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Full Length Article

Enzymatic hydrolysis of microalgal cellulose for bioethanol production, modeling and sensitivity analysis

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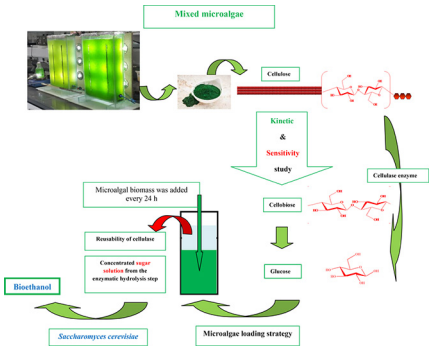
GRAPHICAL ABSTRACT



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ABSTRACT

A kinetic model was developed to describe the enzymatic hydrolysis of microalgal cellulose. In the kinetic model, the factors including product inhibition, temperature, and pH were considered. This model combined two reactions for hydrolyzing algal cellulose to cellobiose and glucose and one reaction for cellobiose breakdown to glucose. Results showed that the highest glucose yield (57%) was achieved at microalgal biomass concentration of 50 g/L, pH 5, and temperature of 50 °C. Moreover, the sensitivity analysis was carried out on each kinetic model parameter. This analysis indicated that k'_3 and k_{m3} in reaction R_3 (cellobiose to glucose) are the most influential parameters during enzymatic hydrolysis of algal cellulose.

Finally, the microalgal biomass loading experiment demonstrated that cellulase could be used thrice without compromising on the glucose yield. Fermentation of concentrated sugar medium with *Saccharomyces cerevisiae* produced ethanol (12.87 g/L) with yield (0.46 g ethanol/g glucose).

1. Introduction

Bioethanol is one of the renewable energy sources that can reduce fossil fuel consumption and environmental pollution [1,2]. Agricultural materials such as starch, corn, rice, wheat, sugar cane, sugar beet, and sweet sorghum are not prospective selections for bioethanol production as the human request for food has yet to be met [3–5]. Lately, process technologies for conversion of other feedstock such as microalgae into biofuel have been considered. Algae grow quickly without need of soil and absorb carbon dioxide from atmosphere for photosynthesis process [6]. In addition, microalgae have short harvesting cycle compared with other feedstocks, which are harvested once or twice a year [4,7,8]. Some studies have reported high levels of carbohydrate accumulation up to 54.3% by microalgae under nitrogen limiting conditions [9,10].

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Table 1
The reaction rates and mass balances.

Stage	Rates	Mass Balances
Cellulose → Cellobiose	$R_1 = \frac{A_1 . e^{\left(-\frac{E_{a1}}{R.T}\right)} . [E_{Cellulases}] . [C]}{\left[\left(1 + \frac{[G_2]}{k_{1IG2}} + \frac{[G]}{k_{1IG}}\right) + [C]\right] . \left[1 + \frac{K_{a2}}{[H^+]} + \frac{[H^+]}{K_{a1}}\right]}$	Cellulose: $\frac{d[C]}{dt} = -R_1 - R_2$
Cellulose → Glucose	$R_2 = \frac{A_2 . e^{\left(-\frac{E_{a2}}{R.T}\right)} . [E_{Cellulases}] . [C]}{\left[\left(1 + \frac{[G_2]}{k_{2IG2}} + \frac{[G]}{k_{2IG}}\right) + [C]\right] . \left[1 + \frac{K_{a2}}{[H^+]} + \frac{[H^+]}{K_{a1}}\right]}$	Cellobiose: $\frac{d[G_2]}{dt} = 1.056R_1 - R_3$
Cellobiose → Glucose	$R_3 = \frac{A_3 . e^{\left(-\frac{E_{a3}}{R.T}\right)} . [E_{Cellulases}] . [G_2]}{\left[k_{m3} . \left(1 + \frac{[G]}{k_{3IG}}\right) + [G_2]\right] . \left[1 + \frac{K_{a2}}{[H^+]} + \frac{[H^+]}{K_{a1}}\right]}$	Glucose: $\frac{d[G]}{dt} = 1.116R_2 + 1.053R_3$
Reaction rate constants	$k_i' = \frac{A_i . e^{\left(-\frac{E_{ai}}{R.T}\right)}}{1 + \frac{K_{a2}}{[H^+]} + \frac{[H^+]}{K_{a1}}}$ (i= 1, 2, 3)	

Table 2
The estimated values of parameters of the model.

