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# Conversion of De-Oiled and Dried Karanja (PongamiaPinnata) Cake into C5 and C6 Sugars by Using Acid Hydrolysis Treatment.

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***Abstract*:** Agricultural bio-mass is the world largest and most potential and promising source of renewable energy and chemicals for upcoming generation. Without depleting the reserves therefore, the present study was aimed to extract sugars from bio-mass using most common processes like acid hydrolysis, where in virtually any acid can be used; however, $H_2SO_4$ diluted (or) Concentrated acid is widely used in this process, since it is least expansive acid. The hydrolysis process converts the cellulose and hemicellulose to sugars, by treating biomass with water in the presence of acid to give sugars. The biomass used in the present work is de-oiled dried Karanja cake that is obtained as a powder from local market. The present work is focusing on acid hydrolysis of biomass to maximize sugar yields from Karanja seed cake. The acid hydrolysis is carried out in a jacketed batch reactor. The conditions employed are 1:10 seed cake to acid solution, 250 rpm, and different acid concentrations ranging from (1-6%(diluted acid) and 30-70%(Conc. acid). the reactions are carried out for 1hr to 6hrs. Hydrolysed product have shown, an average yield of 120±5 mg total sugars/ g of Karanja seed cake and conversion percentage of carbohydrates to sugars was obtained 38% at an (4-5%) diluted acid hydrolysis and 58-60 $^oC$ and 250-300 mg total sugars/g of Karanja seed cake and maximum conversion of carbohydrates to sugars was observed 75% at 40-50% Concentrated acid hydrolysis at 50-55 $^oC$.
***Keywords:*** *Cellulose, jacketed batch reactor, flavonoids.*

## Introduction

Depleting reserves of natural resources and the deletricious effects of fossil fuel burning on the environment have resulted in a great amount of research in the past couple of decades to search for alternative sources that are eco-friendly and renewable to replace fossil fuels (Humbrid et al.,2011). bio based industry is the best substitute to fossil based industry for different classes of bio fuels and bio chemicals from almost all available biomass feed stock. Thus it is required to develop techno economic routs for the production of bio based compounds to make bio-industry competitive in the market. The comparison of bio-industry with fossil based industry dictates that the integration of green chemistry into the bio industry and the use of low environmental impact technologies will result in the sustainable production of biofuels and high value chemicals from biomass. The increasing demand for fuels and chemicals is the driving force for the development and implementation of various technologies in bio-industry for fuels and chemicals that are sustainable, economic, and environment friendly (Cherubini,Francesco et al.,2010).

Biomass is composed of cellulose, hemicelluloses, and lignin as major components. Cellulose is a homo-polymer of glucose subunits (cellobiose) with crystalline structure and hemicelluloses is a hetero-polymer of pentose sugars with amorphous structure, whereas lignin is the highly crystalline and rigid component of biomass. cellulose and hemi-cellulose comprises approximately two-thirds of the dry bio-mass the present work is mainly focuses on acid hydrolysis of biomass for release of sugars take place through two methods either dilute acid treatment or concentrated acid treatment(Kumar, Praveen et al.,2009). Dilute/Concentrated acid hydrolysis is considered as the pre-treatment of biomass for ethanol production. lignin is usually removes after the pre-treatment of biomass and facilitate the hydrolysis of cellulose and hemicelluloses which gives pentose and hexose sugars. Apart from concentrated and dilute acid hydrolysis there are several bio-mass pre-treatment methods: hot water treatment, stream explosion (Chen, Wen-Hua, et al.,2011), ammonia fiber explosion, alkali treatment, organic solvent treatment, enzymatic hydrolysis (Bals, Bryan et al.,2011). Among various pre-treatment technologies acids pre-treatment (i.e. hydrolysis) usingsulphuric acid achieves high sugar yields.

