



KKU Res.j. 2016; 21(2) : 210-213
<http://resjournal.kku.ac.th>

Effect of Nitrogen Source on Ethanol Production from Weeds by a Simultaneous Saccharification and Fermentation Process

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Abstract

The effect of nitrogen source on ethanol production from 2 weeds, small-flowered umbrella sedge (*Cyperus difformis*) and cattail (*Typha angustifolia*), was studied. It was performed through a simultaneous saccharification and fermentation (SSF) process using the ethanol-producing yeast *Saccharomyces cerevisiae* TISTR5339. Both lignocellulosic materials were pretreated by steam explosion at 198°C for 5 minutes then steam exploded pulp was hydrolyzed with Cellic CTec2 (25 FPU/g) to obtain glucose. Different nitrogen sources; ammonium nitrate, ammonium chloride, ammonium sulfate, urea and peptone, were varied under a control fermentation condition. The result showed that the optimal nitrogen source for ethanol production from small-flowered umbrella sedge was peptone with ethanol yield 71.5% and ethanol productivity 0.27 g^l-¹h⁻¹. Ammonium nitrate was found as an optimal nitrogen source for ethanol production from cattail with ethanol yield 39.6% and ethanol productivity 0.11 g^l-¹h⁻¹.

Keywords : *Ethanol, Small-flowered umbrella sedge, Cattail, SSF.*

1. Introduction

Currently, the global warming problem and losing fossil fuel source are the important cause for development of ethanol production process from lignocellulosic materials. By-products from agriculture industry, such as corn cob, sugarcane bagasse and weeds, are interesting raw material sources for ethanol production. Lignocellulosic materials are an abundant

agricultural residues. They contain high cellulose, hemicellulose and lignin. Due to the complex structure of hemicellulose and lignin, it interrupts cellulase enzyme activity to convert cellulose to glucose and subsequent ethanol fermentation step. Pretreatment process using steam explosion is the most popular method for the preparation of material, since it separates lignin and hemicellulose from cellulose, and then result in high glucose concentration

(1). Beside the agricultural residues, weeds are also interesting source in ethanol production. This study focused on two weed types; small-flowered umbrella sedge and cattail. They are most pollution to agriculture field, paddy field and wetland in Thailand. Thus, the application of them for ethanol production would be able to solve the problem, increase their values without effecting food supply like other sources.

2. Materials and Methods

2.1 Raw material

Small-flowered umbrella sedge and cattail were collected from Nakhon Sawan Province, Thailand. They were washed with water to get rid of dust and other debris, milled to 1–2 cm size and sun-dried. After that, they were pretreated by steam explosion at 198°C for 5 minutes. Chemical composition analysis of these weeds were performed according to Technical Association of the Pulp and Paper Industry (TAPPI). Small-flowered umbrella sedge contained (expressed in g/100 g dry weight basis): lignin, 21.07; holocellulose, 72.13; alphacellulose, 40.62, whereas cattail contained: lignin, 16.43; holocellulose, 61.99; alphacellulose, 34.23.

2.2 Microorganisms

Saccharomyces cerevisiae TISTR 5339 was obtained from Thailand Institute of Scientific and Technology Research (TISTR) was maintained in yeast extract-peptone-dextrose (YPD) medium (gl⁻¹: glucose 50; yeast extract 10; peptone 20). After 48 h, the cells were harvested and used as an inoculum for ethanol production.

2.3 Simultaneous saccharification and fermentation (SSF)

The simultaneous saccharification and fermentation (SSF) process was used for

ethanol production. Total working volume was 100 ml in 250 ml Erlenmeyer flask including pretreated dry materials (13.95% small-flowered umbrella sedge and 24.58% cattail), 0.5 gl⁻¹ NaH₂HPO₄, 0.025 gl⁻¹ MgSO₄·7H₂O, 0.5 gl⁻¹ (NH₄)₂HPO₄. Nitrogen sources were varied among yeast extract, peptone, urea, ammonium nitrate, ammonium sulfate and ammonium chloride (1 gl⁻¹). The 0.05 M citrate buffer was applied to control pH at 4.8. The commercially available cellulase enzyme (Cellic® CTec2) was used at 25 FPU/g and yeast cell concentration at 10%. The reaction was incubated on shaking incubator at 150 rpm under room temperature. The sample was periodically taken for further analysis at 0, 6, 12, 24, 48, 72 and 96 h, respectively.

