A Technique to Smartly Re-Use Alkaline Solution in Lignocellulose Pre-treatment

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Alkaline solution was collected from alkali-treatment of steam-exploded rice straw and then reused as is or with addition of NaOH for the next pre‑treatment batch. The as-is-reused solution (without addition of NaOH) showed a significant decrease of alkali-treatment efficiency because NaOH has already reacted with substrates from rice straw in the previous batch. Based on the comparison of alkali-treatment efficiencies between the first-used and as-is-reused alkaline solutions, corresponding reasonable amounts of supplementary NaOH were calculated. The results showed that reused alkaline solutions, with the addition of calculated amounts of NaOH, produce an alkali-treatment efficiency as that of the first-use, while the total amount of NaOH for both batches reduced to around 70 %, implying an impressive saving of chemicals, fresh water, and reduction of volume of waste water.

1. Introduction

For the last twenty years, bioethanol has been receiving an intensive and widespread interest all over the world. Differing from fossil fuels, bioethanol is regarded as a promising renewable fuel, or called as neutral-carbon energy source (Sheehan, 1994). The security of sustainable feedstock is one of the vital trends for bioethanol production. While sugar-based or starch-based bioethanol production disagrees with food security, lignocellulose-based bioethanol can be produced from any kind of popular botanic feedstock, especially agricultural residues (Azuma et al., 1985), which mainly comprise of cellulose, hemicellulose, and lignin (Souza et al., 2014)

In general, cellulose is similar to starch as polymers of glucose, but due to its structural complexity, along with the existence of lignin, lignocellulose has a very strong resistance to enzymatic hydrolysis and inhibit yeast growth. Lignocellulose conversion to bioethanol requires three major processes: physical and chemical pre‑treatment to liberate cellulose and hemicellulose; enzymatic hydrolysis of cellulose and hemicellulose to produce fermentable sugars (Bes et al., 1989); and fermentation of sugars to bioethanol by microorganisms (Talebnia and Taherzadeh, 2006). The high costs of chemicals, enzymes (Koullas et al., 1992), and energy consumed (Ramos et al., 1993;) make commercialisation of lignocellulose-based bioethanol face many obstacles (Sarkar et al., 2012).

Among the chemical pre‑treatment techniques, alkali-treatment of steam-exploded biomass is applied in pilot scales (Sluiter et al., 2008). The use of an alkali cracks ester and glycosidic side chains, leading to structural alteration of lignin, cellulose swelling, partial decrystallisation of cellulose, and partial solvation of hemicellulose (Cheng et al., 2010). In our study employing NaOH as the agent of alkali-treatment, the waste solution after treatment still has a strong basicity, which implies some degree of delignification ability. In this context, the reusability of this solution is investigated based on the following concept.

Consider C1 the initial concentration of an alkaline solution, ATE1 and ATE2 the alkali-treatment efficiencies at the first use and as-is-reuse of solution C1. Consider C2 the concentration of another solution, which has its first-use alkali-treatment efficiency equal to ATE2 as illustrated in Figure1. It is expected that if an amount of NaOH and water added to solution C2 can make up solution C1, the same amount of NaOH and water added to the as-is-reuse solution C1 can restore its treatment efficiency of ATE1.
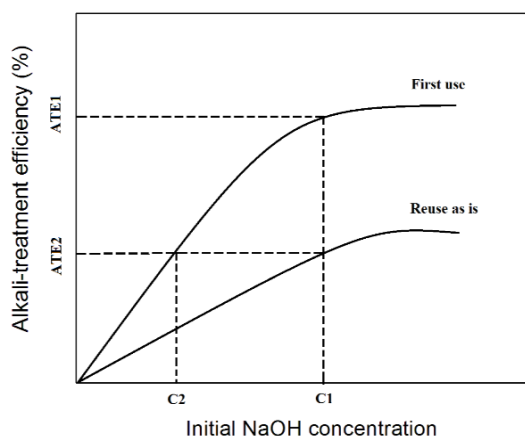
Figure 1: Illustration of the idea of reusing alkaline solution with addition of NaOH and water to restore the alkali-treatment efficiency

2. Materials and methods

2.1 Materials

Rice straw (species Trau Nam Moi) was collected from a farm in Thai My, Cu Chi district, Ho Chi Minh City, Vietnam. It was dried under sunlight after harvesting and then cut into small pieces before steam exploded with a screw-puffing machine. Steam pressure was controlled in a range of 40 - 50 atm and temperature was at 150 °C.