Different biomass feed stock such as bark rich saw mill waste, sugar maple wood extract, oil palm fruit bunch (Rahman, S.H.A. et al.,2007;Heredia Olea et al., 2012) sweet sorghum bagasse, soya been, sugarcane, wheat starch jathropa, Karajan-cake*, nitrogen rich manure etc, has been processed for sugars with help of different diluted/concentrated acids. The acid hydrolysis of bio mass process with varying concentration have showed around 80-85% yield of sugars available in the bio-mass (Kim et al.,2004;Karimi et al.,2006). Preextraction step with water at low/high temperature followed by acid hydrolysis of maple wood gives around 160g/L concentrated wood extract (Hu, Ruofei, et al.,2010).

Different acids likesulfuric acid, hydrochloric acid, phosphoric acid, and H-USY zeolite treated with oxalic acid, employed for the breakdown of crystalline structure of biomass(Zhou, Lipenget al.,2013). different biomass feed stocks and their compositions were showed in table1. The interest in the use of H3PO4 in acid hydrolysis is that after neutralization with sodium hydroxide, it will yield sodium phosphate which will remain in the hydrolyzate and subsequently used as nutrient by microorganisms in fermentation for ethanol production. Therefore, filtration is not needed with the consequent advantage (Orozco, Angela M., et al.,2011)). But hydrolysis with H3PO4 requires higher temperatures and acid loading compared to hydrolysis with sulfuric acid and sodium hydroxide. consider into these factors present work carried out at moderate pressure(-30bar) and high temperature. However, hydrolysis at elevated temperatures and pressure will favors side reactions that result in the formation of fermentation inhibitors such as furfural, 5-hydroxy methyl furfural and acetic acid (Radhakumari, M., et al 2014)

Acidhydrolysed and detoxified sweet sorghum bagasse yielded similar amounts of total potentially fermentable sugars of 56–57% of total sugarsavailable in the sweet sorghum bagasse (390–415 mg sugar/g bagasse) and 44–61 mg totalinhibitors/g bagasse. A mild detoxification was effectively used in the optimized hydrolysates, butdid not have effect an effect in the HCl/ $H_2SO_4$mixture. The acetic acid and HMF significantly decreased in the HCl and $H_2SO_4$ detoxified hydrolysates without any significant degradation ofsugars. The employment of HCl treatment can reduce hydrolysis time to about half compared tothe $H_2SO_4$. However, the use of $H_2SO_4$ allowed the processing of more bagasse solids withoutsacrificing sugar yields. The detoxification with lime at pH 5 effectively reduced levels of aceticacid, HMF and furfural. The sorghum bagasse can be acid-hydrolysed and detoxified to yieldhydrolysates with adequate amounts of potentially fermentable sugars with low amounts ofinhibitors (Heredia-Olea et al., 2011).

The concentrated acid hydrolysis process appears to be an interesting process for hydrolysis of biomass for biofuel and bio refinery applications, with high sugar yields, low levels of fermentation inhibitors, good ferment ability and good robustness towards changes in raw material quality. Taking into consideration of these factors, in the present work karanja (Pongamiapinnata Syn. P.glabra) seed cake has been chosen as the raw material for the synthesis of sugars, because karanja oil and feed cake are not exploited widely due to the presence of toxic components. Karanja trees are widely distributed mainly native westernGhats in India, china,japan, malina, pacific islands and some regions of eastern Asia and it can sustain in harsh weather and neglected soil conditions making it life span more than 80years, and seed kernel contains 27 to 39% oil.

The oil contains toxic flavonoids such as karanj and a di-ketone pongamol as major lipid associates, which make the oil non edible which is used for biodiesel production. The left over seed cake (60-70%) was used as feed stock for the production of sugars by acid hydrolysis method. Prior to hydrolysis, physical treatment such as chipping, grinding and milling, are appliedto reduce the crystallinity of cellulose. It was reported that 30 kwh energy is required for the size reduction of one ton of agricultural biomass to 3-6 mm size which is far greater than the amount of energy produced from biomass (Cadoche.L. et al.,1989). Therefore, in economic point of view physical pre-treatment plays a vital role in bio based industry. As the de-oiled karanja seed cake after drying is available in powered form it is not required to include the size reduction unit in the present process which demonstrates that it is economical and energy intensive to use karanja seed cake for the synthesis of sugars.

