1. Introduction

Xylose and glucose are major components of the hydrolysate from pretreated lignocellulosic biomass, so conversion of xylose and glucose into ethanol is very important for cost-effective production1)–3). Pretreatment of lignocellulosic biomass by physical and/or chemical processes forms acetic acid with the desirable sugars. Acetic acid is usually toxic to microorganisms, so acts as a fermentation inhibitor and reduces sugar consumption and ethanol production, and the concentration is well correlated with that of xylose in hydrolysates from lignocellulosic biomass 4). The inhibitory effects of acetic acid are usually compensated and rapid fermentation (≤ 24 h) promoted5)–7) by the use of high cell density of yeasts (≥ 10 g/L dry cell weight (DCW))5)–7) and moderate adjustment of the pH of hydrolysates that contain acetic acid (≥ 5.5).

Carbon dioxide (CO2) in aqueous solution is hydrated to H2CO3 in equilibrium with bicarbonate anion (H2CO3 ⇌ HCO3− + H+). The pKa value at 25 °C for the dissociation reaction is 6.38, and dissolved carbon dioxide (dCO2) is predominantly present in undissociated form at pH 5.510),11). The CO2 molecule is linear and symmetrical, but the C–O bonds are polarized. More than 50% of acetic acid is dissociated as CH3COO− at pH 5.5 in an aqueous solution. Fermentation of hydrolysates or sugar solutions that contained acetic acid increased concentration of CO2 with ethanol formation, but the pH of the fermented broth remained unchanged at pH 5.5. This phenomenon indicates that the equilibrium reaction tends to progress in accordance with the following formula: H2CO3 ⇌ HCO3− + H+ ⇌ CO2 + H2O.

Fermentation using high density of yeasts can be performed successfully without air sparging in an Erlenmeyer flask with a sponge plug, because sponge plugs are superior to dCO2 removal12),13). In contrast, stirred tank reactors remove product gases from air vents, but the bottom area is small with greater depth. The concentration of dCO2 rapidly increases with ethanol formation, so dCO2 accumulates in the fermented broth.

### Keywords
- Candida intermedia 4-6-4T2, Dissolved carbon dioxide, Continuous chemostat fermentation, Air sparging

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The accumulated CO₂ in the headspace of stirred tank reactors can affect the microbial physiology and may form a blanket (“blanket theory”) without proper ventilation[13]. Thus, aeration through the air vents is likely to be insufficient, and air sparging is required for fermentation using high density of yeasts in stirred tank reactors[13,14]. In this study, the depth of solutions in the Erlenmeyer flask was about 20 mm, but the depth in the spinner flasks was about 65 mm. Other evidence suggests that C. intermedia 4-6-4T2 can be grown in a rich medium with sugars in a 2-L Erlenmeyer flask (1 L working volume, 110 rpm, φ = 70 mm) with a sponge plug without antifoam, but not in the same medium using a 2-L fermenter (1 L working volume, k_L,a = 10-30 h⁻¹) with antifoam. We postulated that the CO₂ produced by cell growth was not removed, but rather contacted and formed protein foams in the medium[15].

Previous studies have described the effects of aeration on ethanol production with cell growth using the volumetric mass transfer coefficient (k_L,a) or oxygen transfer rate (OTR)/oxygen uptake rate (OUR) with changes in volume of fermentation solutions and/or agitation speeds[16–18]. These studies identified the limited oxygenation conditions for xylose-fermenting yeasts such as Scheffersomyces stipites, Spathaspora passalidarum, and C. intermedia used in ethanol production with cell growth[19–23].

Hydrolysates from lignocellulosic biomass also contain furan derivatives such as furfural and 5-hydroxymethylfurfural (HMF) as well as acetic acid. We found that furfural and HMF had negligible effects as inhibitors on fermentation using high cell density of C. intermedia 4-6-4T2 (10 g/L (DCW))²⁴. Consequently, we proposed that unidentified inhibitors may be present in hydrolysates pretreated under severe conditions²⁴.

In the present study, we used a synthetic fermentation solution without nitrogen sources containing 20 g/L xylose and 30 g/L glucose (SF solution) with or without acetic acid for fermentation in spinner flasks, in consideration of the differences in fermentation between Erlenmeyer flasks and stirred tank reactors, to minimize the effects of unidentified inhibitors in lignocellulosic biomass. Then, we elucidated the effect of the accumulated CO₂ in the fermented broth with or without acetic acid on ethanol production and sugar conversion using high cell density of C. intermedia 4-6-4T2 with or without air sparging.

