DELIGNIFICATION OF RICE HUSK BY ORGANOSOLV TREATMENT TO INCREASE ITS IN-VITRO DIGESTIBILITY.

A. Alam,R. Naseer,A.S. Hashmi, S. Naveed* and A. Rahman*

Institute of Biochemistry and Biotechnology, Department of Biosciences
*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan
Corresponding author's e-mail:rahat.naseer@uvas.edu.pk

ABSTRACT: The present study was conducted to analyze the effect of three different organic solvents (Ethanol, Methanol and Acetone) on delignification of rice husk (basmati rice). Two different concentrations (50% and70%) and three different temperatures (160° C, 180° C and 200° C) were used. Two major experimental groups were designed, autocatalytic (without catalyst) and catalytic (with 1% sulphuric acid as catalyst), each with three subgroups and three replicates. Treatment duration was 1 hour for both autocatalytic group andcatalytic group. Our results showed that 70% concentration of each organic solvent was better than 50% for delignification. Treated rice husk was used to test the in vitro digestibility. Result showed a positive correlation between percentage of delignification and digestability, a 20% increase indigestibilitywas observed with 25 % delignification. Hence organic solvents, although expensive can be employed for effective delignification.

Key words: lignocellulosic material, autocatalytic, delignification, organic solvents, in-vitro total dry matter digestibility and sulphuric acid.

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INTRODUCTION

The plant cell wall is composed of cellulose, hemicellulose and lignin, alongside little amount of pectin, protein and dissolvable nonstructural materials, nitrogenous material, chlorophyll and waxes are also present. Lignocellulosic biomass is the most abundant organic material in nature. Almost 10-50 billion tons dry mass is produced representing about 50% of the worldwide biomass yield (Parveen*et. al.,* 2009).

Rice husk (RH) produced during the rice refining process, is considered a waste product and usually makes disposal issue due to its low density and less business interest (Dilip*et. al,.* 2014).

Rice Husk is essentially made up of lignocellulose (72−85 wt%) and silica (15−28 wt%). The prime focus of the research is the optimum use of rice husk in the form of ash and extraction of silica, while the lignocellulose portion is mostly glazed and then wasted. Hence a comprehensive approach is designed to make an optimum use of rice husk by utilizing its lignocellulosic part(Ajay *et. al.,* 2012).

Numerous techniques have been adopted for treating lignocellulosic feedstocks. However, just a few of them appear to be encouraging. These treatment techniques include dilute acid treatment, steam blast (CO_2 blast), pH controlled water treatment, ammonia fiber expansion, ammoniarecycle percolation (ARP) and lime treatment (Asif *et. al.,* 2013). Some survey articles are reported for microbial biomass treatment. The present study will focus on organosolv treatment process. Despite the fact, that organosolv treatment is more expensive than the leading treatment forms. Organosolv treatment can also producea range of useful byproducts, that's why itis more practical for bio refineryof lignocellulose biomass by utilizing every part of biomasswith mimimum environmental effect (Haoran*et. al.,* 2013). Organosolv treatment yields three different parts: dry lignin, a watery hemicellulose stream and a moderately unadulterated cellulose division (Xuebing*et. al*., 2009).

MATERIALS AND METHODS

Rice husk was purchased from a Lahore rice mill and was brought to the Institute of Biochemistry and Biotechnology University of veterinary and animal sciences (UVAS) Lahore. It was dried in the hot air oven at 70°C to a constant weight temperature and was ground up to 2mm particle size. Proximate and fiber analysis of rice husk was carried out to know its composition and profile.(Jancik*et. al.,*2008).

Organosolv Treatment: Two major groups (catalytic and autocatalytic) were designed for organosolv treatment. The catalytic group used 1% sulphuric acid as catalyst and autocatlytic was without catalyst.For each group three different solvents i.e.ethanol, methanol and acetone with two different concentrations, i.e.50% and 70% were used in this experiment. All the treatments were run at three different temperaturesi.e. 160°C, 180°C and 200°C. Reaction duration was 1 hour for both autocatalytic and catalytic(Nahyun*et. al.,* 2010).

Solvent and Water washing: Residue obtained from filtration was washed with respective organic solvent and was placed on water bath to remove the extra organic solvent. After solvent washing, residue was washed with water and kept in incubator at 70°C to dry the residue (Nahyun*et. al.*, 2010).

Estimation of cellulose and lignin: To determine cellulose, 1g treated rice husk labelled as W1 was first treated with ADF solution and the resulting residue was used to determine cellulose. Residue obtained after ADF treatment was weighed and labelled as W2 then washed with 72% w/w sulphuric acid to dissolve the cellulose present in the residue. Itwas washed with water to remove all the acid from residue and wasdried to constant weight and its weight was calculated and was labeled as W3 (Kosan *et. al.*, 2008). The Cellulose was measured through the following equation.

