Asian Journal of Applied Science and Technology (AJAST)
(Open Access Quarterly International Journal) Volume 2, Issue 2, Pages 1089-1099, April-June 2018

# Microwave & Sonication Assisted Pretreatment for the Production of Bio-Ethanol from Waste Paper

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*Article Received: 27 February 2018* *Article Accepted: 29 April 2018* *Article Published: 10 June 2018*

## ABSTRACT

As population is increasing in a geometric progression, there is a continuous depletion of fossil fuels, day by day. Waste paper is an important constituent in the Municipal Solid Waste generated in all over the world. So keeping these two problems, the current study is to utilize waste paper for bioethanol synthesis. This study is to investigate the ethanol production by microwave based alkali pre-treatment and sonication based alkali pre-treatment for the bioethanol production from waste paper using S.cerevisiae. The substrate was immersed in different concentrations of alkali for microwave pre-treatment for 10 minutes at constant power. Sonication based alkali pre-treatment of the substrate was also carried out at different concentrations of alkali for a period of 10 minutes and constant frequency. 689mg/g of biomass, reducing sugar was obtained for microwave based pre-treatment and 721mg/g reducing sugar was obtained for sonication based pre-treatment. Then fermentation using S.cerevisiae and distillation for ethanol was carried out. The results of this study indicate that 53.8% percentage reduction of ethanol was obtained from sonication based alkali pre-treated sample. For microwave based alkali pre-treated sample it was 48.18%. The experimental results indicate a sustainable and time reducing method for the production of bioethanol.

Keywords: Bio-ethanol, waste paper, Saccharomyces cerevisiae, microwave pre-treatment, sonication pre-treatment.

## 1. INTRODUCTION

The twentieth century saw a rapid twenty-fold increase in the use of fossil fuels. According to James Hansen the single most important action needed to tackle the climate crises is to reduce the CO2 emissions from fossil fuels like coal, petrol, diesel etc. Crude oil has also been used as the major resource to meet the demands of the growing population. Hence depletion of these resources started to occur. The production at a global level also decreased from 25 billion barrels to 5 billion barrels. Due to all the above sited reasons, there aroused a worldwide interest for alternative fuels in order to meet the demands of a never ending population.

Until the beginning of the nineteenth century biomass was the predominant fuel, today it has only a small share of the total energy supply. Electricity produced from biomass sources was estimated at 44GW for 2005. So after a series of conclusions based on several tests and the never ending demands it was decided that bio-fuel can be used as the best alternative fuel. These can be directly derived from plants or indirectly from agricultural, commercial, domestic and (or) industrial wastes. The biomass conversion can result the fuel to be in the form of solid, liquid or gaseous. Bio-ethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar (or) starch crops such as corn, sugarcane, (or) cellulosic biomass like waste newspapers (generally derived from non-food sources, such as trees (or) grass) is also being developed as a feed stock for ethanol production [Beukes, N. and B. I. Pletschke ,2010]. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase the octane number and also to improve the emissions released from the vehicles.

Currently bio ethanol is widely used in the United States and Brazil. The only drawback is that the current method of production does not convert lignin portion of plant raw materials to fuel components by fermentation. Bio-ethanol can be produced from many waste materials includes banana peel waste, sugarcane waste (Aiello, C.

A. Ferrer and A. Ledesma)(1996)and waste paper (K. Awamori, Y. Moricava, Y. Ado and S. Takasawa, 1986) (S. Chen and M. Waymann, 1991) and also from range of cellulose (R. Arthe, R. Rajesh, E. M. Rajesh, R. Rajendran and S. Jayachandran, 2008).

In the year 2010 ethanol fuel production reached 86 billion gallons (23 billion gallons out of this was produced from United States itself). Brazil and United states together produce 90% of the total ethanol produced throughout the world. Companies such as Logen, POET and Abengoa are building refineries that can process biomass and turn into bioethanol. The Bioethanol is one of the environment friendly fuels, the effects on environment is less because the Ethanol contains oxygen. With comparison to the conventional gasoline the blends of E10 resulted in 12-25% less emission of carbon monoxide (Ajeetkumar. S, Pushpa. A & Abdul. R., 2014). The sugarcane and corn are the first generation bio-fuels. Due to vast increase in the ethanol production using these crops they cause immoderate pressure on the global food supply. The cellulosic biomass, such as agricultural residue and industrial waste are the most abundant and cheap source of renewable energy in the world.

