

1. Calculations

1.1 the amount of glucose, ethanol yield and polysaccharide utilization ratio

The amount of glucose released by enzymes at certain times gives a way of comparing different enzymes. The amount of glucose released by amylolytic enzymes (cellulolytic enzymes) at 25 hours (24 hours) from 1 kg CR was calculated as followed:

$$\text{The amount of glucose (g per 1kg CR)} = \frac{\text{Glucose(g/L) at 24 h or 25 h} \times \text{Volume(L)}}{\text{Initial CR (kg)}}$$

Ethanol yield was calculated assuming that 1 g of cellulose or starch present in the liquid theoretically gave 0.568 g of ethanol. Ethanol yield was expressed as the percentage of the theoretical yield based on total available sugars. Ethanol yields in different scenarios were calculated as followed:

$$\text{Ethanol yield in scenario A(\%)} = \frac{\text{Ethanol(g/L)} \times \text{Volume(L)} / 0.568}{\text{Initial CR (g)} \times 0.583 + \text{Initial FR (g)} \times 0.482} \times 100\%$$

$$\text{Ethanol yield in scenario B(\%)} = \frac{\text{Ethanol(g/L)} \times \text{Volume(L)} / 0.568}{\text{Initial CR (g)} \times 0.472 + \text{Initial corn (g)} \times 0.752} \times 100\%$$

$$\text{Ethanol yield in scenario C (\%)} = \frac{\text{Ethanol(g/L)} \times \text{Volume(L)} / 0.568}{\text{Initial CR (g)} \times 0.724 + \text{Initial FR (g)} \times 0.482 + \text{Initial Corn (g)} \times 0.86} \times 100\%$$

Polysaccharide utilization ratio compared the three scenarios in term of how good they make use of polysaccharides in materials. Assuming that 1 g cellulose/starch theoretically gave 0.568 g of ethanol, polysaccharide utilization ratio was calculated as followed:

$$\text{Polysaccharide utilization ratio(\%)} = \frac{\text{Ethanol(g/L)} \times \text{Volume(L)} / 0.568}{\text{Initial CR (g)} \times 0.724 + \text{Initial FR (g)} \times 0.482 + \text{Initial Corn (g)} \times 0.86} \times 100\%$$

Where volume in the five equations above was calculated assuming that 0.1 g of water was used to produce 1 g of glucose from glucan.¹

1.2 cellulose concentration, starch concentration and protein

A better understand of the relationship between the ratio of each material and the concentrations of each chemical component will provide a way of achieving these optimum concentrations. At a total substrate loading of 20%, cellulose concentration (C_C) was theoretically computed using the following equation:

$$C_C = f_{FR}L_{FR} + f_{CR}L_{CR} + f_{Corn}L_{Corn} \quad (1)$$

Where L_i is the loading of material i and f_i is the cellulose content in material i . Substituting $L_{Corn}=20-L_{CR}-L_{FR}$, $f_{FR}= 47.1\%$, $f_{CR}=25.0\%$, and $f_{Corn}=11.9\%$ in Eq. (4):

$$C_C = 2.38 + 0.13L_{FR} + 0.36L_{CR} \quad (2)$$

Similarly, for starch concentration (C_S) and protein concentration (C_P)

$$C_S = 15 - 0.28L_{FR} - 0.75L_{CR} \quad (3)$$

$$C_P = 1.49 - 0.037L_{FR} - 0.075L_{CR} \quad (4)$$

2. The detailed description of the Fig. 5

Fig. 5 the concentration range of cellulose and starch in scenario C. (A), the green line presents using sole FR as substrate. In the start and end of the line the loading of FR is respectively 0% and 7%. The grey line presents using sole CR as substrate and the loading of CR ranges from 0% to 20%; (B), the grey line presents using sole corn as substrate and the loading of corn ranges from 0% to 20%; (C), the grey area presents using the mixture of CR and FR as substrates. In this fig. C, FR loading ranged from 0% to 7% while CR loading ranged from 0% to 20%. The red line on the edge of grey area presents that the total substrate loading is 20% and the blank line in grey area presents that the total substrate loading is 10%. In the top edge of grey area, FR loading is 7% while the total substrate loading is not fixed. In the bottom edge of grey area we have 100% of CR, while in the edge in x-axis we have 100% of FR; (D), the grey area presents using the mixture of CR and corn as substrates. In Fig. 5D, both of CR loading and Corn loading ranged from 0% to 20%. The red line on the edge of grey area presents that the total substrate loading is 20% while the ratio of CR and corn is not fixed. In the top edge of grey area, we have 100% of CR and the total substrate loading is not fixed, ranging from 0% to 20%. In the bottom edge of grey area we have 100% of Corn; (E), the grey area presents using the mixture of FR and Corn as substrates, which could be got by vertical translation of grey line in Fig. 5B along with the green line in Fig. 5A. In Fig 5E, FR loading ranged from 0% to 7% and Corn loading ranged from 0% to 20%. The red line on the edge of grey area presents that the total substrate loading is 20%. In the top edge of grey area, FR loading is 7% while the total substrate loading is not fixed, ranging from 0% to 20%. In the bottom edge of grey area we have 100% of Corn and in the last edge in x-axis we have 100% of FR; (F), the grey area presents using the mixture of FR, CR and Corn as substrates, the grey area could be got by putting Fig. 5C, Fig. 5D and Fig. 5E together. The red area on the edge of grey area presents that the total substrate loading is 20% and the ratio of the three materials is changing. In the top edge of grey area, FR loading is 7% and the loading of CR ranged from 0% to 13%. In the bottom edge of grey area we have 100% of Corn, and in the last edge in the x-axis, we have 100% of FR whose loading is from 0% to 7%.

Reference

1 Y. Zhu, M. Malten, M. Torry-Smith, J. D. McMillan and J. J. Stickel, *Bioresour Technol*, 2011, 102, 2897-2903.