2. Material and Methods

2.1. Medium and Culture Conditions

Sugars were purchased from Wako Pure Chem. Ind., Ltd. except for L-arabinose, D,L-arabitol, and antifoam 204 (Sigma-Aldrich). Difco™ yeast extract (YE) and Difco™ yeast nitrogen base without amino acids (YNB) were purchased from Becton-Dickinson. Sponge plugs were purchased from Shin-Etsu Polymer Co., Ltd. The culture methods were described in detail previously. Briefly, an aliquot of C. intermedia 4-6-4T2 maintained in 80 % of Synthetic Defined (SD) minimal medium plus 20 % of glycerol at -80 °C in a deep freezer was transferred to SD minimal medium containing 20 g/L of xylose (10 mL in a 30-mL tube) as the carbon source and cultivated at 110 rpm for 72 h at 30 °C (seed culture). Preculture was performed as follows. The seed culture 10 mL was transferred to a YE-YNB medium in an Erlenmeyer flask (1 L in a 2-L Erlenmeyer flask) with shaking at 110 rpm for 48 h at 30 °C with 20 g/L glucose and 10 g/L xylose as the carbon sources as previously described²⁵. According to the dry cell weight of the culture medium, 1 mL was calculated by the following empirically obtained relationship (1).

\[
\text{Dry cell weight (g/mL)} = \frac{0.00032 \times \text{OD}_{660\,\text{nm}} - 0.00017}{(1)}
\]

Seed cultures and precultures of C. intermedia 4-6-4T2 were observed under a phase contrast microscope (BX51, Olympus Corp.) at 2000 times magnification.

2.2. Fermentation Conditions

Precultured cells were concentrated to 20 g/L (dry weight) by centrifugation (3000 rpm, 2 min). Batch fermentation was performed in an Erlenmeyer flask (100 mL in 200 mL) with a sponge plug (T-38, Shin-Etsu Polymer Co., Ltd.) at 120 rpm for 24 h at 25 °C. The depth of the solution from the bottom of the Erlenmeyer flask to the solution surface was about 20 mm. Synthetic fermentation solution without nitrogen sources containing 20 g/L xylose and 30 g/L glucose (SF solution) plus 5 g/L acetic acid was prepared using 0.1 M phosphate buffer solution (KH₂PO₄/K₂HPO₄) with 20 g/L of xylose and 30 g/L of glucose. The final pH of the SF solution without acetic acid was adjusted to 5.0, and that of the SF solution with 5 g/L of acetic acid to pH 5.5 using 0.1 M phosphate buffer solution (KH₂PO₄/K₂HPO₄) and KOH particles. In sequential batch fermentation, after one cycle of the fermentation period (24 h), cells were collected by centrifugation at 3000 rpm for 2 min, and the separated cells were resuspended in the same SF solution for the next cycle of fermentation without washing with buffer solution or nutrient addition.

The startup-batch and the subsequent Continuous Chemostat Fermentation (CCF) process were performed using a spinner flask (0.36 L working volume in a 0.5-L flask; Wheaton) as illustrated in Fig. 1. The same SF solutions used in sequential batch fermentation were supplemented into the flask, and fermented broth was recovered from the flask at 15 mL/h. The stirring rod length was about 40 mm, and the rate of agitation was 500 rpm at 25 °C. The rate of air sparging was set to one of four conditions (none, 0.056, 0.14, or 0.56 vvm).
During fermentation, the dissolved oxygen concentration (DO) was monitored by a sensor (Fibox3/trace with a probe, PreSens Precision Sensing).

Silicon sponge plugs (N-42, Shin-Etsu Polymer Co., Ltd.) were fitted into both branches of the flask instead of the original plastic caps. Fermenting samples (1 mL) were periodically withdrawn using a polypropylene syringe (SS-01T, Terumo Corp.) with a needle (18G × 90 mm, Terumo Corp.) through the silicon sponge plugs.

The pH of the SF solution with or without acetic acid was finally adjusted to 5-6.5 before fermentation, and not further adjusted during fermentation. All fermentation tests were performed at 25 °C. The pH was checked by a pH sensor (LAQUA twin COMPACT pH METER, B-71X, HORIBA, Ltd.) at every sampling during fermentation.