The second generation biofuels may also include the fuels produced from mixed paper waste which is separated from the municipal solid waste, cash crops Jatropha, Honge, Cotton, Maize etc. can be utilized to produce bio-ethanol. The third generation biofuels can be produced from micro-organisms mainly Algae. The fourth generation biofuels produced from vegetable oil, biodiesel.

In developed and developing countries municipal wastes have become a severe problem during the last century, (Demitrios H et al. ).The shrinking of landfill capacity resulted in rising of landfill costs which is mainly due to the waste paper from the municipal waste. Because of the above concern the waste paper is used as cheap source for the production of bioethanol. Due to the shrinking landfill capacity, the tighter environmental control exists on their siting operation, construction, and of the unwillingness of communities to have new landfill sites nearby. The tighter environmental regulations are responsible for the premature closure of existing landfills and higher costs for constructing new ones, (Alya L et al., 2012). Among the various components the municipal solid waste consists of food waste, wood, leaf, garden or yard trimmings, rubber, textile, leather, metals (ferrous metals or Nonferrous metals), glass and major of paper and paper boards. About 35% to 40% by weight of the municipal solid waste is made of the paper.

## 2. MATERIALS AND METHODS

***Collection of raw material:*** Waste office papers from college campus were collected.

### *2.1 Size Reduction*

The primary step in the ethanol synthesis from waste paper is mechanical size reduction by means of milling, grinding or shredding. Size reduction has been done for increasing the surface area and to improve the biodegradability of lignocellulosic waste paper. It was soaked in water to reduce the hardness of water. For 1kg of waste paper 20litres of water was used for soaking (1:20 ratio). After 24 hours of soaking, the water was filtered and the paper was crushed properly to form a pulp.

### *2.2 Deinking Process*

College campus paper waste mainly consists of black writings. Black ink is made using carbon black. The ink component may affect the biodegradation and yield of ethanol. Deinking process has been done by using concentrated $H_2SO_4$ of concentration 15%. The sample was kept in a magnetic stirrer and for 24 hours it was kept undisturbed. After 24 hours the sample pulp was washed thoroughly with distilled water and air dried overnight. This method is used to make the pre-treatment process inexpensive and effective [Zahid Anwar Et al., 2011].

### *2.3 Alkali based Microwave pre-treatment*

Alkali pre-treatment involves the usage of dilute alkalies like NaOH, $Ca(OH)_2$, $Na_2CO_3$ etc. The purpose of alkali pre-treatment is to remove lignin and a part of hemicelluloses, thereby increasing the accessibility of enzyme action. A laboratory scale batch micro wave system of constant power range is used in this study. 5g of dried waste paper slurry was taken and mixed with dilute alkali reagent in 1:10 solid to liquid ratio. 4 levels of alkali concentrations (1%, 2%, 3%, and 4%) with constant pre-treatment time were conducted. The reaction mixture was added in a reaction vessel and it is placed in the center of the rotating glass plate in the microwave oven. The power output of 600W was set constant for the subsequent four different combinations of pre-treatment.

### *2.4 Alkali based sonication pre-treatment*

Alkali pre-treatment involves the usage of dilute alkalies like NaOH, Ca $(OH)_2$, $Na_2CO_3$ etc. The purpose of alkali pre-treatment is to remove lignin and a part of hemicelluloses, thereby increasing the accessibility of enzyme action. Ultrasound application in is able to reduce the process temperature and time, which eventually improves the energy utilization for sustainable production. A laboratory scale sono-chemical reactor was used in this study. 5g of dried waste paper slurry was taken and mixed with dilute alkali reagent in 1:10 solid to liquid ratio. 4 levels of alkali concentrations (1%, 2%, 3%, and 4%) with constant pre-treatment time were conducted. The reaction mixture was added in a reaction vessel and it is placed in the centre of the rotating glass plate in the sonicator. The frequency was kept constant for subsequent combinations of three different combinations of pretreatment.

### *2.5 DNS assay for estimation of glucose content*

Measuring the amount of glucose in the treated sample could indirectly indicate the production of bio-ethanol [8]. The DNS method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions. The absorbance of the orange to reddish brown color formed was spectrophotometrically estimated. The above assay was conducted for all the pretreated samples.

