EFFECT OF ULTRASONICATION ON BIOGAS AND ETHANOL PRODUCTION FROM RICE STRAW PRETREATED WITH PETHA WASTE WATER AND DAIRY WASTE WATER

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ABSTRACT

In the present study rice straw (RS) was pretreated with petha waste water (PWW) having pH 12-14 and dairy waste water (DWW) having pH 3-5 for biogas and ethanol production. These highly alkaline and highly acidic waste waters were used in replacement of alkali and acid. RS was soaked in PWW, DWW and distilled water (DW) separately. Cow dung (CD) and soil were used as mixed microbial sources and pH was adjusted between 7 to 8 at the time of reactor set-up. Different anaerobic mesophilic batch reactors were set up with and without ultrasonication of the substrate mixtures (RS with PWW, DWW and DW separately). RS was characterised for cellulose, hemicellulose, lignin content and extractives before and after pretreatment. Composition and yield of biogas and ethanol from different mixtures were analysed. Other physical parameters like glucose, chemical oxygen demand and pH change were also analysed. Biogas and ethanol yields were high for DWW pretreated rice straw with ultrasonication (26 %) and PWW pretreated rice straw without ultrasonication (119.5 mg/L) respectively with CD.

KEY WORDS: Rice straw, Pretreatment, Biogas, Ethanol, Ultrasonication

Abbreviations:
RS: Rice straw
PWW: Petha waste waster
DWW: Dairy waste water
DW: Distils water
CD: Cow dung
AD: Anaerobic digestion
COD: Chemical oxygen demand
TS: Total solids
TDS: Total dissolved solids
TSS: Total suspended solids
VS: Volatile solids
US: Ultrasonication/ultrasonic
GC: Gas chromatograph
TCD: Thermal conductivity detector
HAc: Acetic sacid
HPr: Propionic acid
HBr: Butyric acid
VFAs: Volatile fatty acids

1. INTRODUCTION

Fossil fuels deliver about 80% of the universal energy basics and the combustion of fossil fuels results in 73% of carbon dioxide emission globally [1, 2]. Availability of fossil fuels like coal, petrol, diesel and natural gas are limited and is diminishing with time Depletion of fossil fuels and increased energy demand along with greenhouse gas emission is of great concern which integrated the development of a surrogate source of energy [3]. Biofuel production from food crops is not beneficial today as food crops are no more sufficient to fulfil the food requirements of present population. Due to this reason we have to switch over towards lignocellulosic wastes for biofuel production. Energy generation from waste is now in demand because it is doubly beneficial as it limits the waste generation and promotes energy production which is our future requirement.
Bioethanol is an alternate source of energy and can also be blended with petrol in 5-10% ratio which leads to upturn in its demand. The main

source of bioethanol production among sugar crops is sugarcane juice or molasses [4]. Various food crops like barley, wheat, corn stover [5], cassava [6, 7] etc. can also be used for ethanol production but these crops are used as food for animals as well as human beings. Production of ethanol from these crops is expensive so search of cheaper sources for biofuel production is mandatory. Lignocellulosic biomass like wood, grass, tree pruning, rice straw (RS), wheat straw, rice husk etc. can be economically used for bioethanol production [8]. Total potential bioethanol production from lignocellulosic biomass is about 16 times higher than contemporary ethanol production from sugarcane or food crops [9]. It is conveyed that instead of burning gasoline, ethanol burning is able to eradicate the release of sulphur dioxide which is the cause of acid rain [10]. Also use of lignocellulosic biomass for biofuel production results in less CO_2 emission because most of the lignocellulosic waste is disposed off by burning which is environmental unfriendly. With the help of microbial or enzymatic activity this lignocellulosic biomass can be converted into valuable products like biogas and bioethanol.

Biogas is a traditional source of energy and currently most of biodegradable wastes like agricultural wastes [11], food wastes [12], animal wastes, kitchen wastes [13] etc. are used as raw substrate for biogas production. Anaerobic digestion (AD) is the microbial process by which biodegradable wastes can be converted into biogas in absence of oxygen. Biogas is mainly composed of methane and carbon dioxide and major portion comprise of methane which is about 50-70% by volume [14].

