Section A-Research paper

A sustainable pretreatment processes for agricultural residues hydrolysis: Impact to bioethanol biosynthesis by *Saccharomyces cerevisiae* MTCC 3821 strain

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Abstract:

Various agricultural wastes such as sugarcane bagasse, wheat and rice straw were utilized for ethanol biosynthesis by the microbial strain *Saccharomyces cerevisiae* MTCC3821. Application of physical (temperature range from 30 to 120^{o}C with different size of plant biomass with solid ratios) and chemical agents (acidic solution~1.92M to 3.0M; or alkaline solution ~0.125 M to 0.8M) alone or also in combined forms were applied to make effective hydrolysis of plant wastes. Good biomass hydrolysis was obtained at a combined effect of temperature of 120^{o}C and sodium hydroxide (0.25 M) or hydrochloric acid (2.5M) solution. Inhibitory compounds which are formed during hydrolysis process are removed by using activated charcoal which in turn increased the production. Various ethanol concentrations (in 1.2 to 1.45 g/L in different biomass hydrolysates and 4.5 g/L or more titer in case of synthetic media) was achieved during fermentation process and *S. cerevisiae* strain MTCC 3821. It was also observed that ethanol titer depends on feedstock used and also hydrolysis rates of biomasses. This research work results are promising for future researches with promotion to cellulosic ethanol production from cheap agricultural residues. And these waste utilization for this fuel can help to generate revenue to farmers.

Key words: Agricultural wastes, Fermentation, Biofuels, Hydrolysis, Microbial strain, Biosynthesis.

Research Highlights

- This paper discusses the different plant byproducts, generated during crop growth.
- Several pretreatments applied for byproducts hydrolysis for monomers generation.
- Neutralization approach applied for removal of inhibitory compounds in hydrolysate.
- Ethanol/ butanol synthesis from fermentation achieved, a viable option to gasoline

1. Introduction/ background

About 90% of total fuel consumption comes from fossil fuel which impacted both environment and human health via creation of global issues like climate change, greenhouse effect and particulate matter emission. Ever increasing population also led to fossil fuel demand. Due to this, worldwide research is going on to develop more energy secure renewable resources which can replace fossil fuels (Plakandaras et al., 2019). In this context, several researches are going on different nature of biofuel development.

Many countries are utilizing feedstock like sugarcane bagasse, beet sugars, potatoes, molasses and cassava for advanced cellulosic bio ethanol production that involve multistep processes (Zheng et al., 2022).

Plant cell wall is made up of hemicelluloses, cellulose and also lignin components and these are tightly jointed/ bound to each other and it needs to systematically broken down into fermentable sugars. This can be done by two ways like high or low deconstruction processes (Malode et al., 2021). Low temperature deconstruction is preferred than high temperature deconstruction because in later high temperature leads to the formation of other fuel while in low, enzymes can also be used to convert into respective monomers, then to fuel (Zheng et al., 2022, Malode et al., 2021). In the low-temperature technique, plant biomass undergoes effective pretreatment which breaks down the cell wall. And these processes can help to increase the surface area and it uses the chemical/ biological agents assisted hydrolysis for breaking cellulose, hemicellulose and lignin and breaks down into monomeric unit which in turn convert into biofuel (Loow et al., 2016). Due to these treatment processes, these polymers were broken down by enzymatically / chemically processes into simple sugars like glucose/ xyloses with some other monomers as building block components during the hydrolysis process (Merino et al., 2023). And, later due to the contribution of some microbial cells, systems like bacteria, yeast or cyanobacteria, showed their capability to ferment sugars or gaseous intermediates into fuels like ethanol/ other alcohols. Then these can be applied to blend stocks and chemical synthesis (ʻLoow et al., 2016; Merino et al., 2023). We have now discussed the biofuel production from hydrolysis biomass solution via utilization of suitable microbial fermentation and effective microbial systems. Number of research papers have discussed the ability of different microbial systems for capabilities of higher salt and temperature tolerances while compared to *S. cerevisiae* strain [Merino et al., 2023, Loow et al., 2015].

Saccharomyces cerevisiae, Zymomonas mobilis, Klebsiella oxytoca, Escherichia coli, Thermoanaerobacter ethanolicus, Pichia, stipites, Candida shehatae, and *Mucor indicus* are mostly studied for bioethanol production. Normally during the ethanol production process, biomass matter needs to go for systematic pretreatment processes/ steps for cellulose and hemicelluloses (Świątek et al., 2020). And then these should be accessible for further breakdown in subsequent steps like uses of enzymatic hydrolysis. This hydrolysis can convert the celluloses/ hemicelluloses into glucose or xyloses/ other sugars respectively. In later fermentation applications, these sugars (hexoses/ pentoses) are converted into ethanol and then attempted to separate or concentrate from fermentation processes (Maicas, 2020). Ethanol is also separated after fermentation process by recovery technique. Silicate-1, activated carbon molecular sieves and ZSM-5 are used as adsorbents for separating ethanol and, carbon molecular sieve membranes, are found to be most effective for ethanol dehydration. Silica material found to be best suitable for preparation of high performance silicate-1 membrane (Zentou et al., 2019). This membrane have been applied for water and ethanol mixture solution in separation tasks. The ONIOM method was used to check ethanol and butanol adsorption capability in hydrogen-zeolite socony mobil-5 (HZSM-5) with ethanol dehydration (Dzigbor and Chimphango, 2019). Carbon molecular sieves (CMS)-5A can only adsorb 0.008 g/g glucose, 0.0008g/g fructose and 0.011g/g glycerol [Dzigbor and Chimphango, 2019; Xu et al., 2022). And **Fig-1** show the schematic view for plant residues based fuels development.