### *2.6 Fermentation using S. cerevisiae*

Commercially available potato dextrose media (Sigma Aldrich) was used for preparing media for *S.cerevesiae*. Prior to fermentation the pH of the samples should be 4-7 range for supporting the growth of *S.cerevisiae*. Ethanol fermentation was carried out in a 250ml cotton plugged EM flask, contains media (prepared with distilled water) and weighed amount of slurry(filtrate). The contents were sterilized in an autoclave at $120^0$c for 15mins. Batch fermentation was carried out using three acid-microwave pre-treated samples and three alkali-microwave pre-treated samples by using commercially available Baker's yeast. The flasks were marked properly and kept for incubation in a rotary shaker at 120rpm, room temperature. Anaerobic fermentation was performed for a period of 3-4 days.

### *2.7 Distillation*

After the completion of 4days fermentation, the contents were filtered and distillation was carried out in a round bottom flask, at $80^0$C and fractions were collected. Ethanol was evaporated at a temperature of 78-$80^0$C. Alcoholometry method was performed for the estimation of alcohol content in the sample. The solid sludge after fermentation was dried and can be used as a solid fuel.

### *2.8 Detection of alcohol*

Standard Iodoform reaction was performed to confirm the presence of ethanol in the sample. Iodine solution was added to a small amount of sample, followed by sodium hydroxide solution to remove the color of the iodine. Gently swirled the test-tubes a few times. The dark color of the iodine started to fade. A positive result was the appearance of a very pale yellow precipitate of triiodomethane (previously known as iodoform) - $CHI_3$.Apart from its colour, this was recognized by its faintly "medical" smell.

### *2.9 Estimation of Bio-ethanol*

Alcoholometry method was performed for the estimation of ethanol content in the sample. Specific gravity bottles have been used in this study. The standard procedure was followed.

### *2.10 Estimation of percentage of ethanol*

The percentage of ethanol present in the microwave and sonication assisted sample can be calculated using the equation.

% v/v = (Volume of solute)/ (volume of solution) * 100

## 3. RESULTS AND DISCUSSION

### *3.1 Estimation of sugar in the pre-treated sample*

Bio-ethanol was produced by two different pre-treatment methods, in which time and the amount of raw material taken was kept constant. Four different concentration of alkali (NaOH) was used. Estimation of sugar content was done by DNS Assay, which in turn gives the amount of ethanol that can be produced from particular sample.

***3.1.1 Microwave treated sample***

After the DNS Assay, it was found that the sample that was treated with 2% of NaOH contains 579 mg/g of waste paper. Thus the sample that was treated with 2% of NaOH was optimized and more amount of ethanol is expected than the other samples. This may not occur if the weight of the sample or the time spent by the sample in the pre-treatment process changes.

Table 3.1.1: Reducing Sugar (mg/g of waste paper) in the Microwave treated sample

| **Concentration of NaOH** | **Reducing Sugar(mg/g of waste paper)** |
|---|---|
| 1% | 310 |
| 2% | 579 |
| 3% | 516 |
| 4% | 423 |

