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Fermentation of paddy straw and fruit wastes for bioethanol production

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Abstract

A study was carried out for bioethanol production from alkali (2.0% NaOH) pretreated paddy straw by simultaneous saccharification and co-fermentation using *Saccharomyces cerevisiae* and *Candida* sp. The paddy straw was pretreated with 2.0% NaOH by autoclaving at 15 psi for 1 h to degrade the lignin matrix. Mixed fruit solid wastes were used for enhancement of ethanol production from paddy straw. Effect of mixed fruit peel with paddy straw hydrolysate on yield of ethanol was investigated. The addition of mixed fruit peel during fermentation enhanced the quantity of ethanol produced. Different nutrient supplements were also used to carry out for ethanol production. The fermented paddy straw hydrolyzate with 0.3% urea and mixed fruit (apple and pomegranate) peel yielded ethanol content of 2.2 to 3.1% (v/v). The ethanol production increased with the progression of fermentation and reached the maximum value after 7 days of fermentation.

Keywords: Ethanol, fermentation, mixed fruit waste, paddy straw, saccharification

Introduction

Energy consumption has been increasing steadily with population growth and industrial development. Conventional energy sources have difficulty in matching the increase demands. Over the last two decades, a great interest in exploring alternative energy sources has been developed. Biofuel, a clean and renewable energy source, which can be produced through fermentation of sugars, has drawn much attention. The use of lignocellulosic material from agricultural wastes provides low cost fermentative substrate. Ethanol from renewable resources has been of interest in recent decades as an alternative fuel to the current fossil fuels. Lignocelluloses biomass like wood and agricultural crop residue such as straw and sugar beet pulp are potential raw materials for producing several high value products like fuel ethanol and biodiesel. Lignocellulose contains up to 80% of the polysaccharides (Kaparaju *et al.*, 2009) [8]. These renewable raw materials look promising for replacing environmentally unfriendly fossil hydrocarbon raw materials and, hence creating “green” products. In contrast to traditional fuels, fermentation ethanol does not contribute to the greenhouse effect, being a carbon dioxide natural resource. Paddy straw is a by-product of rice production and great bioresource. It is one of the most abundant lignocellulosic waste materials in the world. The paddy straw contains 32-47% cellulose, 19-27% hemicelluloses 5-24% lignin and 18.8% ash (Yoswathana and Phuriphipat, 2010) [18]. The pentoses are dominant in hemicelluloses which contains xylose. Xylose is the most important sugar followed by arabinose and hexoses. The carbohydrate of rice straw involves glucose 41–43.4%, xylose 14.8–20.2%, arabinose 2.7–4.5%, mannose 1.8% and galactose 0.4% (Roberto *et al.*, 2003) [13]. Hemicellulose serves as connection between lignin and cellulose fibers and provides more rigidity to the whole cellulose-hemicellulose-lignin network. India is the second largest producer of paddy in the world after China and has 30,000 varieties of paddy crops. The structural complexity of paddy straw due to the presence of lignin is a major constraint for enzymatic and microbial attacks (Balasubramanian, 2013) [3]. The lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further hydrolysis processes. Therefore, the pretreatment is a necessary process for utilization of lignocellulosic materials to obtain ultimately high degree of fermentable sugars. Bioconversion of cellulosic biomass into fermentable sugar, for production of ethanol using microorganisms, especially cellulose degrading fungi, makes bioethanol production economic, environmental friendly and also renewable (Belal, 2013) [4]. Production of ethanol from lignocellulosic biomass contains three major steps: pretreatment, hydrolysis and fermentation.

Pretreatment breaks the lignin structure and disrupts the crystalline structure of cellulose thus enhancing enzyme accessibility to the cellulose during hydrolysis (Yang and Wyman, 2008) [17]. Sugars generated from hydrolysis of cellulose and hemicelluloses can be fermented either by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and co-fermentation (SSCF). Simultaneous saccharification and fermentation is more favored because of its low cost. A protocol for ethanol production from paddy straw by separate hydrolysis and fermentation has been standardized by Wati *et al.*, 2007 [19] and Malik and Tokas, 2018 [11]. The aim of this study was to enhance the ethanol production from paddy straw using mixed fruit wastes.

Material and Methods

1. Raw materials: Paddy straw was procured from Dabra, Hisar. It was dried at 50 ^{O}C. The paddy straw was grinded and sieved through 0.5 and 2.0 mm sieve.

2. **Pretreatment of Paddy straw:** Paddy straw of mesh size 2.0 mm was immersed in 2% NaOH solution and delignified by autoclaving at 15 psi for 1hr. After cooling, it was washed with tap water 6-7 times to make alkali free and neutralized with 0.1 M acetic acid and dried at 50 ^{O}C.