We discuss some approaches of pretreatment for biomass hydrolysis, neutralization approaches for inhibitory compounds in biomass hydrolysates and finally microbial based conversion of monomers from plant biomass into ethanol/ butanol. Some approaches we discuss the separation of biofuels from broth (Liczbiński et al., 2022). Here, author worked on ethanol synthesis at laboratory scale.

2. Impacts of ethanol production from various sugars sources:

Ethanol biosynthesis was achieved by using of batch fermentation processes and *S. cerevisiae* MTCC 3821 and it has resulted the biomass growth (in OD: 7.0), ethanol titer (4-5 g/L) and productivity (0.0312 to 0.036 ml ethanol/kg. biomass). Some researchers have done the samples analysis after 24 h onward to till 96 h during batch fermentation process and this ethanol and productivity was found to equivalent to 39.1% of the higher yield and it is based on amount/ quantity, released/ generated from glucose as raw material at end of fermentation process (Tesfaw and Assefa, 2014). Acid and alkaline pretreatment efficiency on wheat and rice straw; and also sugarcane bagasse with respect to time was studied. Production of fermented sugar was closely observed with respect to time and form of pretreatment (Beig et al., 2021). This was done under control conditions in laboratory scale via the use of a cast iron cylindrical reactor with a total 1 liter capacity (Meenakshisundaram et al., 2021). And then it was filled with 100g of disc milled feedstock (with 75% moisture contents)/ batch. It was directly heated with a gas burner at a rate of 15 and 20^{o}C/min and then rapidly opened the batch at 220^{o}C and 1.96 M.Pa (Beig et al., 2021; Meenakshisundaram et al., 2021). During experiment for ethanol production at high temperature condition, *Saccharomyces cerevisiae* strain has shown to poor tolerance to high glucose/ reducing sugar and salt concentration in production medium and then it lowered the ethanol concentration with negative performance during fermentation process (Tesfaw and Assefa, 2014; Beig et al., 2021). And **Fig.1** shows the biofuel generation with utilization in transportation vehicles

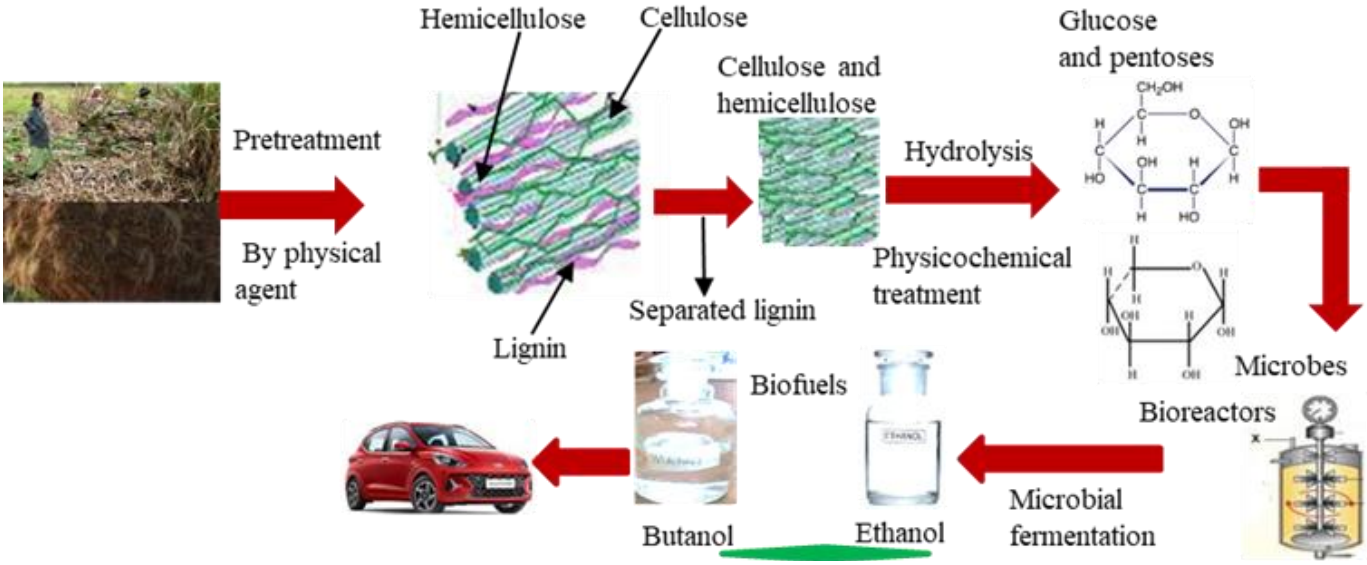

Fig.1. Waste plant biomass hydrolysis and utilization for biofuels like ethanol synthesis.