## 2.Materials and methods

### 2.1 Material

The following chemicals are supplied by Ranbaxy fine chemicals limited and SD fine chemicals company, India: sulfuric acid (purity >98%); Analytical grade sodium hydroxide pellets purified LR (97%

pure with 1% carbonate); DM water, HPLC grade acetonitrile and water. The De-oiled and dried karanja seed cake<1mm was used for acid hydrolysis experiment is obtained from a local producer in Andhra Pradesh (Marathi Agro Limited, Hyderabad, India.

**2.2 Hydrolysis reactions**

Concentrated and diluted acid hydrolysis reaction was performed in a four necked round bottomed glass jacketed reactor of 1L in batch mode shown in **Fig.1**. A maximum temperature of 300 $^0$C can be achieved by this vessel. The temperature of the jacketed reactor was maintained and controlled with poly-science automatic digital refrigerating and heating thermostat. The reaction vessel equipped with a digital overhead stirrer direct control model and a speed of range 50-3000rpm. Classical process flow diagram of acid hydrolysis process shown in Fig.2.

The hydrolysis reactions were carried out with dilute sulphuric acid solution (1, 2, 2.8, 3.8, 4.8, 5.6 and 6.5% $H_2SO_4$) at 58$^o$C temperature and concentrated sulphuric acid solution (30, 40, 50, 60, and70% $H_2SO_4$) at 53$^o$C temperature and with a slurry concentration of 1:10 weight ratio of karanja seed cake to acid solutions. Dilute acid hydrolysis reactions were carried out for 5h and concentrated acid hydrolysis reactions were for 2h. In a typical experiment the reactor was charged with karanja seed cake and acid solution and was heated to the predefined temperature. The reaction was timed as soon as the temperature was reached. During the reaction samples were collected for every 1h. The reaction product was quenched immediately and neutralized with NaOH solution to stop the reaction, separated solid and liquid fractions were using centrifuge is shown in Fig.3.1&3.2**.**Liquid fraction was accurately weighed and analysed to quantify the sugarsformed. Equation1&2 was used to calculate conversion of sugars and yield of sugars from the analysis of hydrolysate of karanja feed

$$\% \text{ Conversion of sugars} = \frac{g\ of\ moomeric\ \ sugars\ \ in\ hydrolyzate}{g\ of\ carbo\ hydrate\ \ and\ \ fiber\ \ in\ seed\ \ cake} * 100 \qquad (1)$$

$$\text{Yield of sugars} = \frac{mg\ \ of\ \ moomeric\ \ \ sugars\ \ in\ hydrolyzate}{g\ of\ \ biomass} \qquad (2)$$

**2.3 Analysis**

**Quantification**

Quantification of sugars of Liquid fraction obtained from hydrolysis of karanja seed cake was done using ***HPLC technique*** with luna 5µ $NH_2$ column as stationary phase and acetonitrile and water in 75:25 volume ratio as mobile phase using RI detector. Authentic samples of glucose, fructose, sucrose, and xylose at different concentrations were analysed for the calibration of sugars from acid hydrolysis experiments and correction factors were obtained. The calibration equation for different components was expressed as:

%wt of the component = $C_f$*(%Area of the component)

**Elemental analysis**

Elemental component is one of the major charactestic of biomass samples. Quantification of sugars from hydrolysis of karanja seed cake was done using a CHNS Analyzer is shown in Table 2.

**3. Results and Discussion**

To produce sugars from de-oiled karanja seed cake it was processed in two different acid hydrolysis reactions with high and low acid concentration ranging from 1%-70%. The acid hydrolysis reactions were performed at <70$^o$C temperature and atmospheric pressure

**3.1Dilute acid hydrolysis**

The results of dilute acid hydrolysis experiments conducted at 58 $^o$C, 250 rpm for 5-6h were presented in this section. From the acid hydrolysis of de-oiled karanja seed cake it was observed that in all experiments fructose, glucose, and sucrose were detected in the hydrolyzate. To study the effect of acid concentration, temperature, and reaction time different experiments were performed.