3. Analysis of Paddy straw

Determination of cellulose, hemicelluloses and lignin

Cellulose, hemicelluloses and lignin contents were estimated by determining acid detergent fiber (ADF) and neutral detergent fiber (NDF) in the sample (AOAC, 1970) [2].

4. Simultaneous Saccharification and Co-fermentation (SSCF): For SSCF, 10% (w/v) of pretreated paddy straw was suspended in distilled water, 1.0 ml of commercial cellulase enzyme was added and 1.0% inoculum of *Saccharomyces cerevisiae* and *Candida* sp. with under different treatments:

A-Paddy straw hydrolysate in water + 0.3% urea

B-Paddy straw hydrolysate in water + 3.0% mixed fruit peel (apple and pomegranate)

C-Paddy straw hydrolysate in water + 5.0% mixed fruit peel (apple and pomegranate)

D-Paddy straw hydrolysate in water + 0.3% urea+ 5.0% mixed fruit peel (apple and pomegranate) +1.0 ml of commercial enzyme-I(Palkonol)

E-Paddy straw hydrolysate in water + 0.3% urea + 5.0% mixed fruit peel (apple and pomegranate) + 1.0 ml of commercial enzyme-II (Palkosoft 720)

5. Estimation of total reducing sugars and ethanol

Total reducing sugars in the sample was estimated by standard Dinitrosalicylic acid (DNS) (Miller, 1959) [12].

Ethanol concentration was determined by spectrophotometric method (Caputi *et al.*, 1968) [5].

Results and Discussion

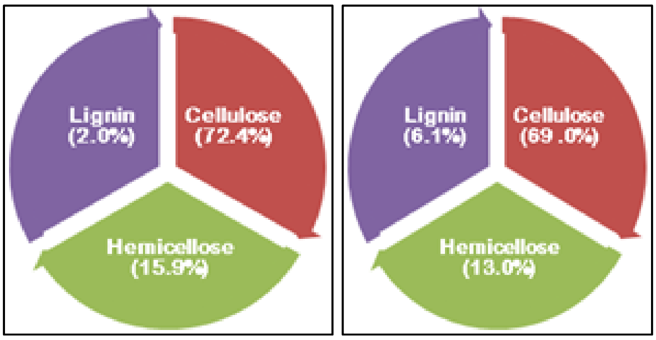
1. Pretreatment of Paddy straw

Paddy straw was procured from Dabra, Hisar was dried at 50 ^{O}C and grinded. Paddy straw of mesh size (0.5mm and 2.0mm) was immersed in sodium hydroxide solution (2.0%) and delignified by autoclaving at 15psi for 1hr. After cooling, it was washed with tap water 6-7 times to make alkali free and neutralized with 0.1 M acetic acid and dried at 50 ^{O}C for further use (Fig-1). Pretreatment of paddy straw with 2% sodium hydroxide at 15 psi for 1 h resulted in 83% delignification (Wati *et al.*, 2007) [19]. In alkali pretreatment, lignin component is dissolved in alkali and removed in liquid fraction while hemicelluloses and cellulose fractions are recovered together in solid fraction (Taherzadeh and Karimi, 2008) [15]. The Rice straw pretreatment was optimized by soaking in 2% H_2SO_4 followed by autoclaving at 15 psi for 90min.It was observed that 25% solubility of cellulose along with decrease in hemicelluloses and lignin contents (Kocher and Kalra, 2013) [9].

Fig 1.A: Untreated Paddy straw, **B:** Pretreated Paddy straw

2. Analysis of Paddy straw

Paddy straw of 0.5mm and 2.0 mm mesh size were delignified and analyzed for cellulose, hemicelluloses and lignin components. Alkali treated paddy straw contained cellulose (69.0% and 72.4%), hemicelluloses (13.0% and 15.9%) and lignin (6.1% and 2.0%) in 2.0mm and 0.2 mm mesh size (Fig.2). Yadav *et al.* (2011) [16] reported 35% cellulose, 22% hemicelluloses and 12% lignin in paddy straw. It was found that lignin content decreased with increase in cellulose content by alkali treatment. The maximum lignin removal was achieved with 0.5 mm mesh size (2.0%) as compared to 2.0 mm mesh size (6.1%).



(A) (B)

Fig 2: Effect of alkali pretreatment on various components of different mesh size paddy straw; A: 0.5 mm mesh size and B: 2.0 mm mesh size

3. Separate Hydrolysis and Fermentation (SHF)

Hydrolysis of delignified paddy straw was carried out using commercial cellulase enzyme (I and II). The enzyme was loaded 1.0% and hydrolysis was carried out at 50 ^{O}C for different time intervals (Fig.2). It was found that the total reducing sugars released increased with increase in incubation period upto 2.5 hrs (Table.1).