The present study work was focussed on:

- The effect of alkaline petha waste water (PWW) and acidic dairy waste water (DWW) on composition of RS, further used for production of biogas and bioethanol from RS.
- Effect of Ultrasonic (US) pretreatment on bioethanol and biogas production after pretreatment with PWW and DWW separately was also investigated.

2. MATERIALS AND METHODS

2.1. Collection and processing of substrate:

2.1.1. RS: In the present study lignocellulosic waste, RS was used as the substrate for biogas and bioethanol production. RS is a good source of cellulose and hemicellulose [15] which can be converted to biofuels. RS was collected from the farms of Dayalbagh, Agra. This RS was chopped into 1-2 cm pieces and dried in a hot air oven at 105 ^{0}C for about 24 h. the oven dried RS was then powdered in a grinder and sieved through 1 mm sieve. 2.5 g of powder RS was subjected to soxhlet extraction with 150 ml acetone at 60 ^{0}C for 4 h for removal of extractives [16]. After extraction RS was analysed for composition using standard protocols as per section 3.3.

2.1.2. PWW: For pretreatment process PWW and DWW were used to pretreat the RS for maximum sugar release. PWW is generated in huge amount during the processing of famous petha sweet and drained as such without any pretreatment [17]. This untreated PWW is highly alkaline and causes nuisance in the society so its use in pretreatment of RS would also help in proper disposal of PWW [18]. This highly alkaline PWW was collected from petha industry situated at narrow streets of Noori Darwaja, Agra.

2.1.3. DWW: Milk processing requires a large amount of water for processing of milk products. DWW is another type of waste water generated in dairy industry during the processing of dairy products and it is acidic in nature. This DWW contains biodegradable organic contaminants which could also be utilised as a good source of nutrients for microbes, used for biofuel production [19]. DWW was collected from local dairy industry, situated at Shahganj, Agra.

2.2. Microbial source:

2.2.1. Cow dung (CD): Mixed consortia CD and Soil were used as inoculum. A number of both aerobic and anaerobic microbes like *Enterobacter*, *Clostridium*, *Rhodeobacter* found in cow dung which can be utilised for bioethanol and biogas production. CD was collected from dairy campus at Dayalbagh, Agra.

2.2.2. Soil: Soil contains a variety of methanogens and methylotrophs which are responsible for biofuel production. Soil was collected from botanical garden present in Dayalbagh Educational Institute, Dayalbagh, Agra. Both the cultures were inoculated in nutrient medium and maintained anaerobically at room temperature (37 to 42 ^{0}C) and renewed regularly with fresh medium after 15 days.

2.3. Pretreatment process: Pretreatment of RS was carried out using highly alkaline PWW having pH 12-14 and acidic DWW having pH 3-5. Small pieces (1-2 cm) of RS were soaked in PWW, DWW and distilled water (DW) separately for one week, after that pH of PWW was decreased to 7-8. The solubilisation of lignocellulosic content of RS resulted in increase in sugar content which was analysed by Dinitrosalicylic acid (DNS) method with respect to glucose as standard ($R^2 = 0.9954$) at 540 nm [20]. Total carbohydrate content was analysed by Anthrone method ($R^2 = 0.9968$) at 630 nm [21] using UV-Visible spectrophotometer (UV-1800 SHIMADZU, Made in Japan). Compositional analysis of RS for cellulose, hemicellulose and lignin content was done using acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) respectively described by standard procedure [22]. The percent yield of reducing sugar was calculated according to the formula [23]:

$$\text{Reducing sugar yield (\%)} = \frac{\text{Reducing sugar in mg/ml} \times \text{Vol (ml)}}{\text{Substrate in mg}} \times 100$$

All the experiments were performed in triplicates and average values are reported here.

2.4. Ultrasonic pretreatment: RS pretreated with PWW, DWW and DW was subjected under ultrasonication (US) bath for ten minutes and checked for sugar content. In US high waves of frequency greater than 20 kHz are released. Ultrasonic cavitation generates shear forces of high intensity that break particle agglomerates (complex structure) into simple form. This technique is helpful in dissolution of solid into liquid, homogenisation, emulsification, cell extraction, cleaning etc. by using high pressure homogenisers and rotor-stator mixers. Thus US used to check the effect on solubilisation of lignocellulosic content of RS which could further affect the biogas and bioethanol production. US pretreated RS was further analysed for reducing sugars to check the effect on solubilisation of lignocellulosic content.