The concentrations of sugars, ethanol, xylitol, arabinol, glycerol, and acetic acid were analyzed by HPLC (Agilent HPLC 1100, Agilent Technologies) with a Bio-Rad Aminex HPX-87H lead column (300 by 7.8 mm) at 45 °C using a refractive index detector. Two peristaltic pumps (Perista pump, SJ-1211, ATTO) were used to control the volume of influent and effluent for the spinner flask. Culture data for C. intermedia 4-6-4T2 was collected occasionally using a 2-L jar fermenter (Mitsuw Frontech Corp.) by detecting the DO, pH, temperature, and dCO2.

3. Results and Discussion

3.1. Morphology of Precultured Candida intermedia 4-6-4T2

Figure 2 shows the morphologies of the seed culture and preculture of Candida intermedia 4-6-4T2. C. intermedia 4-6-4T2 cultured in the SD minimal medium with 20 g/L of xylose as the carbon source for 72 h completely consumed the xylose but did not produce ethanol. More than 50 % of the narrow and thin shaped yeast cells clang together (upper in Fig. 2)26,27, with typical shapes of pseudomycelia (pseudohyphae) grown in the absence of carbon sources27. C. intermedia 4-6-4T2 cultured in the rich medium (YE + YNB) plus 20 g/L of glucose and 10 g/L of xylose as the carbon source for 48 h produced ethanol, and the non-budding yeast cells maintained synchronization at the G1 stationary phase in the cell cycle (lower in Fig. 2)27. The high cell density of the precultured C. intermedia 4-6-4T2 produced ethanol but did not proliferate in the SF solution because of the absence of nitrogen sources.

3.2. Sequential Batch Fermentation

Figure 3 shows the results of sequential batch fermentation that utilized the high cell density of C. intermedia 4-6-4T2 (20 g/L (DCW)). In the absence of acetic acid, the ethanol yield of each cycle was stable (0.4 g/g, 78 % of theoretical maximum) (Fig. 3A). In the presence of acetic acid, the ethanol yield decreased from 80 to 70 % of the theoretical maximum (Fig. 3B).

In contrast, xylose was completely consumed in
cycle 2, but only 50 % of xylose was consumed in cycle 3 with acetic acid (Fig. 3B). The final pH of the fermented broth with acetic acid remained unchanged at pH 5.5 during each cycle of fermentation due to the buffering effect of acetate and phosphate. Consumption of xylose in cycle 3 may have been limited by intolerance of the yeast cells to the effect of acetic acid even at pH 5.5. Acetic acid is transported through both the acetate transporter (ady2p) and the aquaglyceroporin channel with glucose consumption in its undissociated form (CH₃COOH), and protons (H⁺) are released in the cytosol. The decreased pH in the cytosol was compensated for discharge H⁺ from the cell membrane by the proton pump during conversion of ATP to ADP as the cofactor for conversion of xylitol into xylulose. In sequential batch fermentation, the capacity of the mitochondrion decreased due to some stresses. Thus, sequential batch fermentation was limited to two cycles at most with and without acetic acid. Therefore, the practical limit for used cell density for one cycle was 10 g/L (DCW).

3.3. Startup Batch and Continuous Chemostat Fermentation (CCF)

3.3.1. Effect of Acetic Acid on the CCF Process without Air Sparging

Figure 4 shows the results of fermentation without air sparging, using SF solutions with or without acetic acid. *C. intermedia* 4-6-4T2 stably produced ethanol of about 20 g/L (0.4 g/g, 78 % of the theoretical maximum) for more than 240 h (1-cycle startup-batch fermentation and 9-cycle continuous fermentation) without acetic acid at pH 5.0 (Fig. 4A). Xylose was continuously consumed, and the xylitol selectivity from xylose was about 0.18 (Fig. 4A). The pH of the fermented broth decreased to pH 4.0, and the concentration of yeast cells was about 3 g/L (DCW) at the end of the entire process. About 85 % of the yeast cells were lost in the effluent (fermented broth).

In contrast, *C. intermedia* 4-6-4T2 did not efficiently consume sugars or produce ethanol after startup-batch fermentation with acetic acid at pH 5.5 (Fig. 4B). DO fell under the detection level immediately after ferment-
tation began. The ratio of consumed glucose decreased from the start of the CCF process, and most consumed xylose was converted into xylitol but not into ethanol (Fig. 4B). The xylitol selectivity exceeded 0.35 (Fig. 4B). The concentrations of yeast cells were the same in the spinner flask and the Erlenmeyer flask. However, the depth of SF solution in the spinner flask was about 3.2 times that in the Erlenmeyer flask, and the number of yeast cells in the former was 3.5 times greater. The accumulated dCO₂ in the spinner flask may have had severely inhibitory effects on xylose consumption and respiration to convert NADH into NAD⁺ due to shortage of oxygen (Fig. 5; modification of Fig. 6 in ref. 25).