To study the effect of acid concentration experiments were carried out at 58$^o$C temperature for 6h. Conversion of carbohydrates present in karanjacake to sugars was calculated from the predefined formula (1).

Dilute acid hydrolysis of karanja seed cake showed an increasing trend in total sugars(TS) with increasing concentration. however, the total sugar content were decreased after the optimum concentration.

As 40% of karanja seed cake composed of carbohydrates, 25-38% of available carbohydrates were hydrolysed to sugars in dilute acid hydrolysis.to find out the optimum operating conditions we have conducted series of experiments at different concentration, reaction timeFig.3.1.3.The maximum conversion of 37.4% was achieved at 4.8% dilutedacid solution shown in Fig.3.1.1. When acid solution concentration was increased from 4.8% there was a decrease in conversion and therefore yields. The yield of sugars was expressed as mg of total sugars produced per gram of biomass and the yield in dilute acid hydrolysis step was presented in Fig.3.1.2. The maximum yield of ~150-170 mg TS / g Biomass was obtained with 4.8% acid solution at 58$^{o}$C temperature 5-6hrs of reaction.

To study the Effect of temperature on yield of sugars were conducted in three levels of temperature ranging from 58-81$^{o}$C Fig.3.1.4 at optimum dilute acid concentration4.8% for all levels. Interestingly at 81$^{o}$C 185mg TS/g biomass was obtained, its nearly 20-25% more sugars,then operating condition at 58$^{o}$C and 4.8% concentration, the main reason behind this tremendous change is that temperature increases will loosen the crystalline structure of cellulose to release more sugars, after the optimum temperaturecondition total sugar yield is gradually decreases.

**3.2 concentrated acid hydrolysis**

Hydrolysis of karanja seed cake with concentrated sulfuric acid was studied to narrate the effect of acid concentration and temperature on release of sugars at 1:10 weight ratio of de-oiled dry karanja seed cake to acid solution, 250 rpm of mixing intensity, and for 2h of treatment.

The concentrated acid hydrolysis of karanja seed cake was performed at 53 $^{o}$C temperature for 2h with different levels (31, 42, 50, 62, and 73%) of concentrated sulfuric acid solutions. The resultsfrom batch experiments of concentrated acid hydrolysis as conversion of carbohydrates and yield of TS were given by Fig.3.2.1&3.2.2 respectively.In concentrated acid treatment of karanja seed cake the conversion and yield of sugars were increased with increasing acid concentration from 30 to 50% after that a decrease is observedGupta et al.,2009 has explained that any further increase in concentration of acid caused the increase in release of some inhibitors and toxic compounds, and it will lead to decrease of sugar concentration. A maximum conversion of 75% was observed at 50% acid treatment, 53 $^{o}$C temperature after 1h.

The yield of sugars from concentrated acid treatment also followed the same trend as conversion of carbohydrates to sugars. Compared to dilute acid treatment of biomass, concentrated acid treatment showed good conversion and yield with lower operating temperature and in less reaction time. In dilute acid hydrolysis for 5h of reaction yielded only 148 mg sugars/g of biomass whereas 250-300 mg sugars/g of biomass yields were observed with concentrated acid hydrolysis reactions at 53 $^{o}$C in 1h.

The results in the present study are compared with the (PoojaDoshi et.al 2013) who has worked on ethanol production from Karanja oilseed residue. In their work they have achieved 66-67 mg total reducible sugars/g of Biomass, where as in the current study around 120-130 mg total sugars/g of biomass was obtained with dilute acid hydrolysis and 300 mg total sugars/g of biomass was achieved with concentrated acid hydrolysis.

