稲わらからのバイオエタノール生産プロセスにおける同時糖化発酵の窒素源としての蒸留残渣の自己再利用

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Article

Self-reuse of Distillation Residue as a Nitrogen Source for Simultaneous Saccharification and Fermentation in a Bioethanol Production Process from Rice Straw

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Abstract

In this study, the reusability of some portions in distillation residues obtained in a bioethanol production process was investigated as a supplemental nitrogen source for next single batch, focusing on simultaneous saccharification and fermentation (SSF) of rice straw. Distillation residues which obtained in our pilot plant were firstly divided into two parts: liquid and sludge (DR-L and DR-S) and DR-S was further dried and grinded to be powder (DR-P). For the comparison of nutritional effect on SSF between corn steep liquor (CSL) as a control and the distillation residues, compositional analyses (fiber, nitrogen content and amino acids) were performed.

Although the total nitrogen content of the distillation residues was less than a half of CSL, amino acids in DR-P (2.24 %), which can be directly used for the yeast growth, was slightly lower than that in CSL (3.64 %). The performance of SSFs with the distillation residues was monitored. Final ethanol concentration of SSF with DR-L and DR-S (as water and nutrient loading) was 3.52 % (w/w) comparable to that with CSL (3.28 %). The use of distillation residues was concluded to be promising in the bioethanol production, reducing the conventional costly nutrient. In addition, it was suggested that the utilization of the liquid distillation residue would contribute to water recycling.

Key Words: Supplemental nutrient, distillation residue, rice straw, bioethanol

1. Introduction

As a promising renewable energy, bioethanol production from lignocellulosic biomass has been receiving widespread attentions all over the world[1-3]. Three key processes for bioethanol production from lignocelluloses are: pretreatment necessary to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars, and ethanol fermentation. Also, simultaneous saccharification and fermentation (SSF) which can improve ethanol yields by removing end product inhibition and eliminate the need for separate reactors is often employed in various researches[4].

For the ethanol fermentation, yeast (*Saccharomyces cerevisiae*) is commonly employed. Since the growth of yeast as well as the fermentation

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reactions is largely affected by the nutrients level, essential nutrients (e.g. nitrogen, phosphorous, vitamins and trace elements) should be provided at an adequate level for the effective ethanol fermentation (rate and yield)[5-6].

Corn steep liquor (CSL), a by-product of corn starch processing, is one of the most common additive as a nitrogen source used for ethanol fermentation because of its enriched proteins, free amino acids, minerals, vitamins and trace elements[7]. CSL is a low-cost nutrient basically however the actual cost depends on the logistic availability. For example, it should be imported for the use in Vietnam in which no domestic CSL supply is available. Therefore, development of the use of sustainable nutrients which can replace CSL is necessary to implement locally sustainable bioethanol process in Vietnam. Actually, the effectiveness of some agricultural by-products (i.e. soybean residue, potato peel, peanut residue and rice bran) as alternative nitrogen source for bioethanol fermentation was investigated in our previous studies[8-9]. It was found that these alternative materials, is a promising alternative nitrogen source comparable to CSL.

Nonetheless, problems exist that hinder the implementation of sustainable ethanol production from lignocelluloses. Especially, appropriate waste management to deal with large amount of fermentation residues is one of the big issues[10]. On the other hand, fermentation resides may still contain considerable nutrients as well as remaining cellulose and sugars[11] Therefore, a developed concept: internal reuse of process residues for waste reduction and supply of nitrogen source was discussed in this study. We focused on SSF residues remained after distillation so-called "distillation residue". In this paper, the reusability of distillation residue as an alternative nitrogen source for the next single SSF operation (i.e., one-time reuse) was investigated and the contribution of self-reuse of distillation residue to sustainability of small-scale bioethanol production process was also discussed.

2. Experimental

2.1 Feedstock collection and pretreatment

Feedstock of bioethanol production, rice straw was collected from Thai My village, Cu Chi district, Vietnam. After harvesting, it was air-dried for a few days before use. Rice straw cut into 2cm pieces by a cutting machine and then it was puffed by an automatic continuous puffing machine under 15–17 % (w/w) water loading condition of rice straw weight. For alkaline pretreatment, puffed rice straw was immersed into 1% (w/w) NaOH solution at 50 ℃ for 12–24 h. Then alkali-treated rice straw was squeezed by pressing machine equipped with filter for waste water separation at pressure up to 11–13 $kgf \cdot cm^{-2}$. Then, alkali-treated rice straw was neutralized with HCl (to be pH 5–6) and squeezed again and then it was fed into SSF reactor as a substrate.

