PRE-TREATMENT OF RICE HUSKS FOR FUNGAL FERMENTATION

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ABSTRACT

Agro by-products can be sustainable feedstock for the production of various versatile bio-products. The pre-treatment of which is needed for optimal utilization of recalcitrant lignocellulosic biomass. This study investigated the effect of various acids and alkalis on the pre-treatment of rice husks to make it suitable for use in fermentation. Dry rice husks with the weight of 10 g were pre-treated with 1 % (w/v) of five acids and two alkalis with water loading of 10 ml/g of rice husks. The rice husks that was pre-treated with 1 % (w/v) nitric acid produced the highest total reducing sugars in the hydrolyzate (approximately 4.2 mg/ g of rice husks) after 8 hours at 100 ^{o}C followed by pre-treatment with acetic acid (~0.7 mg) and sodium hydroxide (~0.4 mg). Hydrolysis with other acids and alkali produced less than 0.1 mg reducing sugars/ g rice husks. In conclusion, nitric acid was found to be the best hydrolyzing reagent for pre-treatment of rice husks in this study.

Keywords: Rice husks; lignocellulosic biomass; pre-treatment; hydrolyzing reagent

INTRODUCTION

Malaysia generates approximately 30 million tons of crop residues annually, of which 3.4 million tons is consisted of rice crop residue, e.g. rice husks (ESCAP, 1997). The constituents of rice husks are 35.6 ± 0.1% cellulose and 12.0 ± 0.7% hemicellulose (Saha and Cotta, 2008). The crop residue consists primarily of lignocellulosic biomass, in which mostly have limited uses and is commonly disposed as waste or burnt as energy fuel (Muthadhi and Anitha, 2007; Pang *et al*., 2006a). One of the effective measures is to recycle and reuse these agro by-products.

These crop residues are available at low cost and could be sustainable raw materials for the production of versatile bio-products, such as glucose, ethanol (Saha *et al*., 2005; Chang *et al*., 1998), and enzymes (Pang *et al*., 2006b; Alam *et al*., 2005). The benefits of these crop residues could be tapped by means of fermentation by using suitable fungus. For instance, sugarcane bagasse, a residue from sugar refinery, had been used as a substrate for xylanase production by *Trichoderma harzanium* via fungal fermentation (Rezende *et al*., 2002). In general, white rot fungi secrete oxidative enzymes to breakdown lignocellulosic materials. Due to the recalcitrant nature of lignocellulosic biomass, fungi cannot efficiently utilize the crop residue, unless it is pre-treated physically or chemically (Gowthaman *et al*., 2001; Playne, 1984). Therefore, lignocellulosic biomass has to be treated for more efficient use in fermentation.

Kaar and Holtzapple (2000) had reported that chemical pre-treatment processes disrupt lignocellulosic matrix rendering the substrate more accessible to hydrolyze into glucose. The disruption of lignocellulosic matrix can be achieved by partial delignification and solubilization of hemicellulose as illustrated by Gowthaman *et al*. (2001). Typically, dilute mineral acids and alkalis like sulphuric acid, hydrochloric acid, sodium hydroxide, etc. are common reagents to be used in the pre-treatment of lignocellulosic biomass in fermentation studies (Saha and Cotta, 2008; Saha *et al.*, 2005; Chang *et al.*, 1998).

In this study, the effects of different acids or alkalis on pre-treatment of rice husks were investigated in order to determine a suitable pre-treatment chemical that give the best result.

MATERIALS AND METHODS

Substrate

Rice husks were collected from a company in Selangor, Malaysia. The rice husks were immersed in distilled water overnight and washed under running water to remove foreign materials before it was dried in oven at 55^{o}C overnight or until constant weight was achieved.

Selection of Pre-treatment Reagent

The pre-treatment of rough rice husks was done by treating 10 g of dry rice husks in the presence of 1 % (w/v) acid or alkali with a water loading of 10 ml/ g of dry rice husks. Types of acid and alkali used were tabulated in Table 1. The mixtures of rice husks and acid or alkali were pre-treated at 100 ^{o}C for 10 hours. About 5 ml hydrolyzates of the mixtures was taken at 2-hour intervals to be analyzed for their total reducing sugars content by using DNS method (Miller, 1959). Negative control was done by replacing the acid or alkali with distilled water.