2.1. *Detoxification process for biomass hydrolysates*

But in case of sulphite addition in hydrolysate solution for ethanol, biosynthesis was improved from 2.1-2.7 g/L. In the process of detoxification processes, it was involved to precipitate the toxic compounds in the biomass hydrolysate or chemical conversion at high pH solution. In precipitation process, two acids, like livulinic acid and acetic acid in the biomass hydrolysate is reported that worked based on neutralization chemistry principles (Jönsson et al., 2016). During fermentation process, glucose/ xylose mixture in biomass hydrolysate, glucose is first consumed by *S. cerevisiae* and later with the help of isomerase enzyme, we can transformed it into glucose and then it was upgraded the ethanol production (Gandla et al., 2018). We found the second methods to be more effective with production of final

fermentable sugars concentration (3.1g/L). In pretreatment process, huge quantity of inhibitory compounds in fermentable sugar solution was determined and researchers have applied minimization approaches for improving the fermentation process from plant biomass hydrolysate solution via using reducing agent like dithionite and sulphites compounds (Ilanidis et al., 2021). Dithionite addition can help to remove the inhibitory compounds from acidically/ alkaline hydrolysed sugarcane bagasse and it has reduced the inhibitory concentration that confirmed by production of ethanol (titer from 3.2 g/L) (Meenakshisundaram et al., 2021; Ilanidis et al., 2021). Researchers have done technical assessment on acidic preprocessing tasks and it showed the increased concentration of monomeric sugars from waste agricultural residue like sugarcane bagasse/ rice and wheat straw. In this process they found these sources of cheap feedstock carbon material for ethanol biosynthesis and it was sugar recovery of waste biomass sources from 20% to 30% (Lan et al., 2020). This improvement in ethanol titer is due to reduced buffering capacity of sugarcane bagasse and it has provided the improved sugar solubilisation during acid pretreatment process and further other agent treatment like enzymes also (Williams et al., 2018).

Further, the preprocessing step can provide the substitute for the detoxification step with economic evaluation for ethanol production (Lan et al., 2020; Williams et al., 2018). We have applied leaching as a preprocessing step to improve biomass quality via creation of co-products (Zhuo et al., 2018). Normally we can be applied preprocessing operation like blending, sorting, leaching, drying and other quality management approaches that can help in development of "conversion-ready" concept for lignocellulosic feedstock for production of biofuel and bioproducts (Lan et al., 2020; Zhuo et al., 2018). And after treatment material was recovered in a storage tank and wet material cooled to room temperature (Chukwuma et al., 2021). From these studies, they have found optimal pH value: 5.0; temperature: 35^{o}C, sugar concentration: 15 g/L and yeast concentration: 3g/L (Cesaro et al., 2015; Paul et al., 2018). In these inhibitory compounds, furan (furfural/ HMF), carboxylic acids (like levulinic and acetic acids/ formic acid) and also some phenolic compounds (like vanillin, catechol, 4-hydroxybenzaldehyde and syringic acid) are reported that are generated during high concentration of alkaline/ acidic treatment. These inhibitor compounds from lignocellulosic biomass hydrolysate solution have played a critical role in ethanol production by *S. cerevisiae* [Zentou et al., 2019; Zhuo et al., 2018).