2.2 Pre-cultivation of yeast

Before SSF, the dry yeast (*S. cerevisiae*, Ethanol Red™) was pre-cultivated. The pre-cultivation medium (sucrose and CSL) prepared in deionized water was autoclaved at 121 ℃ for 15 min before the loading of dry yeast. The yeast was pre-cultivated in

Table 1 Experiment conditions

SSF	Flask test (125 mL)				Mini-reactor test (20 L)	
	Control	Distilled Residue (DR)			Control	DR
Sample ID	C801	P801	P8015	L800	C1601	PL16015
Straw	8 % (w/w)				16 % (w/w)	
Loading (% w/w)	CSL 0.1 %	DR-P 0.1 %	DR-P 0.15 %	DR-L 125 mL	CSL 0.1 %	DR-L 20 L DR-S 1.25 %
Nitrogen loading* (%, w/w)	0.0036	0.0024	0.0036	na**	0.0036	0.0038**
FPU/g-sample	6.75					

*: Nitrogen loading is based on amino acids measured by Sørensen formol titration method[9]

**: Not available since amino acids in DR-L were not measured. Hence nitrogen loading for PL16015 is based on merely DR-S loading.

the shaking incubator (110 rpm) at 35℃ for 16-24 h. Optical density (OD) of pre-cultivation broth was measured at 600 nm with UV-Vis instrument (Hach® DR 5000). The amount of yeast culture needed for SSF was calculated according to SSF experimental protocol suggested by the National Renewable Energy Laboratory (NREL)[12].

2.3 *Simultaneous Saccharification and Fermentation (SSF)*

As shown in Fig. 1, distillation residues remaining in the bottom of distillation tower was separated into two layers according to density: top layer in liquid state so-called "distillation residue-liquor (DR-L)" and bottom layer containing sludge so-called "distillation residue-sludge (DR-S)". DR-S was dried and grinded to be powder to consider the reuse after storage. The powder is here after termed "distillation residue-powder (DR-P)". DR-S and DR-P were evaluated only as nutrient source. When using DR-L, reusability of water was considered as well as a nitrogen source for SSF. The original distillation residue was obtained in our pilot plant[13].

SSFs with different nutrient sources were carried out in flasks and a mini-reactor. The experimental conditions were summarized in Table 1. Before SSF, all of samples and nutrients were autoclaved (121 ℃, 20 min) and then cooled down to 35 ℃. SSFs were started with loading of pre-cultivated yeast and acremonium cellualse (Meiji Seika Co.; filter paper activity: 360 FPU g^{-1} of enzyme). The flask tests were performed in a shaking incubator (35 ℃ and 150 rpm). Each flask was closed by an S-type bubble airlock in which sterilized water allowed exhaustion of CO_2 while it blocked the entrance of air. SSF with mini-reactor was also performed under a constant-temperature system.

Pretreated rice straw was loaded step-by-step during the SSF to obtain good mixing condition. Before and after each loading, the mixture was sampled and analyzed by HPLC to monitor the glucose and ethanol concentrations.