2.5. Reactor set-up: Reactors were set up for PWW, DWW and DW pretreated RS with and without US using CD and Soil as mixed microbial source. One litre reactor bottles were used for reactor set-up with 500 mL working volume (400 mL substrate + 100 mL inoculum). The pH of all the reactors was adjusted between 7 to 8 as this pH is suitable for the survival of microbes responsible for biogas and bioethanol production. Nitrogen was purged in all reactors for 5 minutes to maintain the anaerobic condition. All reactors underwent simultaneous saccharification and co-fermentation process followed by microbial biogas and bioethanol production. Biogas and bioethanol were analysed by gas chromatograph (GC-5765 Nucon) equipped with thermal conductivity detector (TCD) and potassium dichromate method respectively. Table 1 represents the list of abbreviations used for different reactor conditions in the present study.

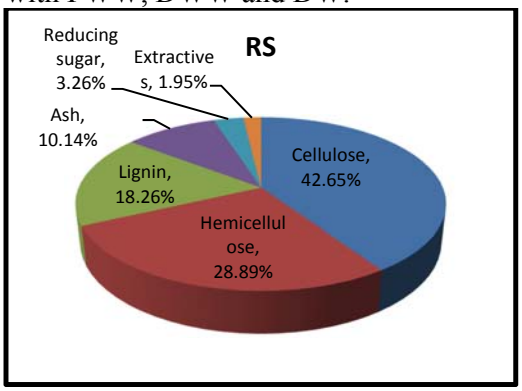
Table 1 List of abbreviations used in different batch reactor set up

Reactor name	Reactor conditions
C1	PWW pretreated RS without US + CD
C2	PWW pretreated RS + 10 min. US + CD
C3	Distil water soaked RS without US + CD
C4	DWW pretreated RS without US + CD
C5	DWW pretreated RS + US + CD
C6	Distil water soaked RS + US + CD
S1	PWW pretreated RS without US + Soil
S2	PWW pretreated RS + 10 min. US + Soil
S3	Distils water soaked RS with US + Soil
S4	DWW pretreated RS without US + Soil
S5	DWW pretreated RS + US + Soil
S6	Distil water soaked RS + US + Soil

3. RESULTS AND DISCUSSION

3.1. Compositional analysis: Pretreatment of RS with PWW was found most effective with about five times increase in the amount of reducing sugars. Pretreatment of RS with DWW increased the cellulose percent with decrease in hemicellulose percent more than PWW because

DWW was acidic in nature and thus solubilised hemicellulose into monomeric sugars resulted in improved cellulose conversion [24]. A little increase in cellulose, hemicellulose and lignin percent was also observed in case of DW, but it was very less as compared to PWW and DWW. Overall PWW pretreatment of RS was more suitable than other two with more solubilisation of cellulose content of RS. Fig. 1 shows percent composition of RS before and after pretreatment with PWW, DWW and DW.



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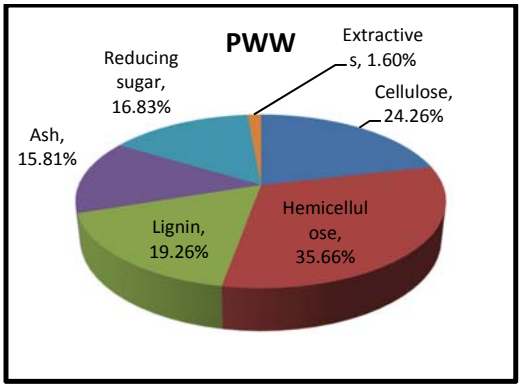

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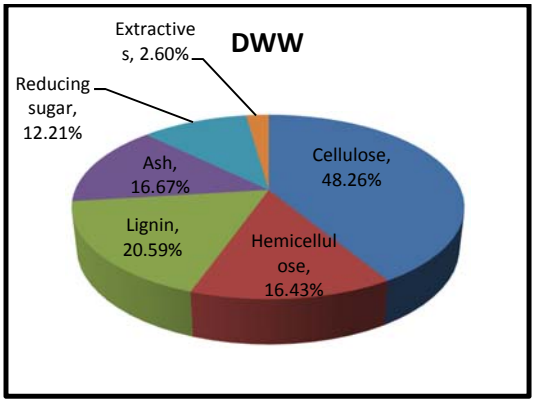