After steam-explosion, puffed rice straw was stored in refrigerator and used within 1 week to minimise microbial growth.

Sodium hydroxide (99.99 wt%), aqueous HCl (37.0 wt%), H_2SO_4 (95 - 98 wt%), and $CaCO_3$ (99.99 wt%) were purchased from Vinachem company (Vietnam).

2.2 Methods

2.2.1 Alkali-treatment

Rice straw after being puffed was mixed with NaOH solution so that 100 g mixture contains 10 g biomass (dry mass). After a definite time of treatment, the rice straw was collected from the mixture by pressing filtration and neutralised with diluted HCl acid until pH reduced to 5.0 - 6.0. After that, the pre-treated rice straw was dried in an oven at 50 °C for 24 hours. The dry rice straw was extensively dried and weighed with an infra-red lamp-balance moisture analyser.

pH of the alkaline solution separated from the treatment mixture was measured with a handy pH analyser. For experiments of reusing this solution to treat another puffed rice straw, NaOH and water can be added or not added but the ratio of biomass to solution must be maintained of 1 : 9 as that of the previous batch.

As mentioned above and illustrated in Figure 1, the amount of added NaOH (M_2, g) and water (M_3, g) to solution C1 after its first use can be solved from the following 2 equations, assuming that this solution has an alkali-treatment efficiency equal to that of fresh solution C2 (Eq(1)):

$$C1 \text{ (wt\%)} = \frac{C2 \text{ (wt\%)} \times M_1 \text{(g)} + M_2 \text{(g)}}{M_1 \text{(g)} + M_2 \text{(g)} + M_3 \text{(g)}} \times 100 \text{ (wt\%)} \tag{1}$$

$$M = M_1 + M_2 + M_3 \tag{2}$$

where M(g) (Eq(2)) is the desired amount of new solution (typically, 90 g solution for 10 g biomass) and M_1 is the weight of solution C1 collected after its first use.

The solution after all alkali-treatment experiments was neutralised until pH 7.0 ± 0.5 with HCl before disposed into the laboratory sewer system.

2.2.2 Fibre analysis

The lignocellulose samples were hydrolysed in a two-step acidic hydrolysis so that cellulose and hemicellulose can be fractionated into glucose and xylose, which can be quantified with HPLC (Shimadzu HPLC analyzer, 2014), employing a column coded SH101, and H_2SO_4 0.005 N solution as the mobile phase. Cellulose and

hemicellulose contents of samples were analysed according to the NREL Laboratory Analytical Procedure (George et al., 2012).

2.2.3 Evaluation of alkali-treatment efficiency

NaOH concentration of alkaline solution to treat puffed rice straw was limited so that cellulose and hemicellulose were not dissolved, while allowing dissolution of other substrates. Assuming cellulose and hemicellulose content in the biomass does not change after alkali-treatment and excluding moisture content by deeply drying samples, the alkali-treatment efficiency (ATE) was defined as the ratio between the mass loss of the biomass after alkali-treatment, and the total weight of substrates other than cellulose and hemicellulose before alkali-treatment. It can be expressed as the following equation (Eq(3)).

$$\text{ATE (\%)} = \frac{\text{Mass loss after alkali-treatment (g)}}{\text{Weight of dried sample before treatment (g)} \times (100\ \% - \text{Cel\%} - \text{Hemi\%})} \qquad (3)$$

where Cel% and Hemi% stand for cellulose content (wt%) and hemicellulose content (wt%) of the dried samples before the treatment.

3. Results and discussion

3.1 Chemical composition

As shown in Table 1, rice straw (species Trau Nam Moi) is an attractive lignocellulosic material for bioethanol production. Rice straw Trau Nam Moi contains 45.7 % cellulose, 17.7 % hemicellulose, and 12.4 % lignin). It has high cellulose and hemicelluloses content that can be readily hydrolysed into fermentable carbohydrates.