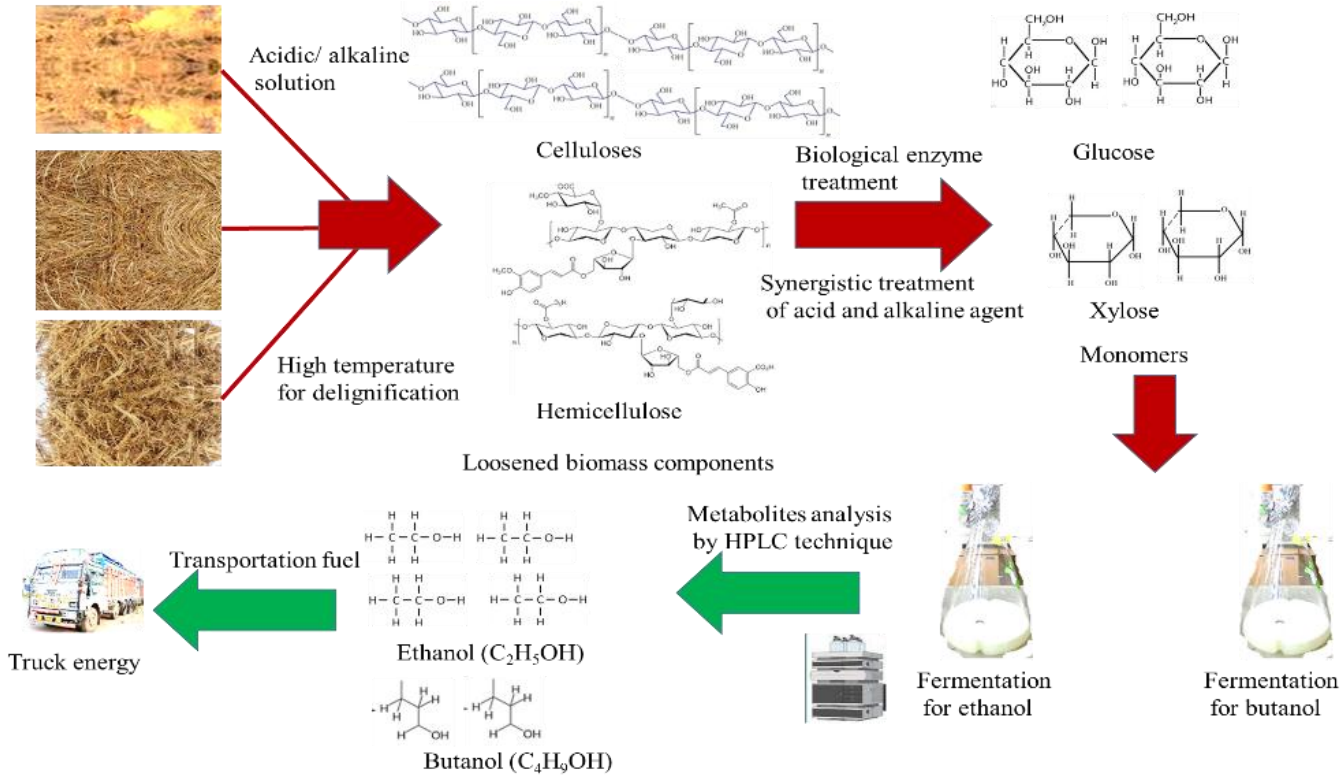

Fig. 2. Plant wastes with respective pretreatment process, used for different biofuels production.

In this context, several researcher have recommended for different approaches/ techniques for removal/ neutralization of such inhibitory compounds (Zhang et al., 2015). Some approaches like fermentation are treated with reducing agents and addition of activated charcoal and also enzymatic treatment with peroxidase/laccase (Shu et al., 2018; Melgar et al., 2017). In wheat and rice straw hydrolysate without/ less concentration of inhibitory compounds are shown with sugarcane bagasse hydrolysate without/ less inhibitory compounds. These biomass hydrolysates have shown a little bit more ethanol titer compared to non-processed hydrolysates (El Roz et al., 2019). **Fig-2** show the plant residues hydrolysed sugars used for bioethanol synthesis.

3. Material and methods for experiments

3.1. *Media compounds/ components and microbial strains*

S. cerevisiae MTCC 3821 obtained from Gitam School of Science was cultured in Yeast Extract Peptone Dextrose media (YEPD). The composition of YEPD media is: yeast extract (10 g/L), peptone (20 g/L), dextrose (20 g/L) and also agar (20 g/L) for plate culture. The yeast strain were incubated at 30^{o}C and then sub-culturing process is done at every 15 d. We have purchased the sodium hydroxide, sulphuric acid/ hydrochloric acid and yeast extract from Merck KGaA, Darmstadt, Germany. Bacteriological peptone was also used for culturing of yeast cells. We have done HPLC analysis for sugars like glucose/ others.

3.2. *Growth media for inoculum preparation for ethanol*

We have taken 3-loopfull of *S. cerevisiae* MTCC 3821 from the petri-plate and then transferred into growth medium (for culture preparation) that contained following components: yeast extract (5.0

g/L), peptone (10 g/L), glucose (5.0 g/L). In synthesis of growth media, 100 ml of growth media is taken 250 ml of Erlenmeyer flask and then it was gone for sterilization process. After inoculation, this flask is kept for incubation period at temperature of 30^{o}C, speed of shaker (200 rpm) and period of 24 h to get exponential phase/ stage of yeast culture. We have kept cell concentration standardization process and then it was kept of 0.3- 0.5 g/L (OD: 1.8-2.5) and this OD was determined by a UV-Vis spectrophotometer at 600 nm. All the experimental procedures were carried out at aseptically and then analysis was done in duplicate runs.

3.3. Synthetic p*roduction media for ethanol*

Production media for ethanol production consists of the following components: Ammonium sulphate (4.0 g/L), glucose (80 -100 g/ L), magnesium sulphate ($MgSO_4$. $7H_2O$: 1.0 g/L) and potassium dihydrogen phosphate (KH_2PO_4: 2.0 g/L) with then pH of medium; 5. All media composition was done to a sterilized level. We have used biomass hydrolysate and other components except glucose in P2 medium and this was used to prepare the production media. Synthetic P2 medium is consisted of yeast extract (1.0 g/L), , ammonium acetate (0.5 g/L), NaCl (10.0 mg/L), monobasic potassium phosphate (0.5 g/L); dibasic potassium phosphate (0.05 g/L), magnesium sulphate heptahydrate (0.2 g/L) , manganese sulphate hepathydrate (10.0 mg/ L), ferrous sulphate heptahydrate (13.0 mg/L), thiamine hydrochloride (1.0 mg/L), biotin (10.0 mg/L), and p-aminobenzoic acid (1.0 mg/L)). We have applied this media for ethanol production during fermentation (for 4 to 5 days) with and without glucose in production media. But synthetic P2 mediums with biomass hydrolysate have shown better ethanol titer and yield.