3.3.2. Effect of Air Sparging on Ethanol Production in SF Solution with Acetic Acid

Figure 5 shows the effects of changes in the rate of air sparging on the concentration of ethanol production, DO, and sugars during the startup-batch and CCF process. Air sparging at 0.056vvm achieved better ethanol production than under the other conditions tested (Fig. 6A). In this case, the monitored DO was about 0.1 mg/L at the beginning of startup-batch fermentation, and about 0.5 mg/L at the end of the CCF process (Fig. 6B). The SF solution did not contain nitrogen sources, so the yeast cells did not proliferate during the fermentation. These results indicate that both aerobic and anaerobic pathways functioned in the CCF process for respiration and ethanol production, respectively, and ethanol was formed in the fermented broth with acetic acid.

Air sparging at 0.14 vvm decreased ethanol production and the consumption ratio of xylose decreased compared to 0.056 vvm (Fig. 6A, 6D). The profiles of the monitored DOs differed completely between these two conditions (Fig. 6B). Air sparging at 0.56 vvm decreased ethanol production more compared to the other two conditions, and the monitored DOs were about 0.5 mg/L at the beginning of the startup-batch fermentation and 1.0 mg/L at the end of the CCF process (Fig. 6A, 6B). In this case, glucose was completely consumed for 120 h, but subsequently the ratio of unconsumed glucose gradually increased (Fig. 6C). Possibly, excess air sparging and acetic acid in the yeast cytosol formed reactive oxygen species (ROS), which caused severe damage to the yeast cells27). Thus, the yeast cells could consume neither glucose nor xylose.

Air sparging at 0.056 vvm caused consumption of more than 80% xylose within one cycle (24-h fermentation period), and the xylitol selectivity was 0.2 (Fig. 6D). Air sparging at more than 0.14 vvm decreased the consumption ratio of xylose compared to 0.056 vvm (Fig. 6D). However, xylitol production and xylitol selectivity were less than at 0.056 vvm (Fig. 7A, 7B). *C. intermedia* 4-6-4T2 precultured in a rich medium with xylose and glucose mixture as the carbon sources could produce ethanol from both xylose and glucose by utilizing the metabolic and catabolic pathways. We previously postulated that glucose is partially converted into 6-phosphogluconolactone through glucose-6-phosphate while producing NADPH from NADP⁺ as the cofactor in the catabolic pathway25).

Yeast NADPH oxidase (YNOp1) localized to the perinuclear endoplasmic reticulum can oxidize NADPH excluding NADH (Fig. 5)25. YNOp1 produces ROS, independently of the production of ROS derived from excess oxygen25. We assumed that NADPH was oxidized by a NADPH-dependent oxidase such as YNOp1 and the decrease of NADPH as the cofactor for xylose reductase to convert xylose into xylitol induced the decrease in the xylose consumption ratio seen with increased volume of air sparging. D-Arabitol and ribitol are intermediates in the pathway of sugar consumption by *S. passalidarum*20. In our research, arabitol was detected but not ribitol. One possibility is that any small amount of ribitol might be detected as the en-
Abbreviations: G6P: glucose-6-phosphate; F1,6P: fructose-1,6-diphosphate; PGI: phosphoglucose isomerase; G6PD: glucose-6-phosphate dehydrogenase; XR: xylose reductase; XDH: xylose dehydrogenase; XK: xylose kinase, ER: endoplasmic reticulum.

Fig. 5 Hypothetical Mechanism of Glucose and Xylose Metabolism in C. intermedia 4-6-4T2

Concentration of ethanol (A); DO (B); glucose (C); xylose (D).

[Symbols] volume of air sparging: none: triangle; 0.056 vvm: diamond; 0.14 vvm: square; 0.56 vvm: circle.