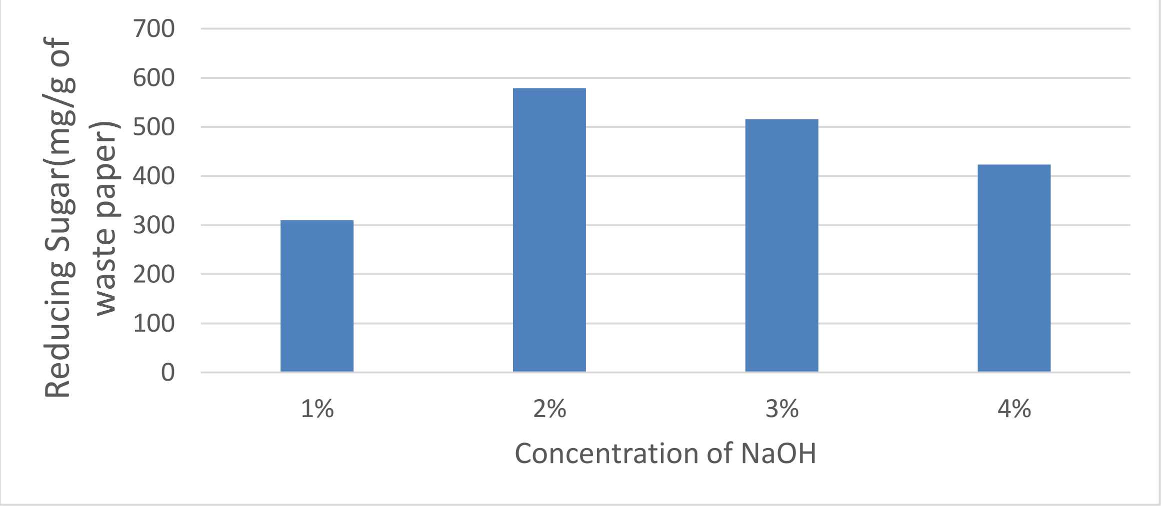


Fig 3.1.1: Reducing Sugar (mg/g of waste paper) versus concentration of NaOH in the Microwave treated sample

***3.1.2 Sonication treated sample***

After the DNS Assay, it was found that the sample that was treated with 2% of NaOH contains 620mg/ g of waste paper. Thus the sample that was treated with 2% of NaOH was optimized and more amount of ethanol was expected than the other samples. This may not occur if the weight of the sample or the time spent by the sample in the pre-treatment process changes.

Table 3.1.2: Reducing Sugar (mg/g of waste paper) in the Sonication treated sample

| Concentration of NaOH | Reducing Sugar(mg/g of waste paper) |
|---|---|
| 1% | 332 |
| 2% | 620 |
| 3% | 539 |
| 4% | 445 |

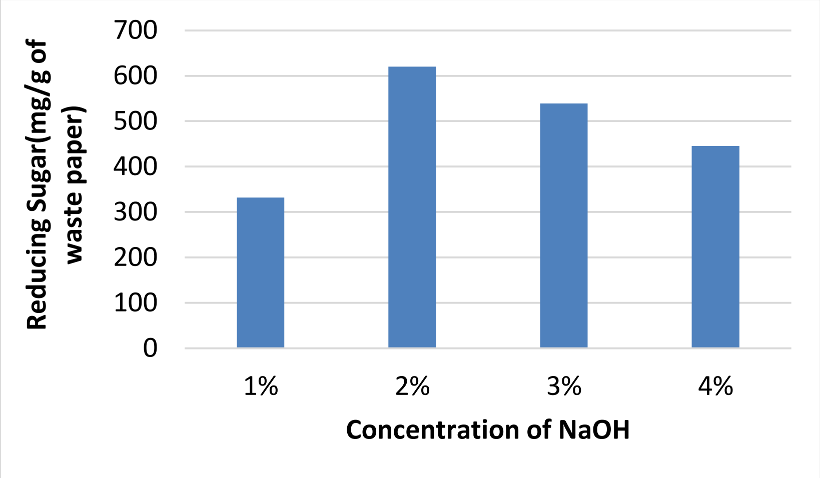


Fig 3.1.2: Reducing Sugar (mg/g of waste paper) versus concentration of NaOH in the Sonication treated sample

Thus from the above results we found that, the amount of sugar present in the sonication assisted sample is more than the microwave assisted sample. Thus the more amount of ethanol can be produced from sonication assisted sample.

### *3.2 Variation of pH during fermentation in pre-treated Sample*

During the fermentation process the pH of the medium to be maintained in the range of 4-7 for supporting the growth of *S.cerevisiae*. The pH of the medium was monitored daily and the pH increases by the end of 3$^{rd}$ day and fermentation comes to end. Yeast survives only in a slightly acidic medium, pH between 4 and 6. The fermented sample was then subjected to distillation.

### *3.2.1 Microwave assisted sample*

| Days | pH |
|---|---|
| 0$^{th}$ Day | 4 |
| 1$^{st}$ Day | 5 |
| 2$^{nd}$ Day | 6.5 |
| 3$^{rd}$ Day | 7 |