3.4. *Biomass hydrolysate for ethanol*

In the process of pretreatment of waste plant biomasses, we had first weighted 50 g of sugarcane bagasse then milled them into smaller and fine particles (sizes:1mm to 2mm). And this milling of sugarcane bagasse has helped to make the biomass hydrolysate solution. And this biomass suspension solution was blended and mixed with concentrated acid/ alkaline solution to make biomass hydrolysate. We have done many experiments for pretreatments with several ranges of acid and alkaline solution to check the hydrolysis rate of biomass and also to check the inhibitory compounds. In this process, we found some optimal condition of sulphuric acid (2% or 0.445 M) solution at temperature of 120^{o}C at 30 min for best hydrolysis of sugarcane biomass and it has yielded a solution with xylose (4.1 g/L) and glucose (5.5 g/L) with some quantities of inhibitory compounds such as 0.4 g furfural/L and 3.35 g/L of acetic acid. We have also gone for proper filtration to remove the solid particles and then it was mixed with synthetic P2 medium in different ratios (30 to 60%). We have applied DNS (3, 5- dinitrosalicylic acid) to determine the total reducing sugars concentration (i.e. residual sugars) generated during the pretreatment process.

Table 1. Approaches for pretreatment and saccharification of different biomass with help of physical, chemical and biological agents and biological agents

Plant biomass	Pretreatment and saccharification steps	Generated monomers/ sugars	References
Straw of rice straw	The combined effects of dilute sulphuric acid and also aqueous ammonia is used as pretreatment process	Ethanol from generated glucose, come from saccharification of straw of rice	(Kim et al., 2013)
Waste nutrient from corn	This waste biomass is low moisture anhydrous ammonia	Good ethanol titer and it is based on glucan/xylan	(Yoo et al., 2011)

stover	(LMAA) solution for effective hydrolysis at high rates	hydrolysis rate in this biomass	
Structural transformation of eucalyptus	Peracetic acid with oxalic acid (PAO) showed synergistic impacts on biomass hydrolysis it increases this biomass hydrolysis rate with good amount of fermentable sugars	High quantities/ amount of glucose (758 mg/g. biomass) is released that increased ethanol titer	(Zhuang et al., 2022)
Sugarcane bagasse	Ferric chloride assisted catalysis with ethylene glycol/ water (FeCl3-EG/ H_2O) used as pretreatment for this biomass hydrolysis	Removal of lignin/ xylan reported with maximum glucose yield	(Wei et al., 2021)
Sugarcane bagasse	Combined effect of ferric chloride catalysed organosolv solution and Tween80 action can be used for pretreatment of sugarcane bagasse biomass	High rate of cellulose conversion (100%) into glucose after these agents as pretreatment	(Zhang et al., 2018)
Stalk of waste tobacco plants as waste biomass the waste residue	Combined effect of alkaline and acid-catalyzed with steam can enhance hydrolysis of this biomass but finally enzyme treatment gained complete hydrolysis of this biomass	Higher in total monomeric sugar yields sugar-to-ethanol	(Yuan et al., 2019)
Wheat straw	Alkaline and mechanical pretreatment of wheat straw with *Pachnoda marginata*	Methane (149%) increased compared to non-pretreated straw.	(Schroeder et al., 2023
Wheat straw hydrolysis	Pretreatment by enzymes or sodium hydroxide solution, help in wheat straw hydrolysis	Methane yield from wheat straw convert into reducing sugars (4 %)	(Vasmara et al., 2017)
Wheat straw hydrolysis reported	Low temperature assisted alkali solution pretreatment enhanced the this waste biomass hydrolysis Further immobilized β-xylanase nanoparticle used for complete hydrolysis	Wheat straw for xylose, arabinose, xylobiose with maximum saccharification (20.6%),	(Hamid et al., 2023)
Wheat straw or poplar chips	Harsh pretreatment and enzymatic hydrolysis for these biomasses are reported	Glucose (12.6 kg) and furfural (2.5 kg) produced from 50 kg of biomass	(Cornejo et al., 2019)
To treat wheat straw as hydrolysis	Ammonium sulphite-based sequential pretreatment is applied for complete hydrolysis of this biomass with good influences	Fungal cellulase-based saccharification for releasing sugars	(Yu et al., 2020)

Fig-3 shows the different concentration of sodium hydroxide (0.125 M to 0.75 M) and hydrochloric acid (1.92 M to 3.0 M). We have used different temperature impacts on biomass hydrolysis. From these temperature impacts we found 120^{o}C is the best and effective temperature for hydrolysis with

help of optimal acidic (2.75M) and alkaline (0.25M) concentration. Inhibitory compounds in pretreatment biomass hydrolysate solution. **Table 1** discusses some pretreaments for biomass hydrolysis. We have determined the several types of inhibitory compounds such as furfural, acetic acid, formic acid, levulinic acid in biomass hydrolysate solution. These compounds were measured in HPLC technique/ device. In HPLC technique, separation and concentration were performed on a Rezex ROA organic acid, HPX-87H (300 x 7.8mm) column and RI detector for separation.

