



## Abridged Production and Revitalization of Bioethanol from Biomass

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Available online at: [www.isca.in](http://www.isca.in), [www.isca.me](http://www.isca.me)

Received 23<sup>rd</sup> March 2014, revised 28<sup>th</sup> April 2014, accepted 14<sup>th</sup> May 2014

### Abstract

*The environmental concern is growing day by day. The emission of the harmful gasses in the environment can be solemnly reduced by the reduction in the usage of the fossil fuels. The alternative is to use a renewable source of energy which could fulfil the growing demands of the fossil fuels. One of the alternatives is the bioethanol but the major concern is availability of raw materials and a very cost efficient production of bioethanol. Here we present a study of production which would fulfil the above two major concerns. We used arum root as the raw material and treated it only with acid to produce bioethanol by the fermentation process. The results have shown the feasibility of producing bioethanol from arum root only using the dilute acid pretreatment. The separation of the ethanol water mixture generated as the fermentation product was done using the pervaporation setup followed by the distillation process to obtain 99.9 % pure bioethanol.*

**Keywords:** Arumroot, *Saccharomyces cerevisiae*, Fermentation, Bioethanol, GC

### Introduction

The amplification on the price of commercial fossil fuels and the requirement to guard the environment from greenhouse gases recommends an experimental survey of the possibilities of using biofuels to substitute them<sup>1-4</sup>. Bioethanol is generally deemed as significant renewable fuel donating to the reduction of pessimistic environmental brunt generated by the worldwide deployment of fossil fuels. Fabrication of renewable fuel from food wastes, agro-wastes, forest wastes, fruits and fruit wastes is of growing attention worldwide<sup>5,6</sup>. It is so because many countries are looking for reducing oil imports, enhanced rural economies and recuperating air quality and also in today's world of commercial fuels where the price and demand of the commercial fuels is increasing day by day, it can be estimated that if this large scale consumption of the commercial fossil fuels continues, ultimately the fossil fuels are going to be depleted<sup>7</sup>. Hence there is a budding demand to enhance the production of ethanol as a fuel from the raw materials which can be reproduced in a short period of time. Therefore in the present situation the production of ethanol from relatively cheaper source of raw materials using proficient mechanism is the only possible way to meet the great demand for ethanol. Since the price of raw material feedstock contributes more than half to the production cost, inexpensive feedstock such as lignocellulosic wastes, highly cellulosic grasses, uncommon food materials and agro-food wastes, are being considered to make bioethanol cheaper and competitive in the open market. USA and Brazil are the top producers and India stands fourth among the bioethanol producers worldwide<sup>8</sup>.

Biomass is a renewable source of energy originated from the wastes of assorted human and non-human commotions. It has abundant resources such as consequences from wood industry, agricultural yields, wastes from jangle and household wastes.

Renewable energy sources such as lignocellulosic biomass, cellulosic forest wastes, fruits and their wastes and typical grasses with high contents of cellulose are able to produce an alternative fuel which is environmentally friendly because the fuel emits less pollution contributing very low quantity of carbon dioxide (CO<sub>2</sub>) to the atmosphere<sup>9</sup>. Ethanol plants also produce highly nutritious food for the animals. They take up the starch and the carbohydrates only from the biomass and the rest nutrients are retained back in the biomass which makes the residue fit for the animal feed. The Carbon Dioxide (CO<sub>2</sub>) produced in the manufacturing route is used to produce beverages and other carbogenated products.

Hence it has taken a lot of interest where bioconversion of biomass deriving artefacts like value-added fuels and compounds offering probable efficient, environmental, and deliberate leads over habitual fossil products are to be produced for the benefit of mankind as well as the environment. Over the last few decades, researches have been carried out constantly to convert lignocellulosic, cellulosic and other raw materials containing poly-carbohydrates to bioethanol<sup>10</sup>. Lignocellulosics are composed of heterogeneous complex of carbohydrate polymers composed of cellulose, hemicelluloses and lignin. Cellulose consists of polymers of glucose having high molecular weight rigidly held together as packages of fibres<sup>11, 12</sup>. A lot of efforts were made for the production of Bioethanol by various people using a wide variety of raw materials such as various fruits, fruit peels, carbohydrate and starch containing materials and the cellulosic and lignocellulosic materials but the analytical methods and the separation methods are of greater concern to provide the purest form of bioethanol. In this paper analysis is done by analytical methods including the Titration method, UV spectrophotometric method and the GC system.