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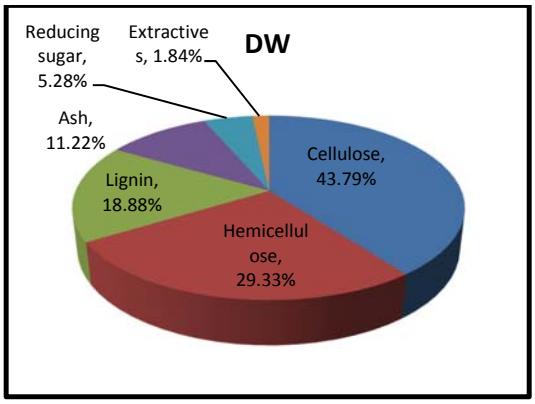
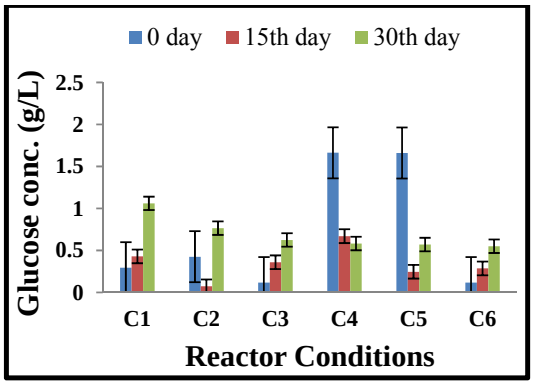


(D)

Fig. 1 Composition of RS; (**A**) Before pretreatment (**B**) After pretreatment with PWW, (**C**) After pretreatment with DWW, (**D**) After pretreatment with DW

3.2. Soluble sugar concentration: Glucose concentration first decreased in all reactors during fifteen days and then further increased after fifteen days. This may be due to the conversion of lignocellulosic content of RS into reducible sugars and consumption by microbes for biofuel production during first run (period of 15 days). After first run, solubilisation of lignocellulosic content was continuous but microbial activity may reduce so sugar was not consumed and resulted in an increase in glucose concentration. It was also observed that glucose concentration was highest for DWW pretreated reactors (C4, C5, S4 and S5) because of presence of milk sugar in DWW. Final glucose concentration was almost same for all reactors but more for reactors with CD than Soil. Total carbohydrate content of RS was analysed by Anthrone method which was 0.6055 g/L, 0.725 g/L and 1.3305 g/L for RS, PWW soaked RS and DWW soaked RS respectively. Reducible sugars were found 0.1859 g/L, 0.0731 g/L and 0.6953 g/L for RS, PWW soaked RS and DWW soaked RS respectively. This shows that PWW pretreatment was more affective for the solubilisation of reducible sugars which were released in the effluent, resulted in more decrease in sugar content of RS. Fig. 2 graphically represents the initial and final glucose conc. during the process.



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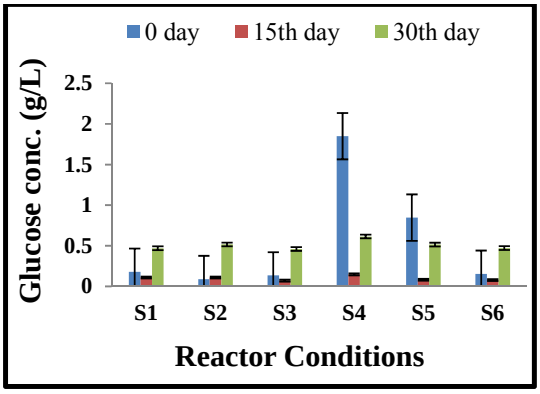
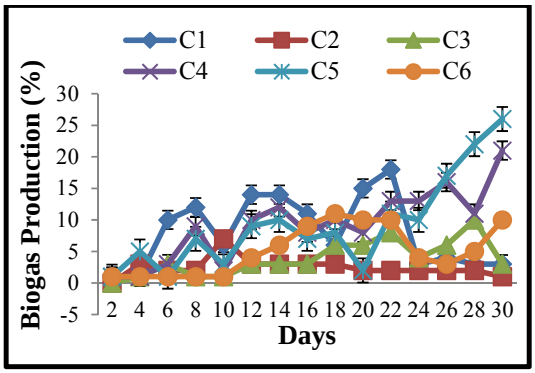