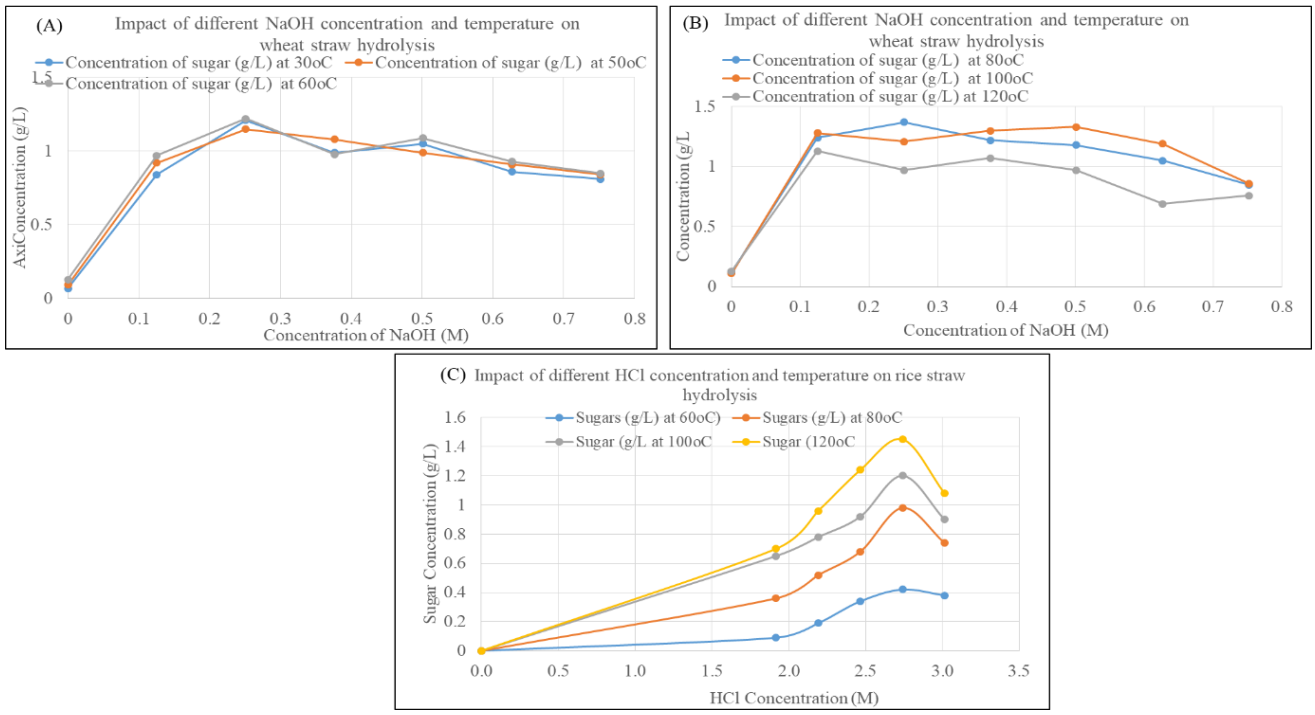

Fig.3. Generation of fermentable sugars from pretreatment of alkaline (A and B: concentration of 0.25 M to 0.75 M) to acidic (C: concentration of 1.92 M to 3.0 M) solution. Combination of high temperature treatment with different time reactions used for two different residues for wheat straw at temperature of 30 to 120^{o}C and rice straw at temperature of 80 to 120^{o}C.

4. Fermentation process for biosynthesis of ethanol and microbial strains

During the experiment, we used the above mentioned production media and then it was added 5 % inoculum in production. After that this culture media was kept on a rotary shaker (150 rpm) under partial anaerobic conditions at 30^{o}C/35^{o}C for 48 h without any pH adjustment. During the experiment, we have done sample analysis at every 6 to 12 h from fermentation broth for bioethanol and residual sugars determination. This ethanol production was compared with commercial glucose in defined media for ethanol production. And then it was gone for ethanol production via application of *Saccharomyces cerevisiae* MTCC 3821. These cultivation have shown the biomass growth (range 2.12 g/L to 2.27 g/L) in wheat and rice straw and sugarcane bagasse (in little bit more quantity compared to other biomass) with comparison to standard / synthetic production media for same biomass estimation (6.50 g/ L and 7. 50 g/ L). We have found the ethanol titer in sugarcane bagasse, and straw of rice and wheat (from 1.1 g/L to 1.2 g/L) their hydrolysate contained the inhibitory compounds are shown in **figure 4 and 5** but ethanol titer was found to high (4 to 5 g/L) in standard media containing high concentration of glucose (range from 20 g/L to 50 g/L). During the alcoholic fermentation process, some of by-products like fatty and volatile organic acid, ester, aldehyde, ketone, terpenes, sulphur compound and also glycerol were generated but these were not properly quantified by analytical techniques (not shown data).

4.2. Fermentation and detoxification for ethanol production

We have attempted the ammonia conditional step and it was tested as a detoxification step but it is not effective in sugarcane biomass hydrolysed liquid fraction, due to the least amount of ethanol production (1 g/ L). **Table 2** shows some biofuels synthesis from biomass In other pretreatment experiment, we again applied the ammonia conditioning step for rice straw biomass hydrolysed liquid fractions after pretreatment to pH of 10 and it was more effective in removing the inhibitors like furfural, HMF and phenolic compounds and it was done by using the sodium hydroxide, potassium hydroxide and calcium hydroxide and ammonia solution than neutralizing to pH; 5.5. This was a well-established step as the one step detoxification method for removing the inhibitors after pretreatment steps for biomass hydrolysis. We have determined the impact of concentrated acid like H_2SO_4/ HCl that has worked as good hydrolysis agents with corrosive and hazardous characteristics and showed better ethanol titer. In the acid hydrolysis process, some disadvantage is found, due to formation of inhibitors from released fermentable sugar. And some portions of released pentoses were found to decompose into furfural and also for hexoses, were converted/ decomposed into HMF. These compounds are reported to yeast cell growth inhibitors with negative impact on bioethanol production. These are known to function for reduced yeast cell growth, ethanol yield and productivity.