Table 1: Acids and alkalis used in the pre-treatment of rice husks

Acid	Alkali
Acetic acid Hydrochloric acid Nitric acid Phosphoric acid Sulfuric acid	Calcium hydroxide Sodium hydroxide

RESULTS AND DISCUSSION

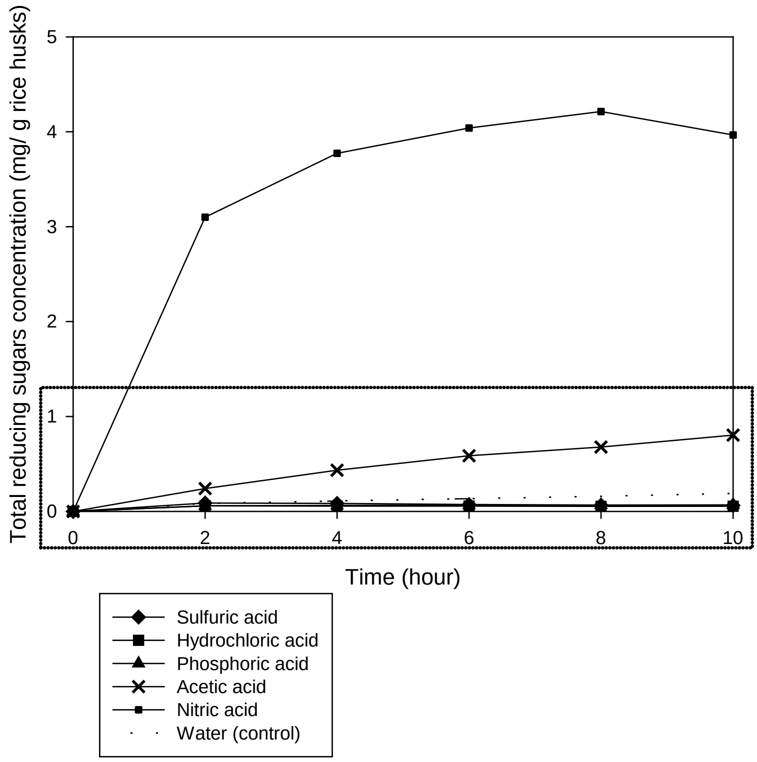
Selection of Pre-treatment Chemical and Its Effect

The effects of various acids and alkali used in pre-treatment of lignocellulosic biomass were evaluated. The most suitable type of pre-treatment agent for treating rice husks in subsequent fermentation was determined. Certain types of acid or alkali hydrolyse specific types of lignocellulosic biomass better due to the variation in lignocellulose contents. For instance, lime hydrolysis of bagasse and wheat straw produced different effects (Chang *et al.*, 1998). Acid and alkali would hydrolyse and partially break down the lignocellulose biomass, which is evident by the release of reducing sugars in the hydrolyzates (Kaar and Holtzapple, 2000).

Out of five acids being tested in this study, nitric acid hydrolysis released the highest reducing sugars from the rice husks, which was approximately 4.2 mg/ g rice husks after eight hours (Figure 1). Hydrolysis of rice husks with nitric acid peaked at the eighth hour and started to reduce at tenth hour. This phenomenon may be due to the degradation of reducing sugars to form by-products after long hour of acid hydrolysis (Vazquez *et al.*, 2007). Thus, extended experiment is recommended to confirm the formation of degradation products from nitric acid hydrolysis. The reagent that produced the second highest amount of reducing sugars from the hydrolysis of rice husks was acetic acid (about 0.9 mg/ g of rice husks). Reducing sugars in hydrolyzate of the acetic acid increased during the course of study and was expected to increase continually after ten hours of pre-treatment (Figure 1). As a whole, nitric acid performed about five times better than acetic acid and many folds better than the other three acids.

On the other hand, hydrolysis of rice husks using sodium hydroxide produced 0.4 mg reducing sugars/ g rice husks, which was about 7 times higher reducing sugars than using calcium hydroxide (Figure 2). The total reducing sugars content of sodium hydroxide treated rice husks increased to about 0.3 mg/ g of rice husks on the second hour and it had slight increment towards the tenth hour of the study.

(a)