(B)

Fig. 2 Reduction in soluble sugar concentration in different reactors: (**A**) With CD, (**B**) With Soil

3.3. Biogas production: US pretreatment was found effective for DWW pretreated RS with maximum 21-26% biogas production. With CD biogas production was highest (26%) for C5 on 30th day whereas in case of Soil biogas production was same for S4 and S5 (25%). This may be due to conversion of milk sugar into biogas along with reducible sugars released from RS. Thus US have an positive effect with CD, without US biogas yield was 21% for C4 reactor. Least biogas production was observed in C3 (3%) and S3 (1%) for DW soaked RS which was taken as blank with respect to PWW and DWW pretreated RS. The results revealed that US increased biogas yield about nine folds and twenty five folds for C5 and S5 respectively. Fig. 3 shows percent biogas production and percent cumulative biogas production in different batch reactors.



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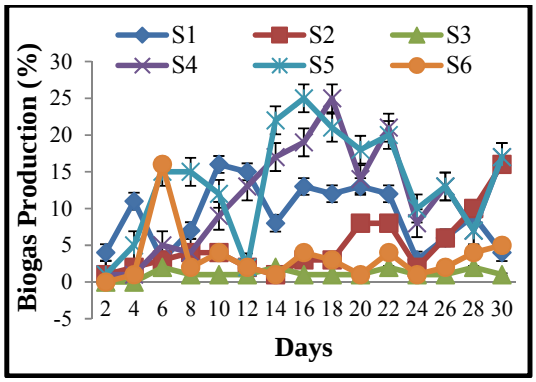

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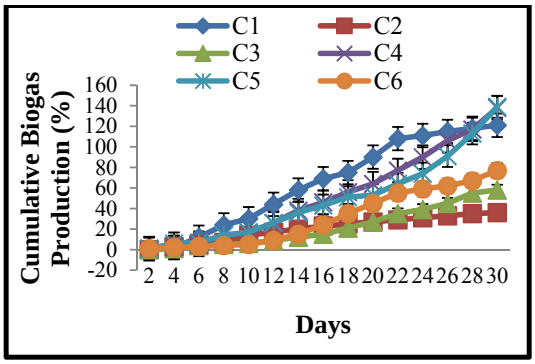

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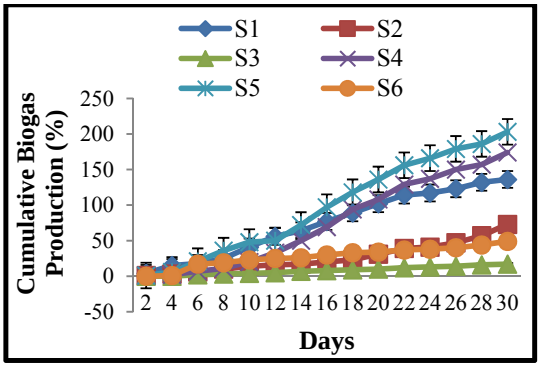


(D)

Fig. 3 Biogas production yields (%) in different reactor set-ups: (**A**) With CD, (**B**) With S, (**C**) % Cumulative biogas production with CD, (**D**) % Cumulative biogas production with Soil

3.4. Bioethanol production: Bioethanol was analysed quantitatively by potassium dichromate method [25]. Ethanol gets oxidised by potassium dichromate into aldehyde in alkaline medium followed by blue colour development. Reaction involved in the process is shown below and absorbance was taken at 584 nm.