Table 1: Chemical composition of rice straw (species Trau Nam Moi)

Component	Content (%)
Cellulose	45.7
Hemicellulose	17.7
Lignin	12.4
Ash	11.3
Dry matter	95.2

3.2 Alkali-treatment time

Alkali-treatment efficiency depends on the treatment time, which needs to be long enough for the dissolution of all non-fibre substrates to get saturated. As shown in Figure 2, when using 0.2 wt%, 1.0 wt%, and 1.8 wt% NaOH solution for alkali-treatment of puffed rice straw, the alkali-treatment efficiency almost did not change after 8, 12, and 16 hours. Since then, for later experiments with NaOH concentration lower than 1.8 wt%, the pre-treatment time was set at 24 hours to ensure the process is completed.
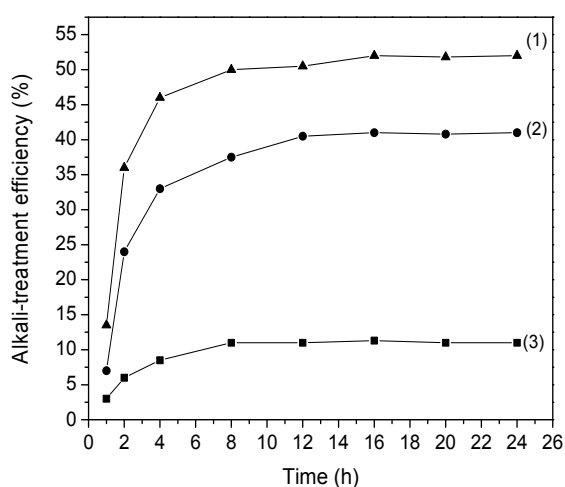

Figure 2: Alkali-treatment efficiency versus time when using (1) 1.8 wt%, (2) 1.0 wt%, and (3) 0.2 wt% NaOH solution

3.3 Concentration of alkaline solution and dissolution of carbohydrates

Although there are numerous studies about fibre dissolution in alkaline solvents, no reference can be found for precise data of the dissolution of cellulose and hemicellulose in biomass during alkali-treatment with 1.0 - 2.0 wt% NaOH solution. To elucidate the fibre loss of rice straw after alkali-treatment, fibre analysis was carried out with samples treated by NaOH at different solution and the results are presented in Table 2.

Table 2. Fibre content of puffed rice straw before and after alkali-treatment

NaOH conc. (wt%)	Sample weight before alkali-treatment (g dry mass)	Before alkali-treatment			Sample weight after alkali-treatment (g dry mass)	After alkali-treatment		
		Cel% (wt%)	Hemi% (wt%)	Fibre mass (g)		Cel% (wt%)	Hemi% (wt%)	Fibre mass (g)
1.0					8.06	48.1	24.5	5.86
1.4					7.55	50.6	26.0	5.78
1.8	10	38.7	21.6	6.03	7.69	50.6	26.0	5.89
2.0					7.48	46.1	24.1	5.25
2.2					7.11	48.3	24.4	5.19

With an acceptable error of ± 5 % for fibre analysis and the mass loss due to small particle of rice straw suspended in the solution, which could pass the filters during collection, it is obvious from Table 2 that the alkali-treatment with NaOH concentration under 2.2 wt% did not cause considerable change in fibre mass included in rice straw. This shows that alkali-treatment with solution concentration lower than 2.2 wt% of NaOH almost removed only substrates other than carbohydrate fibre (Figure 2).

3.2 Alkali-treatment efficiency of reused solutions

As seen from Figure 3, the pH value of alkaline solution after the first use to treat puffed rice straw increased from 8.0 to 11.1 as the NaOH concentration of the starting solution increased. This implies that no matter NaOH in the mixture still remained or all converted to NaOR by reacting with substrates in rice straw, the solutions after the treatment are strongly basic solutions, which can dissolve lignin in rice straw at some degree. As can be seen in Figure 4, graphs (1) and (2), the alkali-treatment efficiency of the alkaline solution reused as is was from 15.1 to 32.2 % corresponding to initial NaOH concentration of 0.8 to 1.8 wt%. Although these values were lower than those at the first use of the solutions (26.8 - 54.1 %), there is no doubt that the solutions after their first uses should be utilised. Based on these results and the idea presented in the introduction above, amounts of NaOH and water to add to the reused solution are calculated and shown in Table 3.
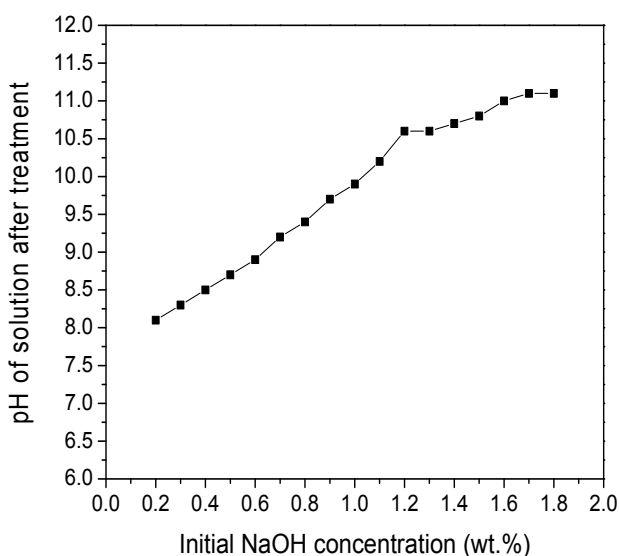