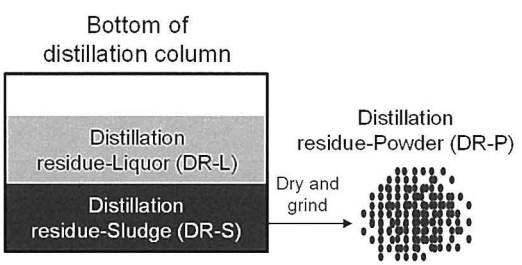

Fig. 1 Classification of distillation residues

2.4 *Analytical method*

Dry matter was determined using an electric moisture balance at 105 ℃. Before compositional analysis, samples were dried at 45 ℃ for 1 day and then grinded to be equal size. Fiber analysis was performed according to two-step acid hydrolysis method suggested by NREL[14]. Starch content of samples was determined by DNS method[15]. During SSF, fermentation broths were sampled to monitor the glucose and ethanol concentration. Prior to the analysis samples were diluted with deionized water approximately 6-fold and centrifuged at 3000 rpm for 10 min. Then the supernatant was filtered through a 0.22 μm filter and injected to high performance liquid chromatography (HPLC) system with an analytical column (SH1011, Shodex Co.) and a refractive index detector (RID-10A, Shimadzu Co.). The column oven temperature was 60 ℃. As the eluent 0.005 M of H_2SO_4 was employed and its flow rate was 1 mL·min^{-1}. To measure the total nitrogen content in nutrient samples, elemental analysis was performed by a CHNS analyzer (EuroEA3000, EuroVector S.p.A.). Amino-acid nitrogen was measures by Sørensen formol titration method[16].

3. Results and Discussions

3.1 *Nutritional facts of distillation residues*

Table 2 shows the composition of feedstock of SSF, control nutrient source (CSL) and distillation residue (DR-P). It can be seen that the glucan content in CLS is almost 4 times more than that in DR-P while lignin and ash content in DR-P were much higher than those in CSL. It can be reasonably expected that CSL, a by-product of corn starch processing still contains much starch and DR-P contains much unusable components for the yeast metabolism such as lignin and ash. Unidentified components shown in "others" are expected to be consisted of mainly protein, amino acid, minerals, vitamins and trace elements[3]. Since fiber analysis did not give critical information about nutritional facts, nitrogen content of CSL and DR-P were analyzed by elemental analysis and

Sørensen formol titration method (Fig. 2).

It is recognized that ethanol fermentation can be improved by presence of nutrients especially a good nitrogen source under anaerobic conditions. Nitrogen is regarded as essential element for the growth of yeast (i.e. cell proliferation) affecting the rate of ethanol production[6]. As can be seen in fig. 2, the total nitrogen content of CSL measured by element analysis was much higher than that of DR-P. However, it is interesting to note that nitrogen contents of both samples estimated by Sørensen formol titration method were not so different from each other. It can be inferred that DR-P may still contain comparable amount of nitrogen (amino-acids) available to yeast.

A previous study[17] which discussed the effects of nitrogen sources on metabolite formation, growth and cell composition of *S. cerevisiae*, suggested that there were several nitrogenous compounds including peptides, proteins and nucleic acids but they could not be directly used by yeast cells for metabolism. Hence, the existence of free amino acids should be an important factor when evaluating the performance of nutrients for the yeast activity. By Sørensen formol titration, the amino-acid nitrogen contents of CSL and DR-P were found to be 3.64 % and 2.24 %, respectively. It was predicted that the performance of ethanol fermentation with DR-P as a nitrogen source would be lower than that with CSL when same amount was loaded. Considering the above results, we employed the amount of amino-acid nitrogen as an index of the dosage of the nutrient materials in the following SSF tests.

3.2 *Effects of distillation residues on the SSF performance*

Figure 3(a) shows the changes in ethanol concentration during the SSF tests in the flask and mini-reactor with different nutrient sources. The final ethanol concentration and ethanol yield for each SSF were summarized in Fig. 3(b). As can be seen from these figures, P801 showed a lower ethanol concentration and yield than C801, possibly due to the lower supply of amino acids. On the other hand, the result of SSF with P8015 was comparable to that of C801. The loading amount of nitrogen as amino acids, was a crucial factor for the utilization of distillation residues as a replacement for CSL.

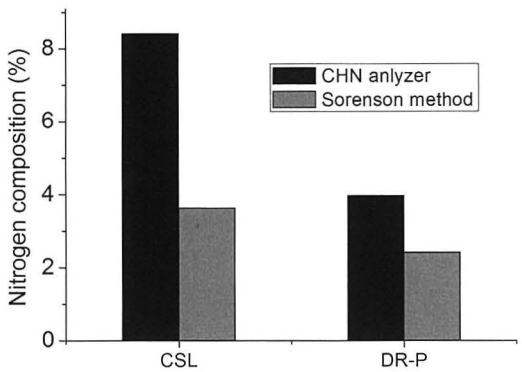

Fig. 2 Nitrogen contents of CSL and DR-P

It was found that a comparable SSF performance could be obtained by loading the distillation residue with an amount of nitrogen as amino acids equivalent to CSL. In addition to the ability to replace CSL with the distillation residues, the effect of DR-L as a replacement of water and nutrient source on SSF was also investigated. As shown in Fig. 3, the performance of SSF with DR-L (i.e., L800) was comparable to that with CSL (C801). In the light of the result, the liquid residue could be successfully recycled to reduce the load of the wastewater treatment. It is difficult to discuss the effect of DR-L on the ethanol fermentation phenomena in detail, since the components in the liquid portion was not measured. In any case, that finding suggested that the use of liquid portion of