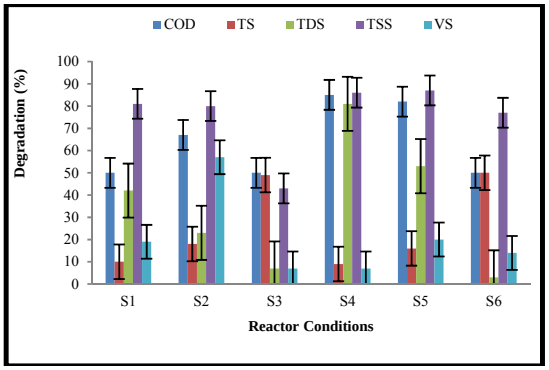
$$3CH_3CH_2OH + K_2Cr_2O_7 + H_2SO_4 \rightarrow 3CH_3CHO + K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O$$

Ethanol concentration was high for reactors with CD as compared to Soil and maximum concentration was obtained for PWW soaked RS without US with CD (C1) and DWW soaked RS without US with CD (C4) which was 119.5 mg/L and 117.2 mg/L respectively. In case of CD there was an increase in ethanol conc. Upto 10th day for US pretreated reactors whereas in case of Soil decrease in ethanol conc. Was observed. This shows a negative effect of US pretreatment on ethanol production for the reactors in which Soil culture was used as reactors undergone less bioethanol production than untreated ones. In case of CD culture positive effect of US pretreatment was observed on bioethanol production. It was also observed that ethanol conc. was increased upto 20th day and then decresed in some cases this may be due to reduction in microbial activity as no microbes were added between the process. Table 2 represents bioethanol conc. in different reactors for 10th, 20th and 30th day.

Table 2 Ethanol yields (g/L) in different reactors with CD and Soil after 10th day, 20th day and 30th day

Reactors	Yields (mg/L) 10th day	Yields (mg/L) 20th day	Yields (mg/L) 30th day
C1	55.0	104.3	**119.5** (Untreated)
C2	62.1	96.1	89.0 (Treated)
C3	33.9	42.5	63.3 (Untreated)
C4	12.8	114.8	**117.2** (Untreated)
C5	33.9	93.7	83.2 (Treated)
C6	24.6	54.1	56.2 (Treated)
S1	58.5	67.9	**107.8** (Untreated)
S2	17.5	71.5	59.8 (Treated)
S3	15.2	67.9	55.1 (Untreated)
S4	7.0	79.7	75.0 (Untreated)
S5	3.5	71.5	72.6 (Treated)
S6	2.3	67.9	57.4 (Treated)

3.5. Degradation in various physical parameters: Fig. 4 shows percent degradation in various physical parameters like chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS), total suspended solids (TSS) and volatile solids (VS). Highest COD degradation was about 85% and 82% for S4 and S5 reactors respectively which also have maximum biogas and bioethanol production. TDS and TSS degradation was also highest for S4 and S5 reactor which was about 81% and 86% respectively. VS degradation was maximum for C6 reactor which was 79% and highest TS degradation was found for C1 reactor which was 60%.



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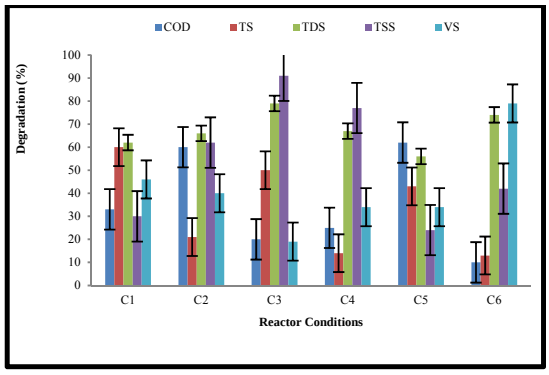
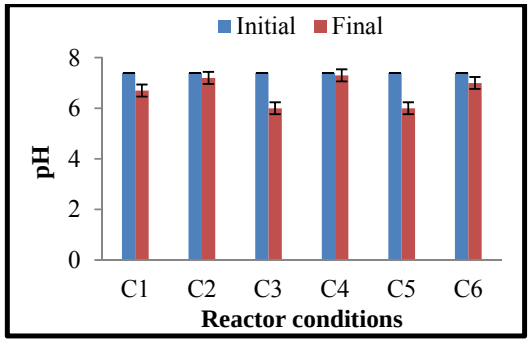


(B)

Fig. 4 Showing percent degradation in various physical parameters: (**A**) With Soil, (**B**) With CD

3.6. Change in pH: Decrease in pH for all reactors was reported this is because of formation of intermediate metabolites like volatile fatty acids (VFAs) during anaerobic digestion process [26]. Maximum decrease was observed for C5 and S5 reactors this may be due to the formation of more VFAs than other reactors as biogas production was maximum in both the reactors. Fig. 5 shows decrease in pH in all reactors.