Fig. 6 Time Course of the Startup-batch and CCF Process by C. intermedia 4-6-4T2 (20 g/L (DCW)) Using SF Solution with Acetic Acid (5 g/L) pH at 5.5 and Various Volumes of Air Sparging
tomer of xylitol at the same retention time in HPLC analysis. The concentration of arabitol increased slightly with higher volume of air sparging (Table 1). *C. intermedia* 4-6-4T2 did not produce arabitol from glucose. Therefore, arabitol was produced only from xylitol. The SF solution did not contain nitrogen sources, and it means that the enzymes and cofactors used for ethanol production were recycled or reproduced *via* salvage pathways in yeast cells. Shortages of cofactor (NAD$^+$) for conversion of xylitol into xylulose resulted in accumulation of xylitol. On the other hand, adequate NAD$^+$ supplied by mitochondrial oxidase (NADH $\rightarrow$ NAD$^+$) through air sparging resulted in conversion of xylitol into xylulose. Xylose consumption was stable, so cofactor (NADPH) for xylose reduction was also consumed and NADP$^+$ was accumulated. Arabitol might be produced using NADP$^+$ as the cofactor in reduction and conversion to NADPH (Fig. 5).

### 3.3.3. Effect of Fixed Air Sparging on Ethanol Production in pH-adjusted SF Solution with Acetic Acid

Sequential batch fermentation for ethanol production has been extensively studied[^5][^6], but continuous fermentation is less well known. Continuous fermentation achieved good results utilizing *Thermoanaerobacter italicus* Pentcrobe 411, with the pH of hydrolysates adjusted to pH 7, and the hydrolysates contained about 5 g/L of acetic acid[^36]. Acetic acid is completely present in the dissociated form (CH$_3$COO$^-$ + H$^+$) at pH 7, and so was not assimilated by the bacteria. Thus, the detrimental effect of acetic acid was not correctly evaluated. Our study of continuous fermentation considered the effect of acetic acid changing the pH of the SF solutions.

Air sparging was fixed at 0.056vvm. The startup-batch and CCF processes were performed with changes in the pH of the SF solutions (pH 5, 5.5, or 6.5). SF solution with acetic acid adjusted to pH 5.0 resulted in concentration of ethanol $\approx$15 g/L (Fig. 8A). Neither glucose nor xylose was effectively consumed (Fig. 8C, 8D). The monitored DO levels were similar for the three conditions, and gradually in-

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### Table 1 Summary of the Start-up Batch and CCF Processes for SF Solutions Containing 20 g/L Xylose and 30 g/L Glucose with/without Acetic Acid Utilizing High Cell Density of *C. intermedia* 4-6-4T2

| Acetic acid [g/L] | Air sparging [vvm] | pH  | Ethanol yield[^a] [g/g] | Ethanol productivity[^a] [g/L h] | Xylitol selectivity[^d] | Arabitol selectivity[^d] | Xylitol (M)/((Xylitol (M) + Arabitol (M))) | Total fermentation periods[^e] [h] | Actually-used cell concentration for one cycle (24 h) [g/L (DCW)] |
|------------------|--------------------|-----|------------------------|----------------------------------|-------------------------|--------------------------|------------------------------------------|-------------------------------|--------------------------------------------------|
| 0.00             | 0                  | 5.0 | 0.41 (80)              | 0.85                             | 0.29                    | 0.06                     | 0.83                       | 240                           | 2.0                                              |
| 5.0              | 0.056              | 5.5 | 0.40 (78)              | 0.83                             | 0.18                    | 0.03                     | 0.86                       | 168                           | 2.9                                              |
| 5.0              | 0.14               | 5.5 | 0.43 (67)              | 0.71                             | 0.15                    | 0.02                     | 0.88                       | 168                           | 2.9                                              |
| 5.0              | 0.56               | 5.5 | 0.38 (55)              | 0.58                             | 0.09                    | 0.06                     | 0.80                       | 144                           | 3.5                                              |
| 5.0              | 0.056              | 5.0 | 0.25 (49)              | 0.52                             | 0.22                    | 0.02                     | 0.92                       | 120                           | 4.0                                              |
| 5.0              | 0.056              | 6.5 | 0.42 (82)              | 0.88                             | 0.15                    | 0.04                     | 0.79                       | 168                           | 2.9                                              |

[^a]: Ethanol yield: ethanol concentration (g)/sugars (g), parentheses: theoretical yield (%).
[^b]: Ethanol productivity: ethanol yield $\times$ 50/24.
[^c]: Xylitol selectivity: xylitol concentration (M)/consumed xylose (M).
[^d]: Arabitol selectivity: arabitol concentration (M)/consumed xylose (M) at the end of total fermentation.
[^e]: Total fermentation periods: startup-batch fermentation (24 h) + periods of the CCF process.
increased in all cases (Fig. 8B). The reason is that the concentration of yeast cells decreased slightly every 24 h (one cycle), and about 70% of the yeast cells were ultimately lost in the effluent (fermented broth).