2.4 Analytical methods

Reducing sugar analysis was performed according to Somogyi-Nelson Method (2,3). Ethanol concentration (gl⁻¹) was analyzed by gas chromatography (GC, Chromosorb-103, GC4000, GL Sciences, Japan) injection temperature 180 °C, flame ionization detector temperature 250 °C and 2-propanol was used as an internal standard. The ethanol yield ($Y_{P/S}$; gg⁻¹), described by Eqn (1), was calculated as the g ethanol produced and g total sugar utilized. The volumetric ethanol productivity (Q_p , gl⁻¹h⁻¹), described by Eqn (2), was calculated by ethanol concentration produced (P; gl⁻¹) divided by fermentation time giving the highest ethanol concentration (4).

$$\text{Ethanol yield (\%)} = \frac{\text{g ethanol produced}}{\text{g total sugar utilized}} \times 100 \quad (1)$$

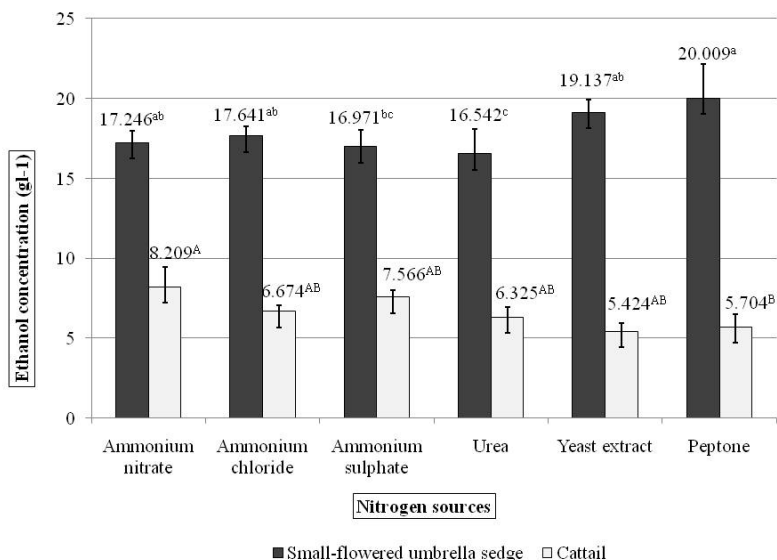
$$\text{Ethanol productivity (gl}^{-1}\text{h}^{-1}\text{)} = \frac{P - P_0}{T_f} \quad (2)$$

P ,	Final ethanol concentration	(gl^{-1})
P_{0p}	Initial ethanol concentration	(gl^{-1})
T_f	Total fermentation time	(h)

3. Results and discussion

Fig. 1 shows the effect of nitrogen source on ethanol production from small-flowered umbrella sedge and cattail by *S. cerevisiae* TISTR 5339. The result indicated that peptone supported the highest production of ethanol (20.01 gl^{-1}) from small-flowered umbrella sedge, with 71.5% ethanol yield and $0.27 \text{ gl}^{-1}\text{h}^{-1}$ ethanol productivity. For cattail, ammonium nitrate was found the optimal nitrogen source to obtain the highest ethanol production (8.21 gl^{-1}), with 39.6% ethanol yield and $0.11 \text{ gl}^{-1}\text{h}^{-1}$ ethanol productivity. The effect of nitrogen on ethanol production from other raw materials were also previously

reported. Laopaiboon *et al.* (5) studied the effect of nitrogen supplementation on ethanol production from sweet sorghum juice. The results revealed that a supplementation with 3 gl^{-1} yeast extract and 5 gl^{-1} peptone increased maximum ethanol production to $120.68 \pm 0.54 \text{ gl}^{-1}$ ethanol, equivalent with $2.01 \pm 0.01 \text{ gl}^{-1}\text{h}^{-1}$ ethanol productivity and $0.51 \pm 0.00 \text{ gg}^{-1}$ ethanol yield. Jones *et al.* (6) and Thomas and Ingledew (7) reported that the addition of nitrogen sources, such as yeast extract, urea and ammonium salts, could improve and accelerate ethanol fermentation from wheat mashes. D'Amore *et al.* (8) reported that supplementation of ethanol fermentation media with excess nitrogen sources could relieve the detrimental effects of high osmotic pressure in media containing high concentration of sugars.



**Different letters indicate significantly different values of each treatment, ANOVA with Duncan's multiple rang test, ($P \leq 0.05$).

Figure 1. Effect of nitrogen source on ethanol production from small-flowered umbrella sedge and cattail by *S. cerevisiae* TISTR 5339. The SSF was performed at 150 rpm and room temperature.

4. Conclusion

This preliminary study showed that the weeds (small-flowered umbrella sedge and cattail) could be used as raw materials for ethanol production by *Saccharomyces cerevisiae* TISTR 5339 through the simultaneous saccharification and fermentation process. The results also showed the effect of nitrogen source on the process. Ethanol production from lignocellulosic materials is also concerned with several parameters. Thus, optimization of the process will be further investigated.

5. Acknowledgements

This research was financially supported by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University.

6. References

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