Table 3.2.1: Variation of pH during fermentation in microwave pre-treated Sample


Online ISSN: 2456-883X

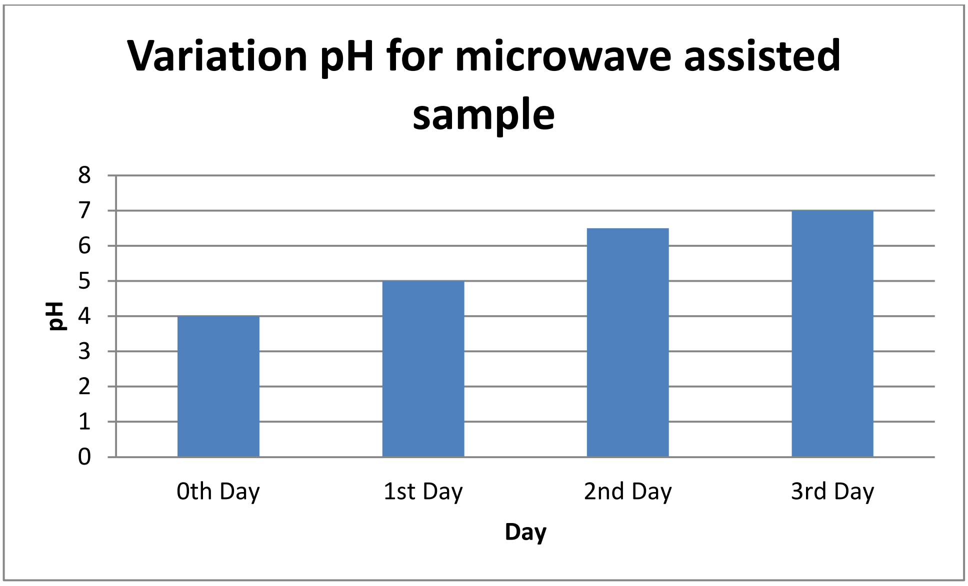


Fig 3.2.1: Variation pH for microwave assisted sample

### *3.2.2 Sonication assisted sample*

| Days | pH |
|---|---|
| $0^{th}$ Day | 3.5 |
| $1^{st}$ Day | 5.5 |
| $2^{nd}$ Day | 6 |
| $3^{rd}$ Day | 6.5 |

Table 3.2.2: Variation of pH during fermentation in sonication pre-treated Sample

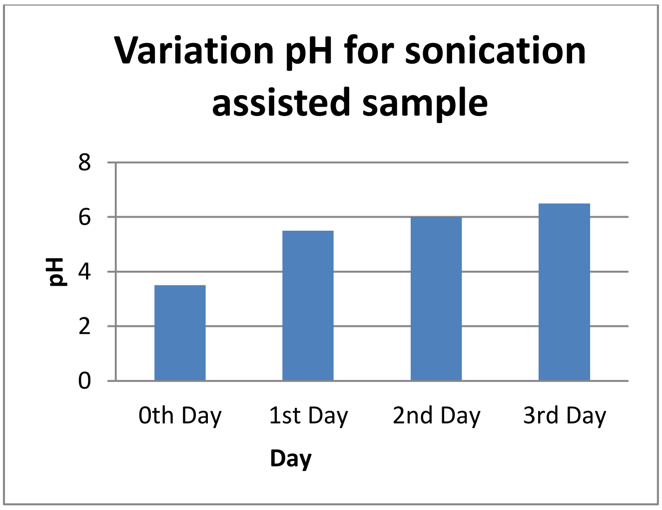


Fig 3.2.2: Variation pH for sonication assisted sample

### *3.3 Variation of temperature during fermentation in pre-treated Sample*

The temperature of the medium to be maintained for support the growth of the *S. cerevisiae.* Thus the temperature was monitored daily during the period of fermentation. Temperature playes a very important role in the production of ethanol as the rate of alcoholic fermentation increases with increase in temperature with in the range. The optimum temperature of ethanol ranges from 25 to 40ºC.

### *3.3.1 Microwave assisted sample*

| Days | Temperature ($^0$C) |
|---|---|
| 0th Day | 38 |
| 1st Day | 36 |
| 2nd Day | 37 |
| 3rd Day | 37 |

Table 3.3.1: Variation of temperature during fermentation in microwave pre-treated Sample