**Conclusion:**

It waspossible to extract moreC5&C6 sugars from de-oiled karanja seed cake treated with dilute acid hydrolysis than concentrated acid hydrolysis by changing temperature. From the dilute acid hydrolysis of karanja seed cake fructose, glucose, and sucrose were observed in the hydrolyzate. Where as in concentrated acid hydrolysis only glucose was present in the product except at 30, 40% acid concentration. Concentrated sulfuric acid treatment gave high TS yield than dilute sulfuric acid treatment. A maximum TS yield of 150 mg TS/ g Biomass in dilute acid hydrolysis whereas 300 mg TS/ g Biomass was obtained in concentrated acid hydrolysis in the studied range of operating conditions.

**Table 1:**Composition of various bio mass feed stocks

| Constituent, % wt | Sweet corn | Switch grass | Potato peels | Soybean | Sugar cane | Wheat straw | Jatropha | Karanja cake* |
|---|---|---|---|---|---|---|---|---|
| Protein | 3-5 | 4-10 | 10-15 | 53.55 | 15-20 | 3.0-10 | 25-40 | 25-40 |
| Carbohydrate | 20-30 | 40-60 | 40-60 | 33.5 | 40-60 | 30-50 | 15-20 | 15-20 |
| Fiber | 15-20 | | 10-15 | 15 | 10-15 | 20-40 | 15-20 | 15-20 |
| Ash | 5-6 | 6-8 | 5-8 | 6 | 1.5 | 6-10 | 3-5 | 4-5 |
| Nitrogen | | | | | | | 4-6 | 4-7 |
| Potassium | 1 | 0.5 | | 2.8 | 1 | | 1.5-3 | 0.5-1.5 |
| Phosphorus | 0.5-1 | | | 0.58 | 0.5 | 0.05 | 1.0-2.0 | 1.0-2 |
| Calcium | <1 | | | 0.2 | <1 | 0.18 | <1 | |
| Magnesium | <0.5 | | | 0.27 | 0 | | <1 | |
| Zinc and other(ppm) | | | | | | | <100 | |
| Sulphur(ppm) | | | | | | | <3000 | |

**Table 2:** Elemental analysis of different bio mass feed stock

| S.No | Element | Wheat Husk(Harmadeep etal.,2013) | Mustard straw(Harmadeep etal.,2013 | Cellulose Pulps(Serani et al.,2011) | Karanja Cake (present work) |
|---|---|---|---|---|---|
| 1 | N | 2.55 | NF | - | 4.8 |
| 2 | C | 41.47 | 41.98 | 41 | 43.6 |
| 3 | H | 5.825 | 5.71 | 6.1 | 6.8 |
| 4 | S | 0.1 | 0.48 | - | NF |
| 5 | O | 37.945 | 36.855 | 52 | 44.8 |