Cellulose percentage = W2 – W3 / W1 x 100

Lignin: After the removal of cellulose,the remaining residue contained lignin and silica. This residue was placed in muffle furnace at 600°C for 4 hours to remove the lignin. Then the weight of residue was calculated and labeled as W4. Lignin was calculated by the following equation (Haoran*et. al.*, 2013).

Lignin percentage = W3 – W4 x 100

In vitro Dry matter digestibility (TDMD)

Collection of rumen fluid: Rumen fluid was collected from a cannulated buffalo at shah purkanjra slaughter houseLahore, Pakistan. The rumen fluid was filtered with two layers of muslin cloth on the large funnel connected with pre-warm insulated thermos flask and was transported to the Laboratory.

McDougall buffer solution: McDougall buffer solution was prepared as per method reported by (McDougall. 1948) presented in table-1, while placing on hot magnetic stirring plate (50^{o}C). The pH was adjusted at 7.5. The Buffer was stored in dark bottles and kept at 39^{o}C in water bath before use.

Table 1: The chemical ingredients of McDougall buffer solution.

Ingredients	g/L distilled water
$NaHCO_3$	9.8
$NaHPO_4$. 12 H_2O	9.3
NaCl	0.47
KCl	0.57
$CaCl_2$ anhydrous	0.04
$MgCl_2$ anhydrous	0.06

After collection the rumen fluid was quickly quantified and was transferred into dark bottle containing buffer solution at 1:2 ratios (one part of the rumen fluid with two parts of the buffer solution), purged with CO_2 to remove oxygen and tightly closed. The bottles containing buffered-inoculum were put on water bath at a temperature of39^{O}C.

About 1g (1 mm ground) mixed substrate sample was put into 500-ml conical flasks and 100 ml of buffered-rumen fluid (2:1) was added to each tube. The tubes were purged with CO_2 to maintain anaerobic condition. The flasks were sealed with rubber stoppers, fitted with pressure release valves and wasincubated in a temperature controlled water bath at 39^{o}C temperature. After 24hours the flasks were collected from water bath and were transferred into ice box to stop fermentation. The liquid and residue were separated by centrifuging each tube at 2500 rpm for 10 minutes. The supernatant was removed and residue was washed with distilled water and was used to determine dry matter digestibility.

Total dry matter digestibility was measured by drying the washed residues in an oven at a temperature of 60^{o}C to remove the excessive water.Afterwashing the temperature was increased to 100^{o}C for overnight. The IVDM digestibility was calculated by the following formula.

Total Dry matter digestibility = W2 - W1 / W3 X100

W2 = weight of china dish with sample after incubation

W1= weight of empty china dish

W3= weight of sample

RESULTS AND DISSCUSSION

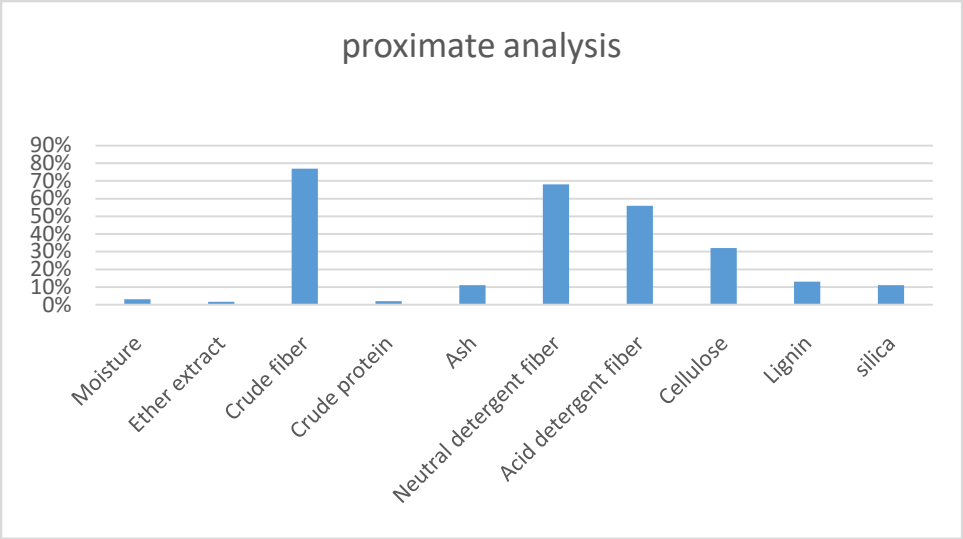
Treatment with organic solvents for the delignification of rice husk was optimized by varying the concentration of solvents, changing the temperature, duration of the treatment and by adding or removing the catalyst. The sample of rice husk used in this study showed cellulose 32± 1.26%, hemicellulose 12± 0.67 %, lignin (13± 0.71%) and ash (11± 0.59 %) as is shown in the Graph-1.

The results showed a higher percentage of lignin and ash as compared to other agricultural roughages as has been reported by(Xuebing*et. al*., 2009) which was the main hindrance in using rice husk in the ruminant feed.

During the treatment with organic solvents, changes in the composition of rice husk were observed, which were in line with the previous studies conducted by (Remsing*et. al*., 2006).