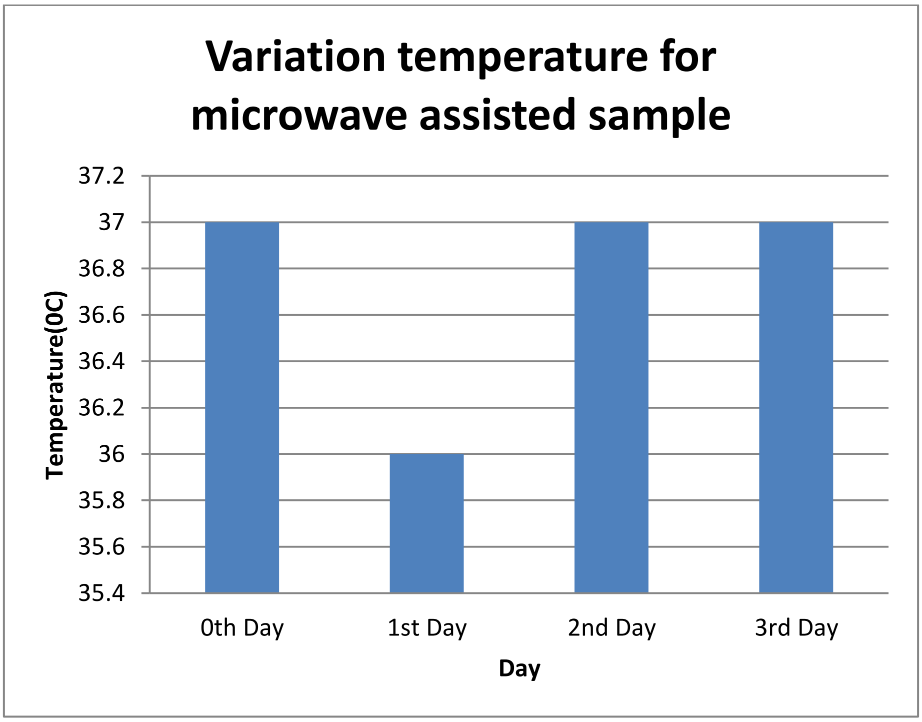


Fig 3.2.2: Variation temperature for microwave assisted sample

### *3.3.2 Sonication assisted sample*

| Days | Temperature($^0$C) |
|---|---|
| 0$^{th}$ Day | 37 |
| 1$^{st}$ Day | 36 |
| 2$^{nd}$ Day | 37 |
| 3$^{rd}$ Day | 37 |

Table 3.3.2: Variation of temperature during fermentation in microwave pre-treated Sample

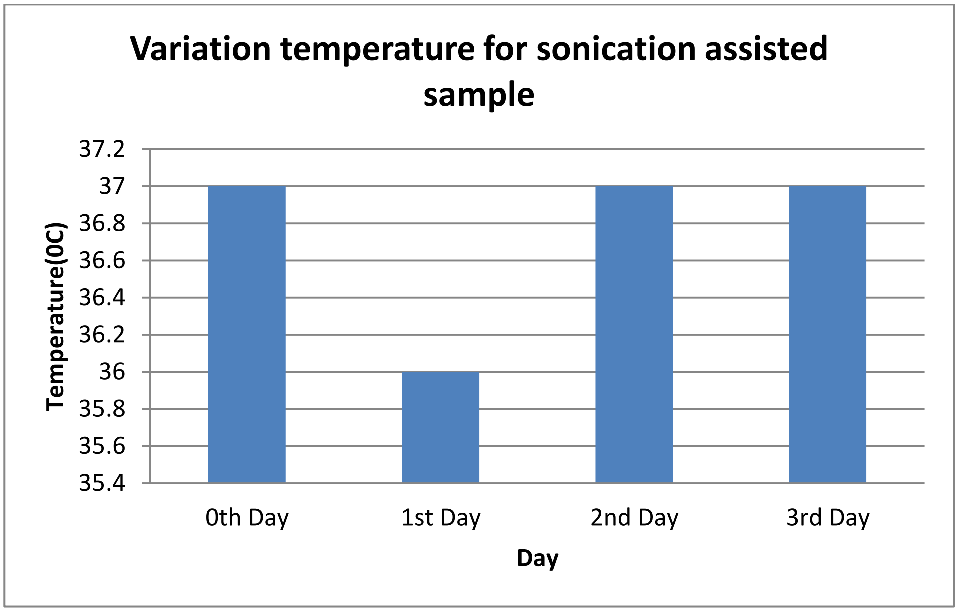


Fig 3.3.2: Variation temperature for sonication assisted sample

### *3.4 Percentage conversion of Ethanol*

### *3.4.1 Microwave assisted sample*

It was found that the sample that was treated with 2% of NaOH, the percentage conversion of ethanol was 48.18%. That was more than the other pre-treated samples as expected.