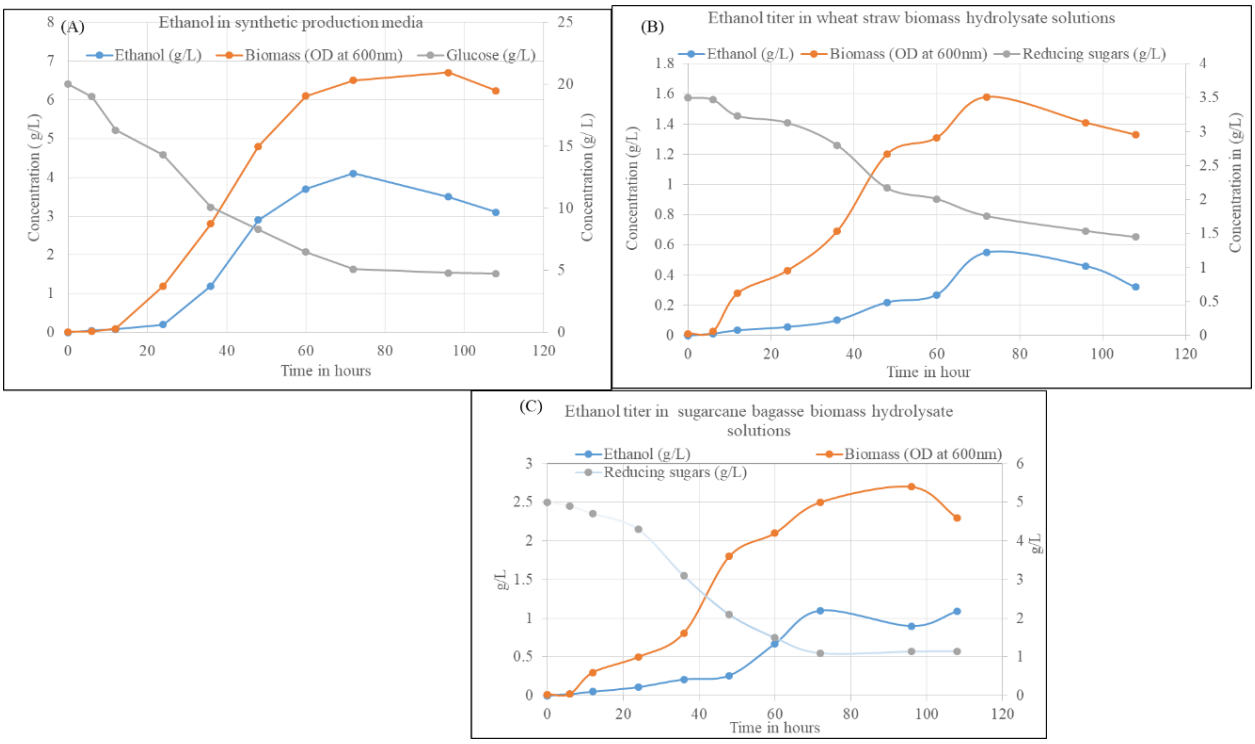

Fig. 4. Ethanol titer in production media containing *S. cerevisiae* and fermentation period was till 108 h. (A) Media with glucose (B) Media with wheat biomass hydrolysate; (B) Media with sugarcane bagasse biomass hydrolysate

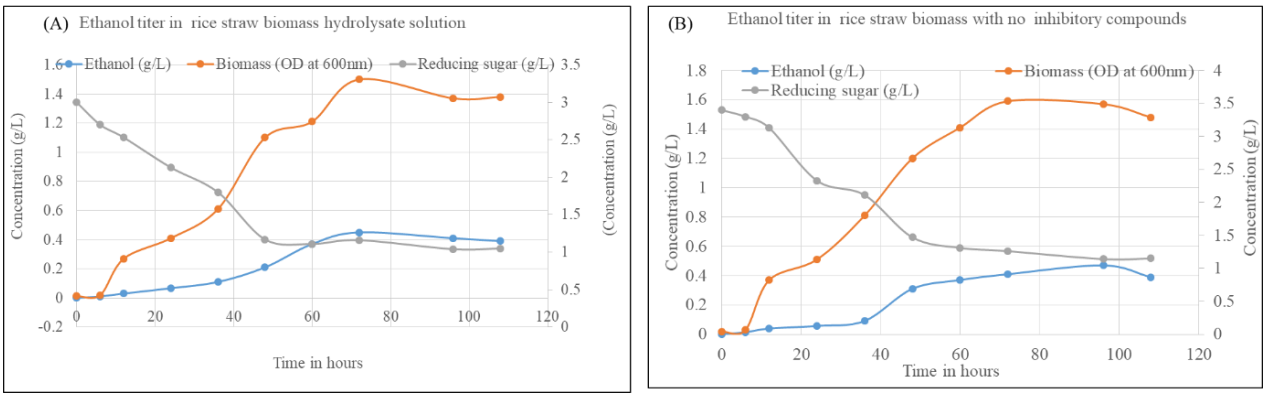

Fig. 5. Ethanol titer in production media containing *Saccharomyces cerevisiae* and fermentation period was till 108 h. A) Media with rice straw hydrolysate without detoxification (B) Media with rice straw hydrolysate with detoxification

Table 2. Different biomass and microbial strains system for different nature of biofuels generation that can provide alternative option to gasoline fuel.