**Bio Fuel – Bioethanol:** The exploitation of ethanol as a fuel carries a record of over thousands of years ago. The oil calamity and the low prices of sugar in the 1970s enforced Brazil to establish a new approach to take-over the situation. The Brazilian government commenced its policy to blend the petroleum-based gasoline with alcohol. Looking at the participation of several groups taking interest into this dramatic move, the government exaggerated the utilization of an assortment of ethanol and gasoline to stimulate common vehicles giving a good result from the environmental point of view<sup>13</sup>. Slowly and gradually many countries followed the trend and bioethanol proved to be one of the best alternative resource of energy with a very low impact on the environment.

Cellulosic and lignocellulosic biomass is copious renewable reserves on earth and incorporates a variety of agricultural residues, forest residues, fruit peels, uncommon food materials used as a food for tribal peoples, high cellulosic grasses, etc. are considered as a waste and is disposed are abundant, cheap, and convenient source for the production of renewable source of energy<sup>14, 15</sup>. Solid-state fermentation of these cellulosic and lignocellulosic raw materials are quite efficient as it provides cheaper substrate, subordinate energy constraints and investment cost, superior volumetric acquiesce and a reduced amount of wastewater fabrication than submerged fermentation.

The first generation ethanol production plants uses corn, sugarcane, fruits and their wastes and other grains as the raw materials for the fabrication of ethanol. Since they are a very important part of human food, they cannot be employed as the raw materials for the enhanced fabrication for the budding demand of bioethanol<sup>16</sup> and hence for the increased demand of the alternative fuel, the second generation of the ethanol production is proposed by the second generation feedstock as the raw materials which include prairie grasses, switchgrass, wood chips, agricultural residue, wood and paper wastes, municipal solid waste, woody biomass bagasse, uncommon food materials and other cellulosic and lignocellulosic wastes<sup>17</sup>.

These second generations' raw material emerges to be a smart feedstock for three foremost reasons: i. They are renewable resources that could be protractedly derived in the future. ii. They ensure to have effectively optimistic environmental possessions ensuing in no net discharge of carbon dioxide and very little sulphur content. iii. They ensure significant economic prospective.

Bio-fuels are liquid or gaseous fuels made from the second generation feedstock from bio-chemical processes<sup>18</sup>. Bioethanol is the cleanest and the most practical renewable source of energy as an alternative of commercial fossil fuels accessible today. Bioethanol diminishes the green house gases' emission considerably when compared directly to gasoline. Emissions of particulate matter, carbon monoxide (CO), air toxics and volatile organic compounds are also

diminished when blended with gasoline displacing venomous aromatics and harmful gas emission. The fuel properties of this alternative renewable source of energy are also excellent. Bioethanol has a superior octane number of 108, extended flammability perimeter, elevated flame swiftness and privileged heat of vaporization when compared to petroleum-based gasoline. These properties help bioethanol to have a higher compression ratio and diminutive combustion time, which tends to show higher theoretical efficiency over gasoline and hence it can be blended with petrol to fuel a car. Greenhouse gases which are principally made up of CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>, H<sub>2</sub>O vapour, and O<sub>3</sub>, are considered to be acting as a catalyst in the acknowledged rising global temperature and can be reduced with the treatment of a fuel comprising of commercial fossil fuel blended with bioethanol as it burns completely reducing the emission of harmful toxic gasses in air<sup>19</sup>.

Disadvantages of bioethanol comprises of its lower energy density, corrosive nature, low flame luminosity, inferior vapour pressure and miscibility in water when compared with commercial fossil fuels, increased emission of acetaldehyde, and increase in vapour pressure when blended with gasoline.

**Arum Root:** Arum root is also famous as dasheen and eddo, is believed to have initiated in the Indo-Malaysian region, possibly in eastern India and Bangladesh from where it broaden eastwards into southern Asia and the pacific islands and westwards to Egypt and the Eastern Mediterranean. Ultimately, it reached to southern and western parts of the globe into East Africa and West Africa<sup>20</sup>. It is also known as Taro in many of the countries. In India, this raw material is commonly known as "Bengali Kochu". It is an underground vegetable which is generally used as a food material for the tribal people and is apersistent, tropical plant principally grown for its non-poisonous starchy corm, and for leafy vegetable. Arum root can be grown in fields where water is copious or in upland conditions where water is furnished by rainfall or incremental irrigation. The plant is unpalatable when unprocessed and judged as toxic due to the existence of Calcium Oxalate crystals.