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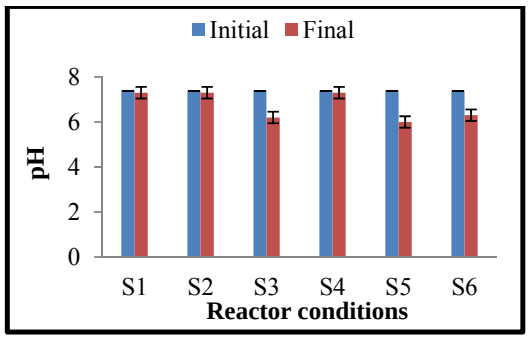
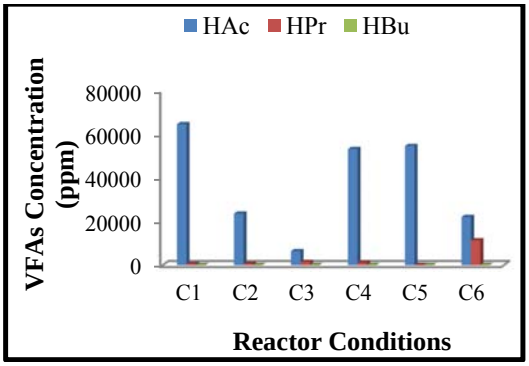


(B)

Fig. 5 Initial and final pH of different reactors during anaerobic digestion process: (**A**) With CD, (**B**) With Soil

3.7. VFAs production during anaerobic digestion: The production of biogas is accompnied by the formation of VFAs [27]. In the present study acetic acid (HAc), propionic acid (HPr) and butyric acid (HBu) were formed during the anaerobic digestion process. The amount of VFAs produced by microbial activity depends upon various conservational factors i.e. pH, reactor temperature, type of inoculum and pretreatment applied [28]. VFAs sam,ples were prepared by centrifuging them at 10,000 rpm [29]. Fig. 6 shows VFAs conc. in various rectors with CD and Soil. Acetic acid conc. was found high for all the reactors which favours the methane and carbondioxide production during the anaerobic digestion process. Reactors in which CD was used as microbial source have produced higher amount of VFAs as compared to reactors in which Soil was used as inoculum. Conc. of acetic acid was high in C1, S1, C5 and S5 reactors in which biogas and bioethanol production was also maximum. This reveals that production of VFAs favours the biogas production especially methane and conversion of bioethanol into acetic acid may result in high amount of HAc production in the reactors in which ethanol conc. was maximum due to oxidation of ethanol by some microbes present in the mixed microbial source.



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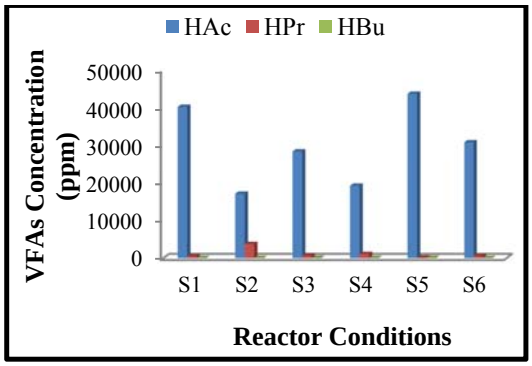

(B)

Fig. 6 Showing VFA's concentration (ppm) in different batch reactors: (**A**) With CD, (**B**) With Soil

4. CONCLUSION

The pretreatment processes reported require excessive amount of expensive chemicals and the present method is a "green" approach in which one waste was pretreated with another waste. Here RS was pretreated with acidic and alkaline waste waters separately. This resulted in enhanced biogas and bioethanol yields. The substrate taken in the study (RS) is found in abundance hence its conversion to biofuel is beneficial as it also helps in solid waste

reduction. The microbial activity of mixed culture taken from CD is more than that from Soil in terms of higher yeilds of biogas and bioethanol.

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