Figure 3: pH values of alkaline solutions after alkali-treatment of puffed rice straw versus the initial NaOH concentration

As seen from Table 3, the weight of solution after the first use with removal of pre-treated rice straw varied almost randomly in a range of 85 - 89 g. This is due to the loss of liquid absorbed in rice straw and the gain of non-fibre substrates from rice straw dissolved into the liquid phase. These little changes in weight can be tolerated for technical calculation.

Table 3: Calculated amount of NaOH and water added to the reused alkaline solutions to restore their alkali-treatment efficiency

Initial NaOH concentration (C1, wt%)	NaOH concentration of first-use solution with alkali-treatment equal to ATE2 (C2, wt%)	Weight of solution after alkali-treatment and rice straw filtration (g)	Amount of NaOH added (g)	Amount of water added (g)	Amount of puffed rice straw to be treated (g)
0.8	0.30	85.8	0.46	3.74	10
0.9	0.50	88.2	0.37	1.43	10
1.0	0.60	88.0	0.37	1.63	10
1.1	0.68	86.6	0.40	3.00	10
1.2	0.74	87.8	0.43	1.77	10
1.3	0.76	89.0	0.49	0.52	10
1.4	0.79	89.4	0.55	0.06	10
1.5	0.83	86.9	0.63	2.47	10
1.6	0.84	88.4	0.70	0.90	10
1.7	0.83	88.1	0.80	1.11	10
1.8	0.85	87.9	0.87	1.23	10

Graph (3) in Figure 4 presented the alkali-treatment efficiencies of the reused solution with addition of NaOH and water as mentioned above. The efficiencies were restored near the same as those of their first-use solutions. This result implies a successful tactic to reuse the alkaline solution after the first rice straw treatment in respect to the efficiencies, which herein is called "reuse-solution alkali-treatment". Table 3 presented a comparison between single-use-solution and reuse-solution alkali-treatments with equal efficiencies at different initial NaOH concentrations in term of amounts of chemicals and fresh water per gram of treated rice straw. When using NaOH solution with concentration from 1.0 to 1.3 wt%, the reuse-solution alkali-treatment significantly saves NaOH and fresh water up to 30 % and 49 %. The volume of waste solution after alkali-treatment can reduce to 50 % while saving of acid used to neutralise the waste liquid can also be equal to the saving of NaOH. The more reusing cycles of the solution, the more savings can be reached. In this study, only one cycle of reuse was investigated because the liquid solutions became dark and viscous.
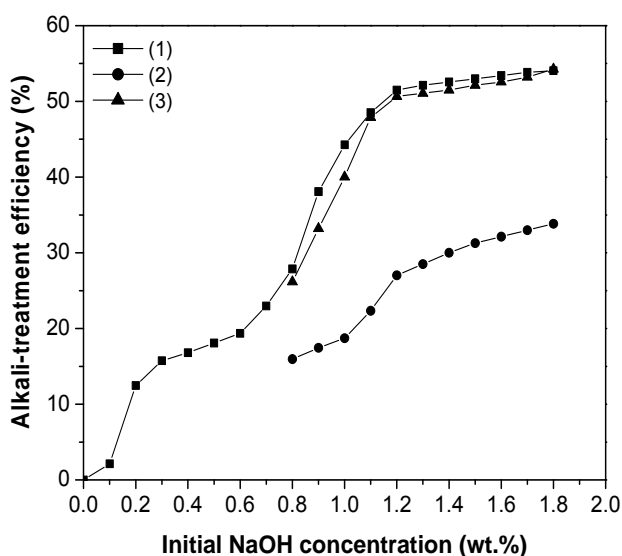

Figure 4: Alkali-treatment efficiency versus NaOH concentration of the first used solution for (1) the first use of alkaline solution, (2) the as is reuse of alkaline solution after treatment, (3) the reuse of alkaline solution after treatment with addition of NaOH and water

4. Conclusion

The solution after alkali-treatment of puffed rice straw in lignocellulosic ethanol production has strong basicity and can be reused with addition of NaOH and water. A novel tactic was developed to determine the suitable amounts of NaOH and water added to the reused solution in order to restore the alkali-treatment efficiency. Experimental results showed that by the reuse-solution alkali-treatment, NaOH, acid used to neutralise the waste, and fresh water can be significantly saved by up to 30 % and 49 %. This technique can help to reduce lignocellulosic production cost in term of chemicals and waste management.