**Fig.1.** Four necked jacketed batch reactor for acid hydrolysis treatment

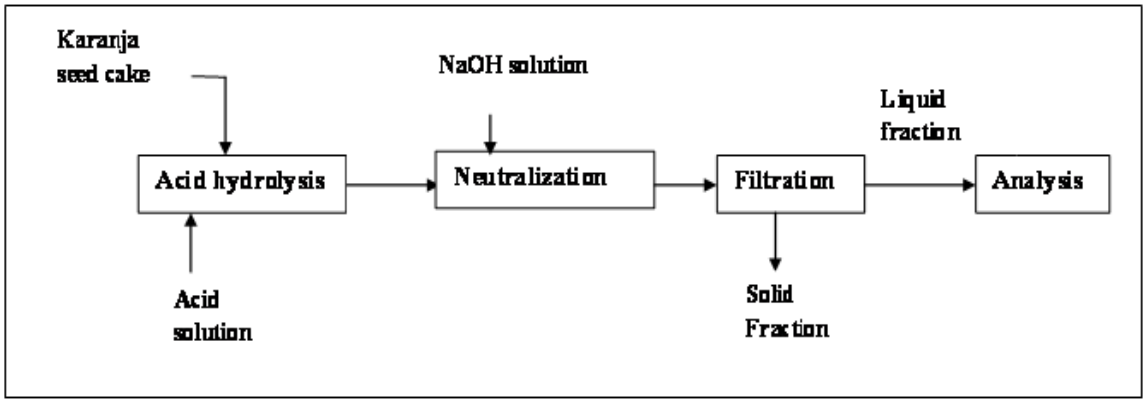


**Fig 2:** Flow Diagram of Acid Hydrolysis

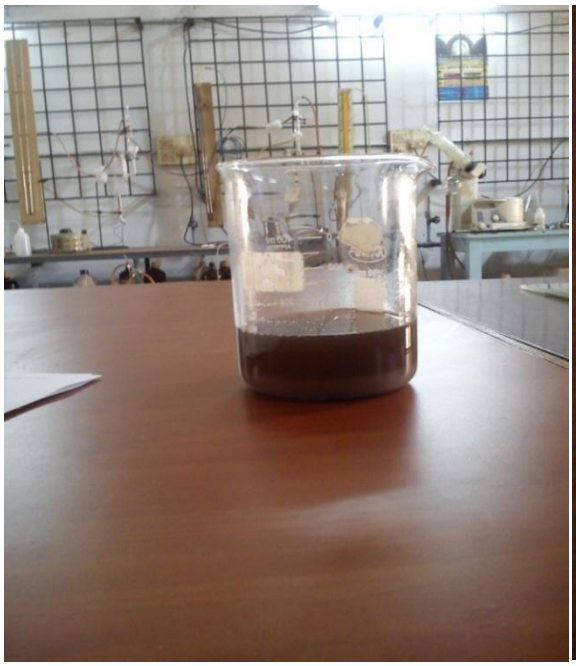

**Fig 3.1:** After Reaction Liquid Fraction.

**Fig 3.2:** After Reaction Solid Fraction

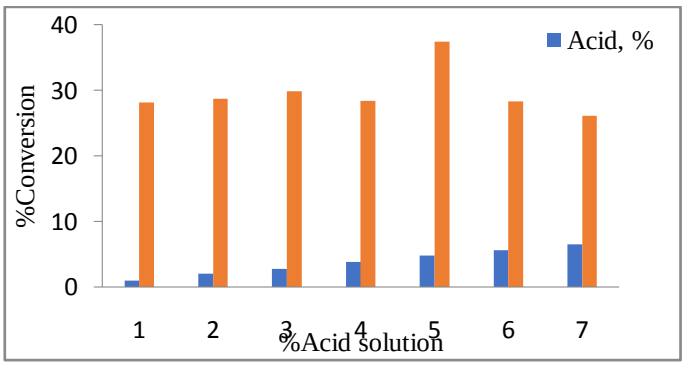


**Fig. 3.1.1:** Conversion of carbohydrates to sugars in dilute acid hydrolysis at 57-58$^{o}$C temperature, 1:10 slurry concentration, 250 rpm mixing intensity, and for 5-6h of reaction.

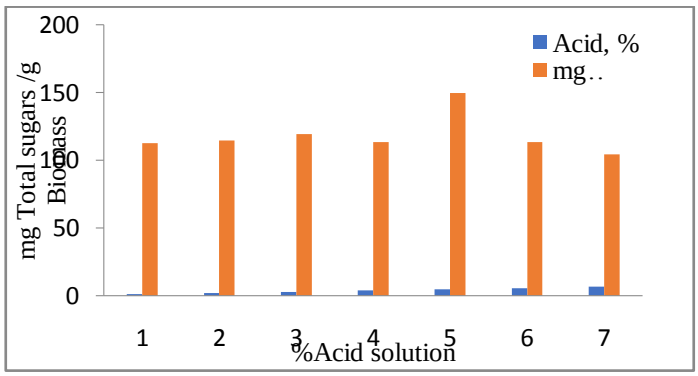


**Fig. 3.1.2:** Yield of total sugars in dilute acid hydrolysis at 57-58°C temperature, 1:10 slurry concentration, 250 rpm mixing intensity, and for 5-6h of reaction

