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Optimal trade-off design of integrated fermentation processes for ethanol production using genetically engineered yeast

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ABSTRACT

In this study, we considered a multi-stage integrated extractive fermentation with cell recycling for ethanol production using the genetically engineered *Sacchromyces* yeast 1400 (pLNH33), which can utilize glucose and xylose as carbon sources to produce ethanol. Each stage consists of a stirred-tank bioreactor, a cell settler and an extractor. A generalized mathematical model was formulated to express the multi-stage integrated process. The aim of the optimization problem was to obtain the maximum overall productivity and conversions subject to the interval inequality constraints for the residual glucose and xylose concentrations and the total sugar supply. A fuzzy goal attainment method was applied to the multiobjective problem in order to achieve the maximum satisfaction for all design requirements. From the computational results, the integrated extractive fermentation with cell recycling (involving the extraction of ethanol from the extractor *in situ* to alleviate product inhibition) led to an optimal overall productivity that was 8.0% higher than that obtained by the method of continuous fermentation with cell recycling, and about 13-fold higher than that obtained by the method of continuous fermentation without cell recycling.

1. Introduction

Ethanol, one of the most important bio-fuels, can be produced by converting the sugar content of raw materials (e.g., corn, potatoes, beets, sugarcane, and wheat) to alcohol [1–4]. Today, there is heightened interest in ethanol as a transportation fuel. Ethanol production from renewable resources can improve energy security, reduce the accumulation of carbon dioxide, and decrease urban air pollution. When blended with gasoline, "neat" ethanol, as opposed to petroleum, would aid in stabilizing the concentration of smog-forming compounds in the atmosphere. Obtaining ethanol from lignocellulosic materials holds great promise as a new industry in the world and has the potential for making a significant contribution to the solution of major renewable energy and environmental problems [5–8].

Lignocellulosic feedstocks like wood, waste paper, agricultural residues and fast-growing energy crops have been identified as economical starting materials for ethanol production. These raw materials contain glucose and xylose as the major fermentable sugars. Although production of ethanol from the fermentation of hexose and pentose has been studied for many years, there are still several bottlenecks for the economical production of fuel ethanol. The fermentation of xylose to ethanol represents the main bottleneck in the production process. Several articles have reported the development of genetically engineered strains that utilize pentose and hexose as substrates in the production of ethanol [9–12].

Achieving a high ethanol production rate requires high cell concentrations in the bioreactor and maximization of the dilution rate. Continuous fermentation can increase productivity; however, it cannot be carried out in high cell density culture, which results in a low ethanol concentration and a significant loss of residual substrate [13]. To increase the efficiency of the ethanol fermentation process, various cell culture methods have been investigated [14–17]. A cell-recycling fermentation coupled with membrane filtering modules (for achieving a higher ethanol concentration) has gained considerable interest in recent years [18–21]. However, a high ethanol concentration may poison viable microorganisms and abrogate the fermentation process. Extractive fermentation is an alternative technique used to reduce end product inhibition by removing the fermentation product *in situ*. This technique is very simple and can be easily implemented with a large-scale fermentation system [22–26]. However, the toxicity of the organic solvent used in the removal of the end product is always a problem [27]. A biocompatible solvent should be employed to alleviate the poisoning of the microbe [28].

Lin and Wang [29] have introduced a multi-stage, integrated continuous fermentation process; each stage consists of a mixed tank, a bioreactor, a cell-recycling unit and an extractor used to pro-

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Nomenclature

b_l bleed ratio at the lth stage bioreactor
D_1 dilution rate at the first stage (h^{-1})
$D_{E,l}$ solvent dilution rate at the lth stage (h^{-1})
E_l extraction efficiency at the lth stage
F_l feed flow rate at the lth stage (m^3/h)
$F_{E,l}$ solvent flow rate at the lth stage (m^3/h)
f_k objective function and inequality constraint, $k = 1, \ldots, 6$
K extractive distribution coefficient
K_g, K_x saturation coefficient for cell growth on glucose and xylose, respectively (kg/m^3)
K'_g, K'_x saturation coefficient for ethanol production on glucose and xylose, respectively (kg/m^3)
K_{ig}, K_{ix} inhibition coefficient for cell growth on glucose and xylose, respectively (kg/m^3)
K'_{ig}, K'_{ix} inhibition coefficient for ethanol production on glucose and xylose, respectively (kg/m^3)
p_l ethanol concentration at the lth stage (kg/m^3)
p_{mg}, p_{mx} maximum ethanol concentration for cell growth on glucose and xylose, respectively (kg/m^3)
p'_{mg}, p'_{mx} maximum ethanol concentration for ethanol production on glucose and xylose, respectively (kg/m^3)
$s_{g,l}$, $s_{x,l}$ glucose and xylose concentration at the lth stage (kg/m^3)
s_{f_l} feed sugar concentration at the lth stage (kg/m^3)
$s^L_{g,r}$, $s^U_{g,r}$ lower and upper bounds of the residual glucose (kg/m^3)
$s^L_{x,r}$, $s^U_{x,r}$ lower and upper bounds of the residual xylose (kg/m^3)
s^L_t, s^U_t lower and upper bounds for the total sugar supply for the process (kg/m^3 h)
t time (h)
x_l, $s_{g,l}$, $s_{x,l}$, p_l cell, glucose, xylose and ethanol concentration inside the bioreactor at the lth stage (kg/m^3)
$x_{e,l}$, $s_{ge,l}$, $s_{xe,l}$, $p_{e,l}$ cell, glucose, xylose and ethanol concentration in the effluent at the lth stage (kg/m^3)
Y_{p/s_g}, Y_{p/s_x} yield coefficient for ethanol from glucose and xylose, respectively
z operation variables in the optimization problem