| Concentration of NaOH | % Conversion of Ethanol |
|---|---|
| 1% | 37.56 |
| **2%** | **48.18** |
| 3% | 43.5 |
| 4% | 37.4 |

Table 3.4.1: Variation of % Conversion of Ethanol for microwave pre-treated sample

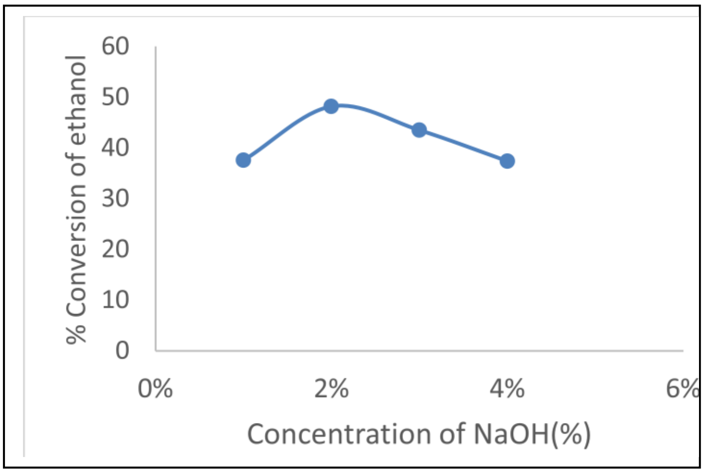


Fig 3.4.1: Variation of % Conversion of Ethanol for sonication assisted sample

### *3.4.2 Sonication assisted sample*

It was found that the sample that was treated with 2% of NaOH 53.4% of ethanol that is more than the other pre-treated samples as expected.

| **Concentration of NaOH** | **% Conversion of Ethanol** |
|---|---|
| 1% | 42.33 |
| **2%** | **53.4** |
| 3% | 40.13 |
| 4% | 38.33 |

Table 3.4.2: Variation of % Conversion of Ethanol for sonication pre-treated sample

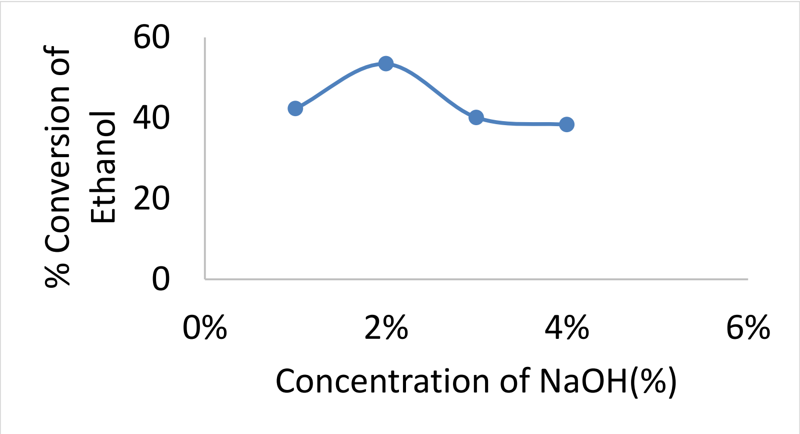


Fig 3.4.2: Variation of % Conversion of Ethanol for sonication assisted sample

Thus in the above results it was found that sonication treated sample yields more amount of ethanol than the microwave treated sample. In another study, the yield of 0.218g per gram of cellulosic waste mixture (office paper, newspaper and cardboard in 1:1:1 ratio) was reported (Iuliana Leustean, 2009).

## 4. CONCLUSIONS

Based on the above experimental results, it was clear that the substrate waste paper that was sonication assisted pre-treated with alkali has more capability to produce bio-ethanol as compared to microwave assisted alkali pre-treatment. It can improve sugar output and the percentage ethanol conversion to the maximum. The study has proved that pre-treatment of waste paper substrate with ultra-sonication can be useful for the intensification of bioconversion both in nature and under production condition. The percentage conversion of ethanol from sonication assisted pretreated waste paper was 53.4%. The purity of ethanol obtained from this can be raised further by doing second distillation. This method does not liberate any harmful fuel emissions during synthesis. Hence the utilization of waste paper as thereby production of biomass based fuels enhances the economic potential.

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