Table 2 The composition of alkali-treated rice straw (RS), control nutrient source (CSL) and distillation residues (DR-P)

Materials	Compositions (%)				
	Glucan	Xylan	Lignin	Ash	Others
RS	51.23	23.52	18.31	2.37	4.57
CSL	60.02	15.47	1.05	3.36	20.1
DR-P	15.51	15.72	31.41	20.04	17.32

the distillation residue contributed to the water recycling in the SSF process. Similar result was obtained from SSF with DR-L and DR-S in the mini-reactor (PL16015). The advantage of the utilization of distillation residues as a replacement of nutrient source and water is discussed in the following section.

3.3 Practical flows for the utilization of distillation residues as a replacement of nutrient source and water for SSF

The fundamental assumptions for the internal reuse scenarios of distillation residues to recycle nitrogen and water are as followings:

1) Distillation residues will be reused for one-time to prevent the accumulation of fermentation inhibitors (e.g., acetic acid and glycerin).
2) DR-L is considered as only water source.
3) DR-S or DR-P with equivalent amount of nitrogen (as amino acids) to CSL has same effect on the SSF performance.
4) The amount of distillation residues will be same regardless of types of nitrogen source (i.e., CSL, DR-S and DR-P).

Upon the assumptions, two possible options for the internal reuse of distillation residues including the conventional flow without internal reuse can be described as shown in Fig.4. For the option 1, total DR-L and a portion of DR-S (with equivalent amount of nitrogen as amino acids to CSL) will be reused for the next single SSF. The rest of DR-S will be disposed. For the option 2, the reuse flow is same as option 1 except for that the rest of DR-S will be stored as DR-P until the second SSF. Then DR-P will be reused for the following single SSFs with the

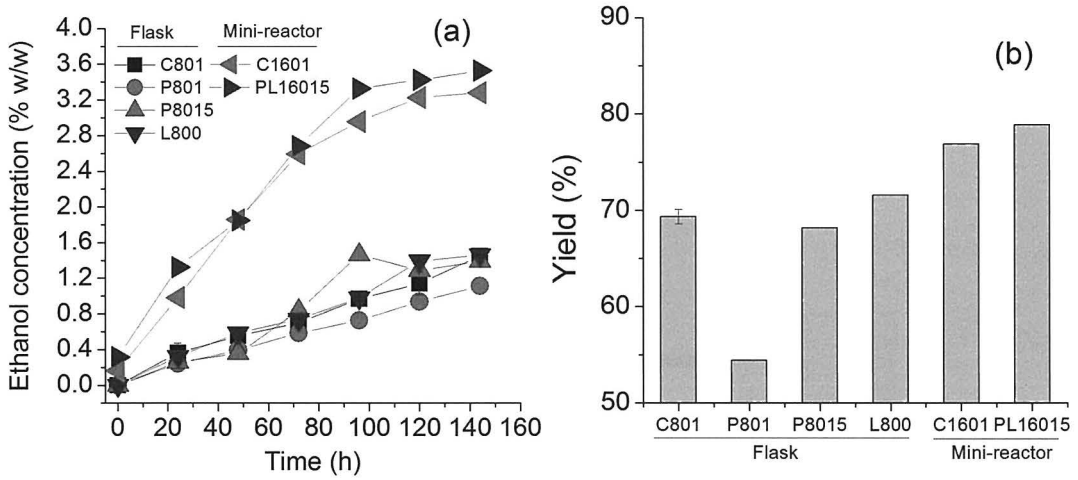

Fig. 3 Variation of ethanol concentration during the SSF (a) and final ethanol yield at 144 h (b)