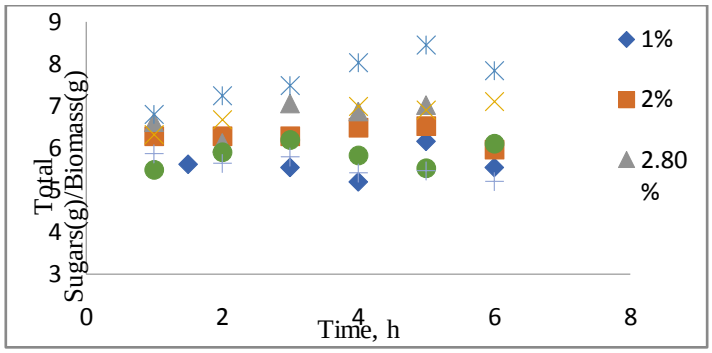


**Fig. 3.1.3:** Total Sugars VS with time

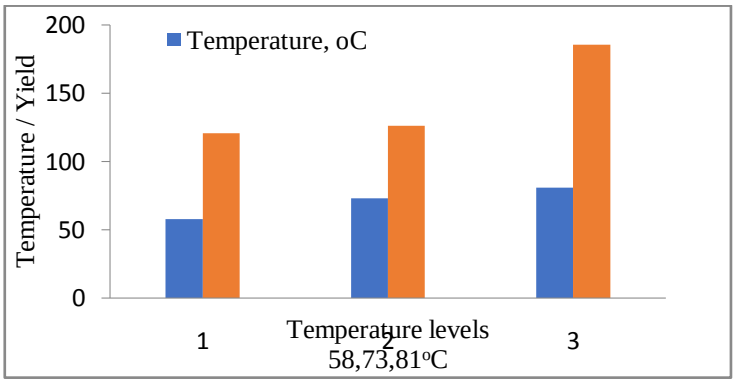


**Fig. 3.1.4** Total Sugar yield from 5% dilute acid hydrolysis at different temperatures

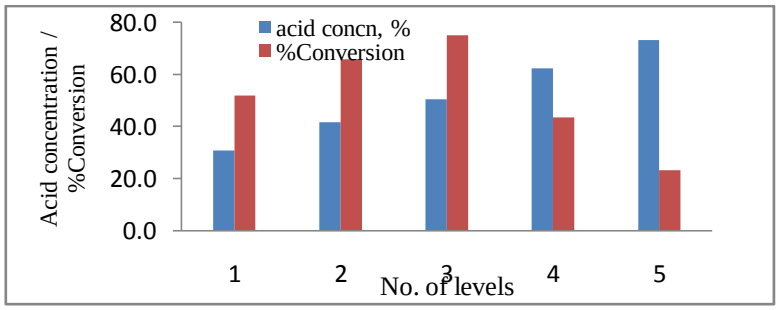


**Fig. 3.2.1** Conversion of carbohydrates to sugars in concentrated sulfuric acid hydrolysis

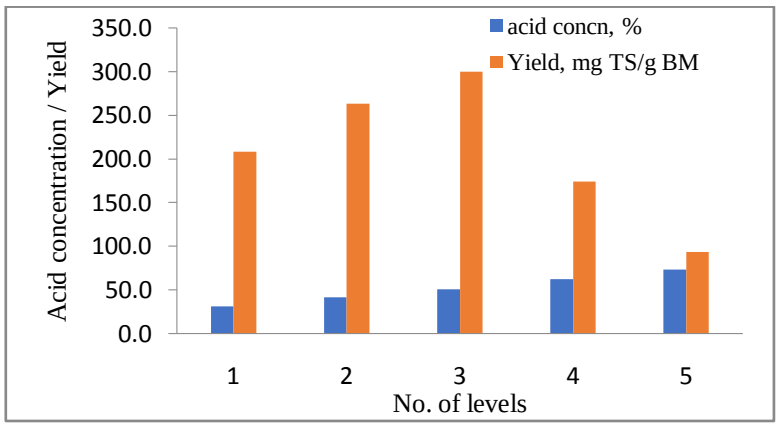


**Fig. 3.2.2:** Yield of TS in concentrated sulfuric acid hydrolysis

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