Fig 2: Hydrolysis of delignified paddy straw

Table 1: Effect of Cellulase concentration on total reducing sugars released from delignified paddy straw at 50 ^{0}C at different time intervals

Time (h)	Total reducing sugars (%)	
	Enzyme-I	Enzyme-II
1.5	50.1	52.0
2.0	62.1	63.8
2.5	63.2	67.0
3.0	60.0	64.6

4. Simultaneous Saccharification and Co-fermentation (SSCF)

Sugars produced as a result of hydrolysis were fermented to ethanol by yeast. Efficient hexoses fermenting yeast strain *Saccharomyces cerevisiae* HAU-1 and pentose fermenting yeast strain *Candida* sp were checked for alcohol production. For fermentation studies, yeast biomass after 24hr growth in sucrose containing medium was harvested and inoculated to hydrolysate @ 1% (v/v). For enhancing ethanol production from paddy straw its co-fermentation was studied in urea (0.3%) and 5% mixed fruit peel (apple and pomegranate) as cheap nitrogen source and sugar supplement. Fermentation was carried out at 35 ^{0}C over a period of one week. It was observed that maximum ethanol (3.1%) was produced with co -culture of *Saccharomyces cerevisiae* HAU-1 and *Candida* sp. with 0.3% urea, 5.0% mixed fruit peel (apple and pomegranate) and 1.0 ml of commercial enzyme-II at 35^{0}C under stationary condition (Table-2).

Table 2: Ethanol production from paddy straw supplemented with different nutrient at 35 ^{0}C under stationary condition

Treatment	Ethanol (%)
A-Paddy straw hydrolysate in water+ 0.3% urea	2.2
B-Paddy straw hydrolysate in water+ 3.0% mixed fruit peel (apple and pomegranate)	1.5
C-Paddy straw hydrolysate in water+ 5.0% mixed fruit peel (apple and pomegranates	1.8
D-Paddy straw hydrolysate in water+0.3% urea+ 5.0% mixed fruit peel (apple and pomegranate) +1.0 ml commercial enzyme-I	2.4
E-Paddy straw hydrolysate in water+0.3% urea+5.0% mixed fruit peel (apple and pomegranates)+ 1.0 ml commercial enzyme-II	3.1

Simultaneous Saccharification and co-fermentation of delignified paddy straw supplemented with urea (0.3%) with co-culture of *Saccharomyces cerevisiae* and *P. tannophilus* showed maximum production of ethanol 310 ml/kg after 72 hr incubation at 35 ^{0}C (Goel, 2012) [6]. The fermentation of the dilute acid pretreatment hydrolysate of mixed fruit pulps (banana and mango) showed maximum ethanol production of 35.86% corresponding to a fermentation efficiency of 70.31% at 48 hr of incubation (Arumugam and Manikandan, 2011) [1]. Kandari and Gupta (2012) [7] used vegetable and fruit peel wastes for valuable products. The maximum production of alcohol was produced within 36 hrs of fermentation in papaya peels extract followed by banana (5.90%) and apple peel extract (4.94%). In case of turnip peel extract the alcohol produced was 1.5% (w/v). Singh and Bishnoi (2012) [4] reported an ethanol yield of 0.50, 0.47and 0.48 (g/g) by *Saccharomyces cerevisiae*, *S. stipitis* and by co-culture respectively, using hydrolyzate of pretreated rice straw. The co-culture of *Saccharomyces cerevisiae* and *S. stipitis* produced 25% more ethanol as compared to *S. cerevisiae* alone and 31% more ethanol than *S. stipitis* alone. The maximum production of ethanol was produced within 72 hrs of fermentation in PSH + sapota peels (3.9% v/v) followed by PSH + kinnow peels (3.6%) and PSH+ papaya peels extract (3.1%) (Malik, 2015).

Conclusion

In the present study, the paddy straw was pretreated with 2.0% NaOH by autoclaving at 15 psi for 1h to degrade the lignin matrix. The maximum lignin removal was achieved with 0.5 mm mesh size (2.0%) as compared to 2.0 mm mesh size (6.1%), respectively. Effect of mixed fruit peel with paddy straw hydrolysate on yield of ethanol was investigated. Supplementation of 0.3% urea and 5.0% mixed fruit peel (apple and pomegranate) to paddy straw suspended in water resulted in production of 2.2 to 3.1% ethanol by *Saccharomyces cerevisiae* and *Candida* sp. Mixed fruit wastes were proved as one of the potential raw material for ethanol production from paddy straw. The addition of mixed fruit peel during fermentation enhanced the quantity of ethanol produced.

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