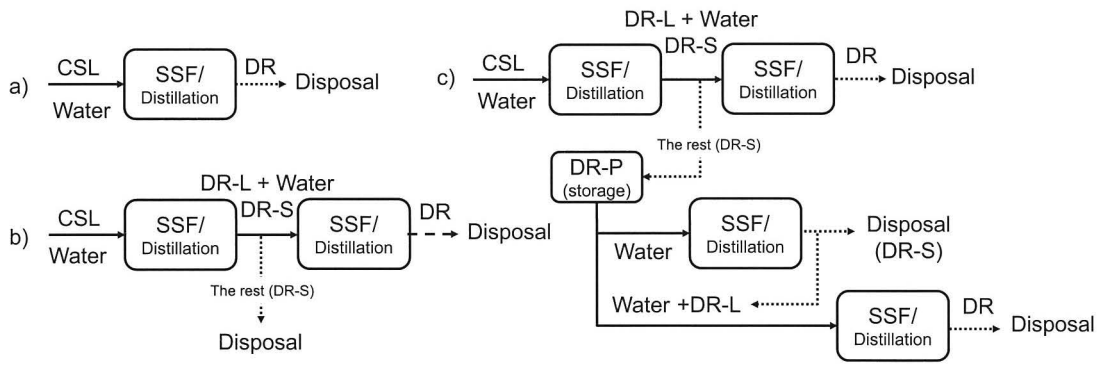

Fig. 4 Possible options for the internal reuse of distillation residues (a: conventional flow without reuse; b: option 1; c: option 2)

same material flow as option 1.

First, the conventional flow (Fig. 5) was estimated through the fundamental material flow data obtained in our previous study[13]. In this calculation, we considered that 6000 ton (wet basis) of rice straw (the annual production amount in Thai My village, Cu Chi District, Ho Chi Minh City) will be used as a feedstock for bioethanol production per year.

Figure 6 shows the estimated flows of

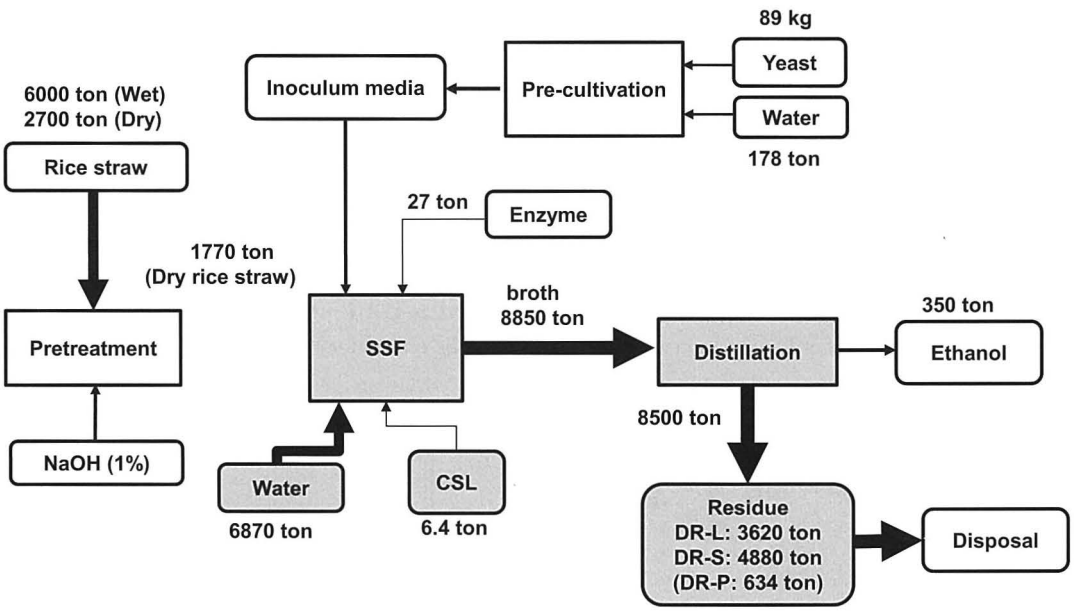

Fig. 5 Material flows of bioethanol production process from rice straw with CSL based on our previous study[13]

