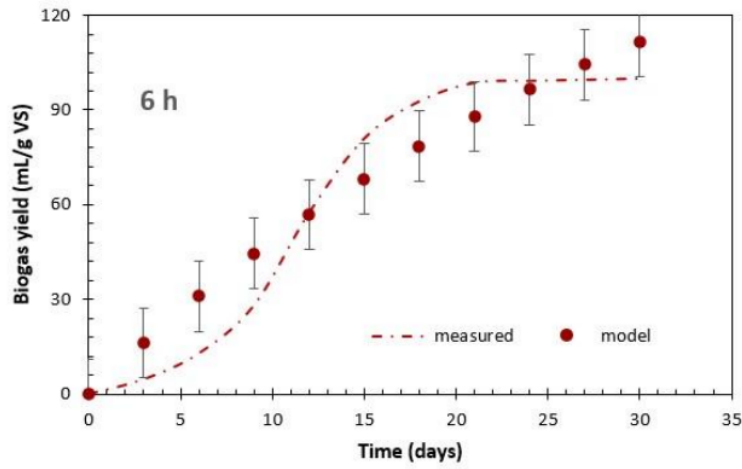
142 The result showed that the longer pretreatment led to a lower pH. The initial pH of 6 h-pretreated
 143 corn stover was slightly lower than the 12 h and 24 h- pretreated corn stover. Pretreatment at lower
 144 pH generated greater yield signifying a higher degree of solubilization of complex organic material
 145 into particular monomers (Dasgupta & Chandel, 2020). Therefore, pretreatment of 6 hours produced
 146 greater biogas yield than pretreatments of 12 hours and 24 hours (see Figure 2). The final pH of
 147 digestates remained constant with the initial pH for all different soaking durations. It indicates that
 148 the biogas performance was stable.

149 3.3. Effect of pretreatment soaking on kinetic parameters

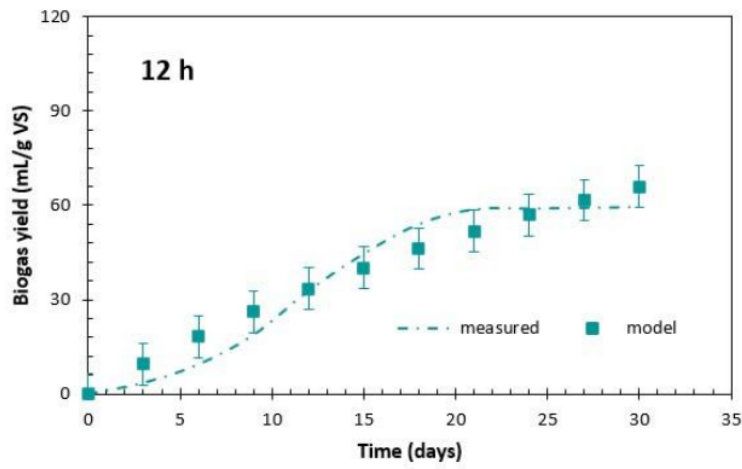
150 ¹⁸ The kinetic parameters obtained from the models are summarized in Table 1. The first-order kinetic
 151 and logistic models performed well with the determination coefficients (R^2) higher than 0.9. The
 152 logistic model got a higher R^2 (0.9923-0.9987) compared to the first-order kinetic model (0.9752-
 153 0.9884). The logistic model demonstrates less difference between the predicted and measured yield
 154 (0.12-1.48%).

155 Figures 4 and 5 the results of the non-linear fitting of the model for the soaking durations employing
 156 ⁵ the first-order kinetic model and the logistic model, respectively. The logistic model fitted the
 157 experimental results more closely than the first-order model. The maximum biogas production rate

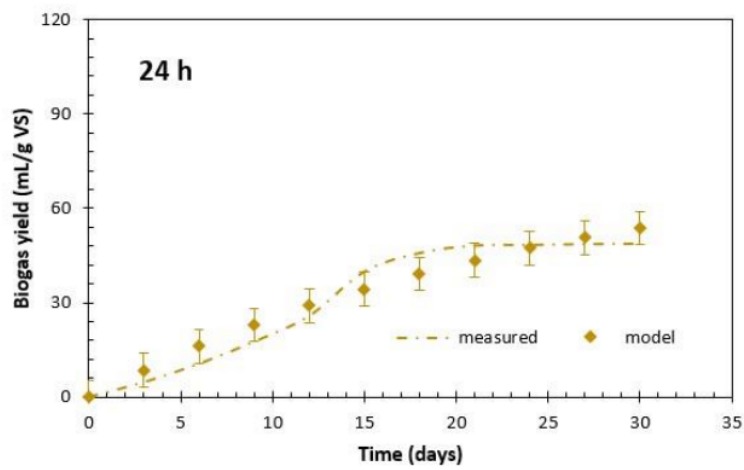
158 (R_m) values varied between 3.93 and 28.94 mL/g VS/d. The highest R_m was obtained by the 6 h-
159 pretreated corn stover, while the lowest R_m was obtained by the 12 h-pretreated corn stover. The first-
160 order kinetic model's biogas rate constant (k) varied between 0.029 and 0.038 day⁻¹. The highest k
161 was estimated for the 24-h pretreated corn stover. This result is contrary to the logistic model, which
162 estimated the 6 h-pretreated corn stover obtained the highest R_m .