References

Azuma J., Asai T., Isaka M., Koshijima T, 1985, Effects of microwave irradiation on enzymatic susceptibility of crystalline cellulose, Journal of Fermentation Technology, 63, 529-536.

Bes R.S., Gas G., Molinier J., Vidal P., Mathieu J., Mora J.C, 1989, Enhancement of poplar cellulose susceptibility to cellulase enzyme hydrolysis by ozonation, Ozone: Science & Engineering, 11, 217-226.

Cheng Y.S., Zheng Y., Yu C.W., Dooley T.M., Jenkins B.M., VanderGheynst J.S., 2010, Evaluation of high solids alkaline pretreatment of rice straw, Applied Biochemistry and Biotechnology, 162, 1768-1784.

George J.M.R., Carlos M., Vinícius F.N.S., Edgardo O.G., Adilson R.G., 2012, Mass balance of pilot-scale pretreatment of sugarcane bagasse by steam explosion followed by alkaline delignification, Bioresource technology, 111, 447-452.

Ibrahim M.M., El-Zawawy W.K., Abdel-Fattah Y.R., Soliman N.A., AgblevorF.A., 2011, Comparison of alkaline pulping with steam explosion for glucose production from rice straw, Carbohydrate Polymers, 83, 720-726.

Koullas D.P., Christakopoulos P., Kekos D., Macris B.J., Koukios E.G., 1992, Correlating the effect of pretreatment on the enzymatic hydrolysis of straw, Biotechnology and Bioengineering, 39, 113-116.

McIntosh S., Vancov T., 2010, Enhanced enzyme saccharification of sorghum bicolor straw using dilute alkali pretreatment, Bioresource Technology, 101, 6718-6727.

Ramos L.P., Nazhad M. M., Saddler, J.N., 1993, Effect of enzymatic hydrolysis on the morphology and fine structure of pretreated cellulosic residues, Enzyme and Microbial Technology, 15, 821-831.

Rolz C., de Leon R., de Arriola M.C., de Cabrera S., 1986, Biodelignification of lemon grass and citronella bagasse by white-rot fungi, Applied and Environmental Microbiology, 52, 607-611.

Sarkar N., Gosh S.K., Bannerjee S., Aikat K., 2012, Bioethanol production from agricutural wastes: An

Sills D.L., Gossett J.M., 2011, Assessment of commercial hemicellulases for saccharification of alkaline pretreated perennial biomass, Bioresource Technology, 102, 1389-1398.

Sluiter A., Hames B.R., Ruiz R., Scarlata C.J., Sluiter J., Templeton D.W., Crocker D., 2008, Determination of structural carbohydrates and lignin in biomass, National Renewable Energy Laboratory, Colorado.

Sheehan J., 1994, Bioconversion for production of renewable transportation fuels in the United States: A strategic perspective. Chapter. In: Himmel J.B.M., Overend R. (Eds.), Enzymatic Conversion for Biomass Fuels Production, American Chemical Society, Washington, DC, USA.

Souza E.L., Liebl G.F., Maragoni C., Sellin N., Montagnoli M.S., Souza O., 2014, Bioethanol from fresh and dried banana plant pseudostem, Chemical Engineering Transaction, 38, 271-275.

overview, Renewable Energy, 37, 19-27.

Szczodrak J., Fiedurek J., 1996, Technology for conversion of lignocellulosic biomass to ethanol, Biomass and Bioenergy, 10, 367-375.

Talebnia F., Taherzadeh M.J., 2006, In situ detoxification and continuous cultivation of dilute-acid hydrolyzate to ethanol by encapsulated *S. cerevisiae*, Journal of Biotechnology 125, 377–38

Wyman C.E., Dale B.E., Elander R.T., Holtzapple M., Ladisch M.R., Lee Y.Y., 2005, Coordinated development of leading biomass pretreatment technologies, Bioresource Technology, 96, 1959-1966.

Zhang Y.H., Schell D.J., McMillan J.D., 2007, Methodological analysis for determination of enzymatic digestibility of cellulosic materials, Biotechnology and Bioengineering, 96, 188-194.