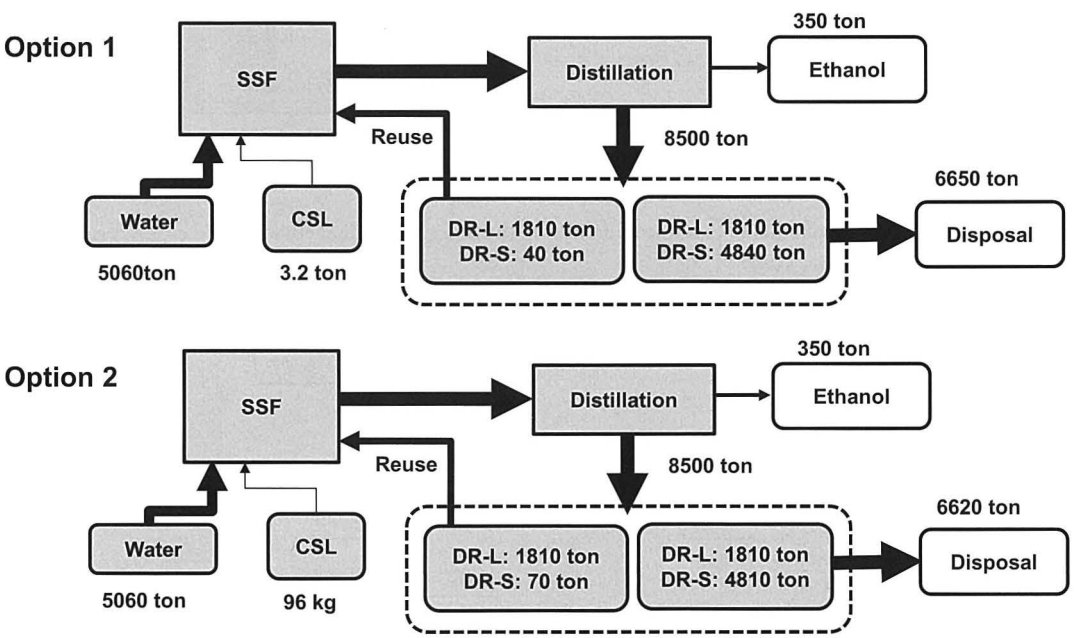

Fig. 6 Estimated flows of bioethanol production process from rice straw considering the two options for the internal-reuse of distillation residues

bioethanol production process from rice straw considering the two options for the internal-reuse of distillation residues. According to the material flow shown in Fig. 5, 6870 ton of water and 6.4 ton of CSL will be consumed per year to obtain 350 ton of ethanol from 6000 ton of rice straw. At the same time, 8500 ton of distillation residues will be disposed. As can be seen from Fig. 6, the reuse of DR-L contributes to the water saving of 1810 ton (26.3 %) as well as the reduction of same amount of wastewater. Although, the internal reuse of distillation residues cannot significantly reduce the sludge wastes compared to the conventional flow, it can largely save CSL demands (option 1: 50.0 %, option 2: 98.5 %). Considering this, the reuse of distillation residues (especially, DR-S) for other purposes should be discussed to reduce the sludge wastes largely. With respect to the reuse of distillation residues for other purposes, it is thought that DR-P certainly has the merit of storage.

4. Conclusions

The feasibility of the use of distillation residues as a replacement of conventional nutrient sources (i.e., CSL) was investigated in this study. Through the SSFs with various nitrogen sources, it was clearly confirmed that distillation residues with equivalent weight of nitrogen (amino acids) to CSL have considerable effect on SSF to CSL. Furthermore, DR-L was also effective nutrient source as well as water source for SSF. It was expected that the internal reuse of DR-L and DR-S can reduce the demand of CSL by 98.5 % and recycle 26.3 % of water. Accordingly reuse of distillation residues can be promising option to enhance the sustainability of small-scale bioethanol production process.

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Reference

1) Kang, Q., L. Appels, T. Tan, and R. Dewil (2014) Bioethanol from Lignocellulosic Biomass: Current Findings Determine Research Priorities. The Scientific World Journal, Volume 2014, Article ID 298153, doi:10.1155/2014/298153

2) Joshi, B. M. R. Bhatt, D. Sharma, J. Joshi, R. Malla and L. Sreerama (2011) Lignocellulosic ethanol production: Current practices and recent developments. Biotechnology and Molecular Biology Review, 6, 172-182.

3) Kumar, S., S. P. Singh, I. M. Mishra and D. K. Adhikari (2009) Recent Advances in Production of Bioethanol from Lignocellulosic Biomass. Chem. Eng. Technol., 32, 517-526.