Parameter	Temperature (condition: pH 5)			pH (condition: Temperature 50 °C)		
	30 °C	40 °C	50 °C	4	5	6
k'_1 (h^{-1})	1.12	3.18	8.76	3.56	8.76	2.73
k'_2 (h^{-1})	1.30	4.10	10.71	4.35	10.71	3.34
k'_3 (h^{-1})	1.10	3.80	8.60	3.49	8.60	2.68

Carbohydrates in microalgal biomass are chiefly cellulose without lignin. The absence of lignin makes hydrolysis of microalgae easier compared to lignocellulosic materials [11].

Some studies have examined the ethanol production using the pure culture of macroalgae [12–15] and microalgae [16–18]. Cultivation of microalgae in pure culture will increase operating costs because of the need for the sterile condition. Therefore, usage of a mixed microalgal culture dominates these problems [19,20]. Hydrolysis process for carbohydrates extraction from algae can be performed using acids or enzymes. Enzymatic hydrolysis has various advantages compared to acid hydrolysis, including low utility consumption, less corrosion problems, higher glucose yield without sugar-degradation and inhibitory products production. In contrast, the enzymatic hydrolysis increases the cost of producing ethanol [21,22]. Some researchers have investigated carbohydrate extraction from a pure culture of algae [12–18,23]. Besides, Hwang et al. investigated the different pre-treatments of both filamentous and cyclotella algal cells for bioethanol production [24]. To author's awareness, there is only one research on the kinetics of enzymatic hydrolysis algal cellulose by R. Harun and M. K. Danquah [25] in which the kinetics parameters of hydrolysis was obtained using Michaelis–Menten's model. However, their study did not consider pH effects on enzyme kinetics and sensitivity analysis of kinetic parameters. In addition, their study investigated the kinetics of enzymatic hydrolysis of a pure culture of algae of *Chlorococcum* sp.

In the present study, the kinetic modeling and sensitivity analysis of enzymatic hydrolysis of microalgal cellulose using cellulase was studied. Furthermore, the microalgae loading strategy was used to obtain a concentrated sugar solution from the enzymatic hydrolysis step, leading to high volumetric bioethanol productivity, the principal factor affecting the cost-effectiveness of full-scale applications. Subsequently, the bioethanol yield of the fermentable sugars derived from enzymatic hydrolysis step was investigated through cultivating *Saccharomyces cerevisiae* yeast.

2. Materials and methods

2.1. Microalgae

Mixed microalgae (collected from a freshwater located in Oskou, East Azarbayjan, Iran) have been precultured in our previous study [26], and then inoculated into glass photobioreactor with a working volume of 10 L, illuminated with eight white LED lamps with a light intensity of about 260 μmol. m^{-2}. s^{-1}. The aeration rate in the photobioreactor was 8 vvm. The temperature and pH were 25 ± 1 °C and 9, respectively. When the nitrogen source in the photobioreactor medium was depleted, the CO_2 was bubbled into the medium with the rate of 0.1 vvm. The medium composition of microalgae was the same as explained in our previous paper [26].

At the end of the cultivation, the algae were harvested from the photobioreactor and then oven dried at 80 °C for 48 h. These dried algae were ground into powder by Planetary Ball Mill and consumed for enzymatic hydrolysis by cellulase.

2.2. Enzymatic hydrolysis of microalgal cellulose

Cellulase from *Trichoderma reesei* obtained from Novozymes, Denmark was used for enzymatic hydrolysis of algal cellulose. The enzymatic activity of this enzyme was 0.04 U/mg corresponding to 0.054 mg protein/mg. For the enzymatic hydrolysis experiments, the dried algal powder was loaded into citrate buffer with the concentration of 25, 50, 75, and 100 g/L. These samples were autoclaved at 121 °C for 15 min and then mixed with constant cellulase concentration (0.416 mg protein/mL) in a 100 mL-Erlenmeyer flask with 50 mL working volume. The effects of variation of temperature in the range of 30–50 °C and pH in the range of 4–6 on enzymatic hydrolysis were investigated. The Erlenmeyer flasks were incubated in a shaker at 150 rpm for 72 h. The analysis samples were taken at different times during enzymatic hydrolysis of algae. These samples were centrifuged at 4000 × *g*, then the supernatant was taken and cellobiose and glucose concentration were measured.

2.3. Bioethanol production

For bioethanol production, *S. cerevisiae* has been precultured in our previous study [26]. After microalgal biomass loading experiment, pH of the microalgae hydrolysate was adjusted to 6.5 to achieve an appropriate pH range for bioethanol production. Afterwards, 10% V/V of yeast pre-culture cells were added in the fermentation medium, which contained hydrolysates of microalgae. The fermentation experiment was performed anaerobically at 30 °C shaken at 150 rpm for 24 h.