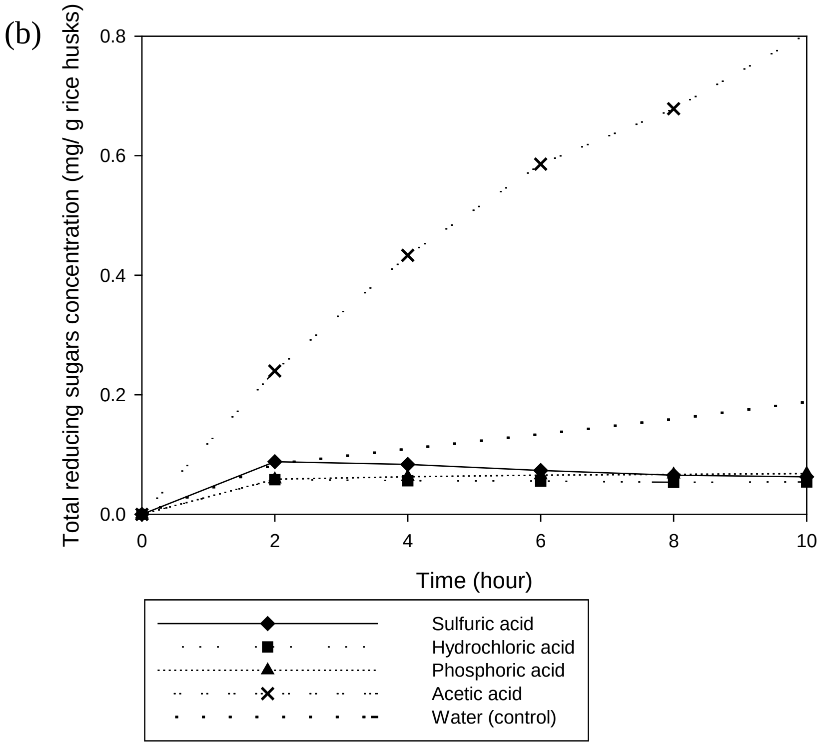


Figure 1: The effect of different acids on the hydrolysis of rice husks. Note: Graph (b) is the magnification of the boxed section in graph (a).

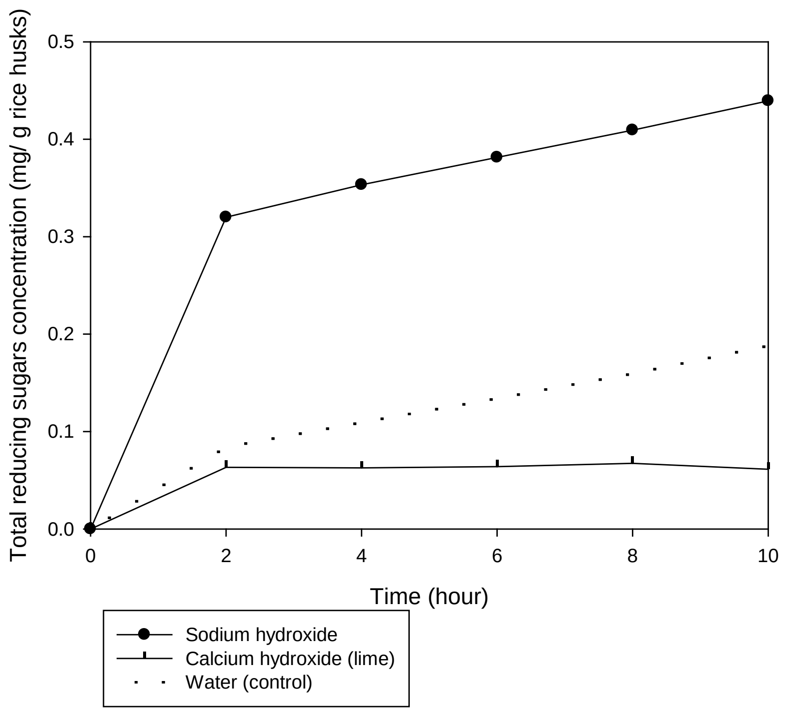


Figure 2: The effect of different alkalis on the hydrolysis of rice husks.

Table 2: Total reducing sugars produced after 10 hours of pre-treatment.

Reagents	Total reducing sugars (mg/ g rice husks)
Acetic acid	0.80
Hydrochloric acid	0.05
Nitric acid*	3.97
Phosphoric acid	0.07
Sulfuric acid	0.06
Calcium hydroxide	0.06
Sodium hydroxide	0.44
Water	0.19

* *Highest yield was 4.2 mg/ g rice husks after 8 hour.*

The production of reducing sugars from hydrolysis of rice husks using selected acids and alkalis were compared and tabulated in Table 2. The table indicated that nitric acid had efficiently hydrolysed rice husks to produced 3.97 mg reducing sugars/ g rice husks. The other acids and alkalis showed low hydrolysis of rice husks, i.e. production of less than 0.1 mg reducing sugars/ g rice husks after ten hours of pre-treatment. The performances of these reagents were not satisfactory as the production of total reducing sugars were lower than the control experiment that is around 0.2 mg/ g rice husks. The hydrolysing effect of water was found to be three times better than the hydrolysing effect of sulphuric acid, hydrochloric acid, phosphoric acid and lime. The higher value obtained from the control experiment demonstrated that treatment with hot water possibly hydrolysed a large fraction of hemicellulose and smaller fraction of cellulose as reported by US Dairy Forage Research Center (1997).

CONCLUSION

The effects of the selected acids and alkalis on the pre-treatment of rice husks were in the descending order of nitric acid, acetic acid, sodium hydroxide, water, phosphoric acid, sulphuric acid, calcium hydroxide and hydrochloric acid. Nitric acid was found to give the best performance in the hydrolysis of rice husks and produced approximately 4.2 mg reducing sugars/ g of rice husks after eight hours of pre-treatment. Therefore, it could be a suitable reagent for the pre-treatment of rice husks prior to fermentation as compared to the other reagents under investigation.

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BRIEF BIOGRAPHY OF PRESENTER

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