4) Olofsson, K., M. Bertilsson and G. Lidén (2008) A short review on SSF - an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnology for Biofuels, 1:7, doi:10.1186/1754-6834-1-7

5) Lau, M. W. and B. E. Dale (2008) Cellulosic ethanol production from AFEX-treated corn stover using Saccharomyces cerevisiae 424A (LNH-ST). Proceeding of the National Academy of Sciences of the United States of America, vol.106 no. 5, 1368-1373, doi: 10.1073/pnas.0812364106.

6) Bafrncova, P. and D. Smogrovicava (1999) Improvement of very high gravity ethanol fermentation by media supplementation using Saccharomyces cerevisiae. Biotechnology Letter, 21, 337-341.

7) Peraira., F and P.M.R. Guimaraes (2010) Optimization of low cost medium for very high gravity ethanol fermentations by Saccharomyces cerevisiae using statistical experimental designs. Bioresource Technology, 101, 7856-7863.

8) Vu, L.V.K., P.N.U. Tran, D.T. Phan, D.J. Seo, K. Mochidzuki and D.Q. Nguyen (2013) Agricultural residues as alternative supplemental nutrients for Bioethanol fermentation process from rice straw. Journal of Science and Technology, Vietnam, 5B, 32-36.

9) Mochidzuki, K., S. Kobayashi, H. Wang, R. Hatanaka and H. Hiraide (2015) Effect of Rice Bran as a Nitrogen and Carbohydrate Source on Fed-Batch Simultaneous Saccharification and Fermentation for the Production of Bioethanol from Rice Straw. Journal of the Japan Institute of Energy, 94, 151-158.

10) Wang, Z. and W. Lin (2012) Pyrolysis of the lignocellulose fermentation residue by fixed-bed micro reactor. Energy, 43, 301-505.

11) Sorensen, H. R. and S. Pedersen (2005).Efficiencies of designed enzyme combinations in releasing arabinose and xylose from wheat arabinoxyland in an industrial ethanol. Enzyme and Microbial Technology, 36, 773-784.

12) National Renewable Energy Laboratory, Technical Report NREL/TP-510-42628.

13) Tran, P.N.U., L.V.K. Vu, D.Q. Nguyen, T.K.P. Le, D.T. Phan, K. Mochidzuki, S. Kobayashi, D.J. Seo and A. Sakoda (2013) Energy balance of Small Scale Biorefinery Process. Environmental Science, 26, 489-496.
14) National Renewable Energy Laboratory, Technical Report NREL/TP-510-42617, 42618, 42620 and 42622
15) Miller, G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, 426-428.
16) Nguyen, D.L. (2003) Biochemistry Experiments. National University-Ho Chi Minh City Publisher.
17) Cooper, T.G. (1982) Nitrogen Metabolism in Saccharomyces cerevisiae. J.N. Strathern, E.W. Jone and J.R. Broach (Eds) The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression, Cold spring harbor laboratory press, New York.

稲わらからのバイオエタノール生産プロセスにおける同時糖化発酵の窒素源としての蒸留残渣の自己再利用

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摘　　要

本研究では，稲わらからのバイオエタノール生産プロセスにおける蒸留残渣を同時糖化発酵の窒素源として自己再利用する可能性を検討した。バイオエタノール製造のパイロットプラントで得られた蒸留残渣より，糖やタンパクなどの水溶性成分を含む上清の液体層（DR-L）と未分解炭水化物やリグリンなどを含むスラリー層（DR-S）に分画し，さらにDR-Sを乾燥して粉末にしたDR-Pを得た。繊維，窒素，アミノ酸含量などを考慮した上で，粉末コーンスティープリカー（CSL）と比較しながら，各蒸留残渣サンプルの栄養源としての効果を同時糖化発酵で評価した。

DR-Pの窒素含量はCSLの半分以下であったが，酵母の増殖に利用可能なアミノ酸含量はDR-Pが2.24%でCSLの3.64%と大きな差はなかった。また，DR-LとDR-Sを仕込水とCSLの代りに用いた同時糖化発酵では3.52%の最終エタノールが得られ，これはCSLを用いた場合の3.28%と比較して遜色のない値であった。これらの結果から蒸留残渣を自己再利用することで，同時糖化発酵における栄養源を代替することが可能であるとともに，水の循環利用にも貢献できることが示された。

キーワード：栄養源，蒸留残渣，稲わら，バイオエタノール