Maximum delignification i.e33.333% ± 1.304 was obtained with 70% ethanol treatment at 180 ◦C for 1 hour time, with the addition of 1% sulphuric acid as a catalyst. The lowest percentage of delignification was observed with 50% acetone i.e 7.664% ± 1.304 without catalyst.

The potential of organic solvents for delignification as shown in this study was found to be in line with previous studies carried out by (William *et. al.*, 2011).



Graph .1.Proximate analysis of rice husk.

Table 1.Effect of different solvents on catalytic and autocatalytic delignification.

Sr.no	Organic Solvent concentration	Mean of delignification (autocatalytic)	Mean of delignification (catalytic)
1	Ethanol 50%	15.33±1.026	25.33 ±1.304
2	Ethanol 70%	23.00±1.026	33.33 ±1.304
3	Methanol 50%	7.667 ±1.026	15.33 ±1.304
4	Methanol 70%	15.33 ±1.026	23.00 ±1.304
5	Acetone 50%	2.66 ±1.026	7.66 ±1.304
6	Acetone 70%	7.66 ±1.026	15.33 ±1.304

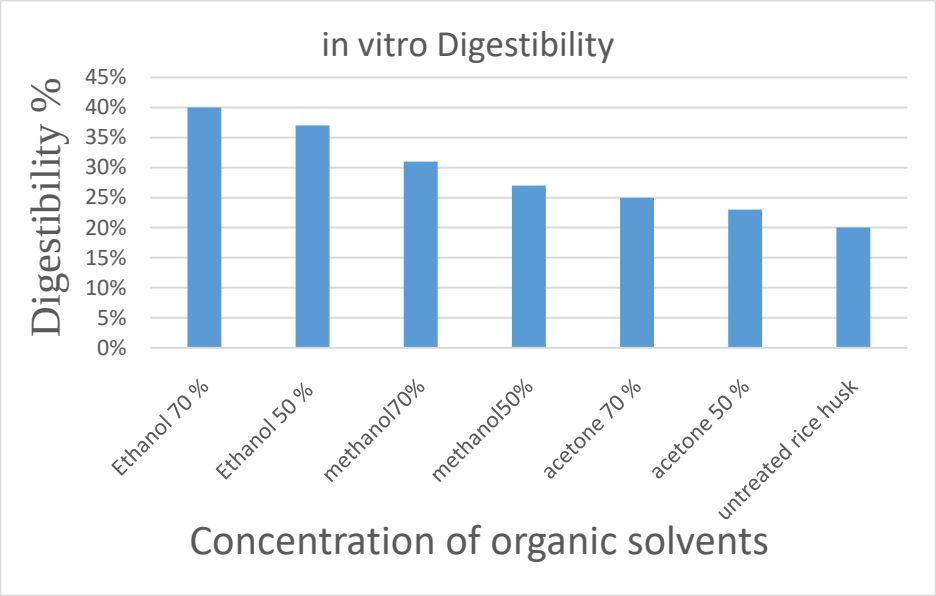
While studying the effect of catalyst on delignification, it was found, that in the presence of a catalyst, it effected delignification significantly i.e33.333% ± 1.304 with catalyst and 23± 1.026 % without catalyst. It was reported by various studies that the catalytic delignification and subsequent hydrolysis proved to be an effective procedure for effeicient conversion of lignocellulosic substrate into useful products (Long *et.al.*, 2011).

Table.2 Effect of temperature on the delignification of rice husk.

Delignification (Without Catalyst)			
Sr .no	**Temperature**	**Mean**	**Std.Error**
1	160° C	11.500	±.725
2	180° C	19.167	±.725
3	200° C	5.167	±.725
Delignification (With Catalyst)			
1	160° C	19.167	±.922
2	180° C	29.333	±.922
3	200° C	11.500	±.922

Table -2 shows that delignification process is very much temperature dependent. The delignification increased sharply from 11.50% to 19.16% (without catalyst) and 19.16% to 29.33% (with catalyst), when the reaction temperature was elevated from 160° C to 180° C. When the temperature was further increasedto 200° C, a decrease in delignification occurred. The results of the present study are in accordance to previous studies reported by (Xuebing*et. al*., 2009).

In-vitro digestibility of treated rice husk was also tested. As it was a well-established fact that lignin concentration of roughages was negatively correlated with digestibility as has been reported by (Kenneth *et. al.*, 2001). The results shown in graph-2 showed an increase in digestibility i.e from 20% to 40% with the increased degree of delignification, which was very much in line with the previous studies carried out by (Ang*et. al.*, 2012).



Graph 2.In-vitro digestibility of rice husk

Conclusion: It is concluded from the present work that 70% concentration of each organic solvent is useful for delignification.However ethanol gave the best results at 180 ◦C. Furthermore, digestibility can be improved with 70% ethanol treatment at 180 ◦ C in the presence of 1% solution of 98%sulphuric acid for 1 hour.

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