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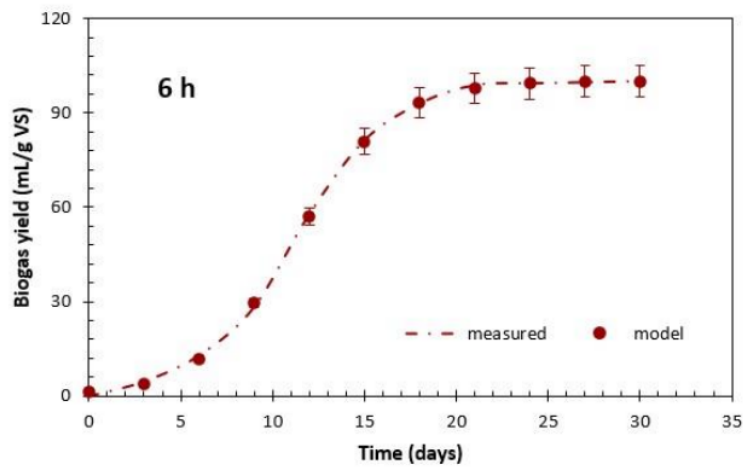


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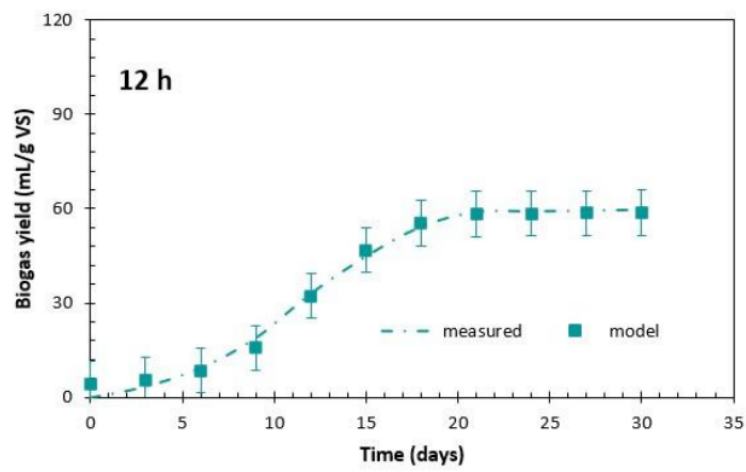


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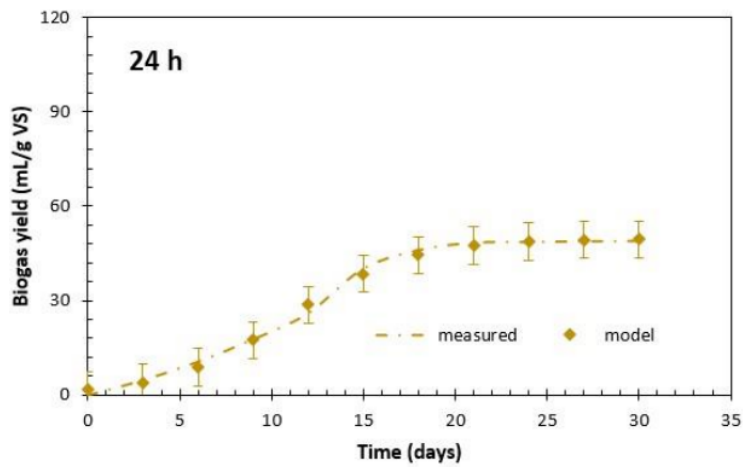
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Figure 4. Biogas yield using the first-order kinetic model



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Figure 5. Biogas yield using the logistic model

178 The lag phase time (λ) of the three pretreated corn stovers ranged from 14.12-47.56 days. (Otohrise
179 et al., 2022) discovered the values of λ within 5.3-9.6 days when applying the logistic model to the
180 anaerobic digestion of goat dung and pawpaw seed. (Opurum, 2021) reported lower λ (1.39-4.05 days)
181 obtained from the batch anaerobic digestion of cabbage waste. Results may diverge due to the reliance
182 on numerous factors and variables influencing anaerobic digestion, such as substrates, pH, inoculum,
183 co-digestion, substrate/inoculum (S/I) ratio, and types of pretreatment (Casallas-Ojeda et al., 2021).

184 **Table 1.** Kinetic parameters model for First-order and Logistic for different soaking durations

Model	Soaking duration (hours)	¹⁹ B_0 (mL/g VS)	k (day ⁻¹)	λ (day)	R_m (mL/g VS/d)	R ²	RMSE	Difference
First-order	6	187.27	0.030	Not calc.	Not calc.	0.9844	11.19	10.36%
	12	112.42	0.029	Not calc.	Not calc.	0.9851	5.63	9.88%
	24	78.68	0.038	Not calc.	Not calc.	0.9752	4.38	9.27%
Logistic	6	300.43	Not calc.	14.12	28.94	0.9963	0.78	0.12%
	12	199.22	Not calc.	47.56	3.93	0.9923	1.95	1.16%
	24	148.85	Not calc.	14.54	11.47	0.9987	1.34	1.48%

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The RMSE value ranged from 4.38 to 11.19 for the first-order kinetic model and 0.78 to 1.95 for the
logistic model. Based on the statistical values, it can be evaluated ¹¹ that the logistic model gave a better
fit to the experimental results with the higher R² and smaller RMSE values.

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