Greek symbols

α_l V_l/V_1, the volume ratio of the lth bioreactor to the first bioreactor
β_l F_l/F_1, the ratio of the overall feed flow rate at the lth stage to that at the first stage
$\chi_{g,l}$, $\chi_{x,l}$ glucose and xylose conversions at the lth stage
$\delta_{x,l}$, $\delta_{s,l}$, $\delta_{p,l}$ separation factor for cell, substrate and ethanol at the lth stage
ε_l recycle ratio for the lth cell settler
ϕ_g, ϕ_x power of ethanol inhibition for cell growth on glucose and xylose, respectively
φ_g, φ_x power of ethanol inhibition for ethanol production on glucose and xylose, respectively
η_D aggregation function
$\eta_k(f_k)$ membership function for each of the objective functions
λ linear combination ratio for the fed glucose concentration to the fed sugar concentration
μ_g, μ_x specific cell growth rate for yeast 1400 (pLNH33) on glucose and xylose, respectively
μ_{mg}, μ_{mx} maximum specific growth rate coefficient for yeast 1400 (pLNH33) on glucose and xylose, respectively (h^{-1})
ν_g, ν_x specific ethanol production rate for yeast 1400 (pLNH33) on glucose and xylose, respectively
ν_{mg}, ν_{mx} coefficient of maximum specific ethanol production rate for yeast 1400 (pLNH33) on glucose and xylose, respectively (h^{-1})
π_l ethanol productivity at the lth stage (kg/m^3 h)
$\zeta_{x,l}$ discarded factor for cell at the lth stage
$\zeta_{sg,l}$, $\zeta_{sx,l}$, $\zeta_{p,l}$ condensed factor for glucose, xylose and ethanol at the lth stage

Subscript

* optimal solution

duce lactic acid. A membrane filter was employed in the integrated process so that the filtrate was assumed to be cell-free. In this study, we will introduce a modification to the integrated process that uses the genetically engineered *Saccharomyces* yeast 1400 (pLNH33) to produce ethanol. The yeast 1400 (pLNH33) has a self-flocculating characteristic that enables us to use a cheaper settler as a cell separator to replace the expensive membrane separation unit; the yeast can also utilize xylose and glucose to produce ethanol. In this study, we reformulate the mathematical model to describe characteristics of the integrated extractive fermentation process using hexose and pentose to maximize ethanol productivity. Several design parameters, such as the dilution rate, the fed sugar concentrations and the bleed ratio, should be considered in the integrated process. Sensitivity analysis is applied to determine which operation variables are the most relevant in the process. The fuzzy goal attainment method will be introduced to design the integrated extraction fermentation processes.

2. Process formulation

A schematic drawing of the multi-stage, integrated extractive fermentation process is shown in Fig. 1. Each stage consists of a stirred-tank bioreactor, a cell settler and an extractor. The sterile glucose, xylose and nutrient media are well stirred in the mixing tank to form a homogeneous substrate, which is continuously fed into each bioreactor. The genetically engineered *Sacchromyces* yeast 1400 (pLNH33) [30] can utilize the glucose and xylose to produce ethanol. Small amounts of the outlet of each bioreactor are fed into the next bioreactor, but the rest flows into a cell settler while maintaining a constant temperature of 42 °C throughout. The yeast 1400 (pLNH33) quickly flocculates at that temperature. The density of self-flocculated yeast is greater than that of the broth, so the yeast settle down to the bottom. The original characteristics of the yeast 1400 (pLNH33) are restored and they are recycled back to the bioreactor when a constant temperature of 30 °C is maintained. As a result, the bioreactor can retain a high cell density culture. The clear fluid is overflowed into an extractor to take off the ethanol. A biocompatible solvent, such as an isopropyl pentyl ketone, is added to the extractor to extract ethanol [31]. The solvent should be biocompatible, inert to the reaction, stable under the liquid-phase reaction conditions, easy to separate from ethanol and able to induce phase splitting. The raffinate phase in the extractor, containing some unconverted substrate, ethanol and solvent, is also transferred to the next bioreactor.