The CCF process successfully proceeded if the pH of the SF solution was adjusted to exceed pH 5.5 (Fig. 8A, 8C, 8D). Under these conditions, xylose was effectively consumed, and the xylitol selectivity from xylose was <0.2 (Fig. 9A, 9B).

However, if the pH of the SF solutions with acetic acid was adjusted to pH 6.5, acetic acid is mostly present in the dissociated form (CH₃COO⁻ + H⁺), so acetic acid was not transported into the yeast cytosols. Consequently, the inhibitory effect of the acetic acid was almost negligible. Taken together with the findings of the CCF process without acetic acid, only a small volume of air sparging (less than 0.056 vvm) is required for SF solution at pH 6.5.

4. Conclusion

The inhibitory effect of dCO₂ in the fermented broth with or without acetic acid on ethanol production, xylose consumption and xylitol selectivity was investigated using a spinner flask with sponge plugs and air sparging.

We found that a startup-batch and CCF process that utilizes the high cell density of *C. intermedia* 4-6-4T2 (20 g/L (DCW)) was effective for stably producing ethanol and achieving rapid fermentation of xylose and glucose. The average cell densities in this process compared to those of generally used sequential batch fermentation were one-fifth without acetic acid and one-third with acetic acid.

These results are important for the development of efficient ethanol production processes for hydrolysates from lignocellulosic biomass, especially those containing acetic acid.
Concentration of xylitol (A); xylitol selectivity (B).

Fig. 9 Time Course of the Startup-batch and CCF Process by C. intermedia 4-6-4T2 (20 g/L (DCW)) Using SF Solution with Acetic Acid (5 g/L) and Various pH of SF Solutions with Air Sparging (0.056vvm)

References

1) Lau, M. W., Dale, B. E., Proc. Natl. Acad. Sci. U. S. A., 106, 1368 (2009).
2) Binder, J. B., Raines, R. T., Proc. Natl. Acad. Sci. U. S. A., 107, 4516 (2010).
3) Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Environ. Microbiol., 13, 12 (2020).
4) Sarks, C., Jin, M., Sato, T. K., Balan, V., Dale, B., Biotechnol. Biofuels, 7, 73 (2014).
5) Santos, S. C., de Sousa, A. S., Dionísio, S. R., Tramontina, R., Fuller, R., Squina, S. F., Rossell, C. E. V., da Costa, A. C., Ienczak, J. L., Bioreourc. Technol., 219, 316 (2016).
6) Tran, P. H. N., Ko, J. K., Gong, G., Um, Y., Lee, S., Biotechnol. Biofuels, 13, 12 (2020).
7) Ahmed, F., Yan, Z., Bao, J., Bioreourc. Bioprocess., 6, 24 (2019).
8) Jones, R. P., Greenfield, P. F., Enzyme Microb. Technol., 4, 210 (1982).
9) Jpn. J. Petrol. Inst., Vol. 64, No. 4, 2021.
要　旨

高濃度 Candida intermedia 4-6-4T2 を利用した連続ケモスタット発酵プロセスによる キシロースおよびグルコースからのエタノール生産におけるアスピージング効果

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リグノセルロース由来のバイオマスからのエタノール生産において、発酵時間の短縮、生産速度の増加は、費用効果の高いエタノール生産工程である。酵母濃度の増加は、通常、エタノール生産速度を増加させるが、発酵液中の溶存 CO₂（dCO₂）の濃度も増加させる。蓄積した dCO₂ はエタノール生産および生産速度を低下させることができる。この影響を解明し、24時間での迅速発酵のために連続ケモスタット発酵（CCF）工程を適用した。培養器に窒素不含有培養液（20 g/L キシロースおよび 30 g/L グルコース+5 g/L 酢酸）を 15 mL/h で充填し、発酵プロセスを 15 mL/h で回収した。蓄積した dCO₂ を排出する条件として、アスピージング量 0.056 vvm が CCF 工程でのエタノール生産に最も効果的であり、初期回分発酵（24時間）および6回-CCF 工程（144時間）におけるエタノール収率は 0.4 g/g。Candida intermedia 4-6-4T2 の1回当たりの菌体濃度は実質約3 g/L（細胞乾燥重量）となり、連続回分発酵の 1/3 であっ

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