Plant biomass	**Microbial fermentation and microbes**	**Generated biofuels**	**References**
Rice straw pretreated by NaOH	Anaerobic digestion for lignocellulosic biomass conversion is reported	Total biogas (446.3 mL/g. VS) and methane (263.5 mL/g V.S) yields with metal removal (95%)	**(**Xin et al., 2019)
Saccharum munja and *sugarcane bagasse*	Pretreatment process with saccharifcation is done for *Saccharum* biomass and it used *Sporotrichum thermophile* organism for complete enzymatic hydrolysis	Utilization of co-culture of *S. cerevisivae* and *Pichia stipitis* can be used to produce the high quantities of bioethanol (31.0 g/L)	(Bala and Singh, 2019)
Rice straw	Report on saccharification process is given with its temperature of 60^{o}C and pH of 5. This was done for 49 h period that released high amount of reducing sugar (729 mg/g. biomass)	Fermentation by *S. cerevisiae* ethanol (18. g/L) after 72 h of period at process parameters like temperature (30oC), pH: 6 and speed rate for this biomass hydrolysate utilization (	(Singh and Kumar, 2020)
Wheat Straw	Auto-catalysis and sulphuric acid assisted catalysis with hydrothermal process (at 160 to 190^{o}C) used for this biomass complete hydrolysis	The highest yield of glucose and xylose for ethanol 270 L / ton dry wheat straw	(Ilanidis et al., 2021)
Wheat straw as a fermentable saccharides	Advances in pretretament process resulted in complete hydrolysis of celluloses and hemicelluloses components during the ABE process for advanced biofuels development	Biobutanol production from lignocellulosic biomass via ABE fermentation	(Guo et al., 2022)

Potato peel wastes (PPW)	The enzymatic hydrolysis process of PPW biomass with 75% treated ethanol and at 180^{o}C can result with rate of glucose generation (38 g/L) in pretreatment process	This treatment by microbial stain can successfully ferment to ABE (11.6- 25 g/L)	(Abedini et al., 2020)
Pine plant waste matters	Life-cycle water based footprint analysis can utilize as useful tools that is thermochemical method of pretreatment	Total water use (146 L. water/ MJ) is found for biobutanol that uses the pineapple wastes	(Li et al., 2022)
Waste banana peels	Application of combined influence of sodium hydroxide and sulphuric acid solution is found in pretreatment process that resulted high rate hydrolysis	Higher butanol yields from high quantity of sugar (20g/L) solution with batch fermentation process is reported with uses of *S. cerevisiae* and *Pichia* species at 72 h of fermentation	(Mishra et al., 2020)

6. Conclusions

And we have gone for selection of particles of optimum range (size: 1 to 2 mm) with additional solid residues in alkaline / acidic solution. We found the acidic solution (of 2.75 M concentration) and alkaline solution (of 0.25M concentration) for best and effective agents for rice and wheat straw and sugarcane bagasse. We have performed several experiments for biofuels production like ethanol (synthetic/ glucose and biomass hydrolysate in production media with concentration ~ 4 to 5 g/L and 1.0 to 2.15 g/L respectively). From these efforts, we can provide more benefits to farmers via reducing waste matter accumulation with promotion of biofuels. This effort has more scope for alternative options for sustainable and renewable energy sources for the world.

Abbreviations

$(NH_4)_2SO_4$: Ammonium sulphate; **ABE:** Acetone, ethanol and butanol; ***C. acetobutylicum****: Clostridium cetobutylicum;* **$CaCl_2$:** Calcium chloride; **CBM-S:** *Clostridium* basal medium –S; **CMS-5A:** Carbon molecular sieves; **DNS**: 3, 5- Dinitrosalicylic acid; **$FeSO_4.7H_2O$:** Hydrous ferrous sulphate; **g/L:** Gram per liter; **H_2SO_4:** Sulphuric acid; **HMF:** Hydroxy methyl-furfural; **HPLC:** High performance liquid chromatography; **HZSM-5:** Hydrogen-zeolite socony mobil-5; **KH_2PO_4:** Potassium dihydrogen phosphate; **M:** Moles; **$MgCl_2$:** Magnesium chloride; **$MgSO_4.7H_2O$:** Hydrous magnesium sulphate; **$MnSO_4.H_2O$:** Hydrous manganese sulphate; **MPa:** Megapascal; **NaCl:** Sodium Chloride; **RCM:** Reinforced *Costridial* medium; **RI:** Refractive index; **RSD:** Relative standard deviation; ***S. cerevisiae:*** *Saccharomyces cerevisiae;* **TYA:** Tryptone yeast agar;

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