Arum root consumption is inadequate because it acquires long time to cook and it have a propensity to irritate when peeling or eating tubers that are not appropriately cooked and hence making it suitable for the production of bioethanol in a large quantity to meet the growing demands of an alternative energy source as it is readily available in every part of the Earth and also abundantly renewable<sup>20</sup>. Key producing countries are Nigeria, Ghana, Kenya, Malawi, India, and Indonesia.

However, study using selective extraction of polysaccharides specified that they included about 70% of the hemicellulosic polysaccharides and an adequate amount of fermentable carbohydrate which implies arum root is one of the crops which are dominantly fit for the production of the bioethanol.



**Figure-1**  
Arum root used for this research



**Figure-2**  
Crushed arum root



**Figure-3**  
Arum root paste



**Figure-4**  
Arum root paste after acid pre-treatment



**Figure-5**  
Bioethanol Solution Yield

Generalising the process of fabrication of bioethanol from the second generation raw materials, the biochemical process of converting the biomass to bio-fuel bioethanol engages primarily four steps<sup>21</sup>: i. Pre-treatment to breakdown either lignin or hemicellulose bondage to release entrapped cellulose. ii. Hydrolysis of carbohydrate polymers to fabricate free sugars for the upcoming fermentation process. iii. Fermentation of hexose, pentose, glucose and fructose sugars to produce ethanol, iv. Separation of ethanol from other impurities primarily water.

**Pre-treatment:** Biological translation of lignocellulosic raw materials to bioethanol is extensively encumbered by the structural and chemical intricacy of biomass, which makes these stuffs confront to be utilized as feedstock for bioethanol fabrication. Cellulose and hemicellulose, after hydrolyzing into their elemental sugars, can be renovated into bioethanol through healthy recognized fermentation. Pre-treatment is entailed to alter the raw materials' macroscopic and microscopic size and structure as well as its sub-microscopic structural and chemical composition to assist swift and efficient hydrolysis of carbohydrates to fermentable sugars. On the whole, the ultimate

purpose of pre-treatment is to shatter down the shelter outlined by lignin and hemicellulose, dislocates the crystalline configuration and shrinks the extent of polymerization of cellulose; however feedstock adaptability is another factor which is to be judged when opting for a pre-treatment process. Some of the most commonly used pre treatment processes are listed below.

**Dilute acid pre-treatment** - Among all categories of chemical pretreatments of raw materials for production of bioethanol, hydrolysis using diluted acid is accounted as one of the mainly exercised and matured techniques. The most frequently applied acid is sulphuric acid ( $H_2SO_4$ ). Its impact with biomassic raw material encourages hemicellulose breakdown in fermentable sugars. On the other hand, acids such as hydrochloric acid (HCl), phosphoric acid ( $H_3PO_4$ ), nitric acid ( $HNO_3$ ), and Oxalic acid have also shown competent results<sup>22</sup>.

**Steam-explosion or Hydrothermal expansion**- This process is also known as auto-hydrolysis, in which merely steam is exploited. In this scheme, raw materials are hastily warmed through high-pressure drenched steam for a particular time period to support the hemicellulose hydrolysis. This procedure is completed by quick liberation of pressure, which delivers the biomass endure a volatile de-compression.

**Liquid hot water (LHW) pre-treatment**- Pressure is employed to retain water in the fluid phase at lofty temperatures in liquid hot water pre-treatment. LHW pre-treatment has been accounted to have the probable to augment cellulose digestibility, sugar extortion, and pentose revitalization, with the benefit of fabricating prehydrolyzates enclosing modest or no obstacle to sucrose fermentation.

**Mechanical comminution**- Raw materials of bioethanol production can be technically reduced to smaller size by various processes comprised of fragmenting, pulverizing and granulating processes. In addition to these, attrition and disk refining were also used for pre-treatment.

**High energy radiation**- Assimilation of raw materials has been improved by the employment of high energy emission techniques such as  $\gamma$ -ray, ultrasound, electron beam, pounding electrical pitch, UV, and microwave warming. Conversely, these high energy radiation processes are typically time-consuming, energy-exhaustive, and simultaneously pricey.

**Alkaline pre-treatment**- It utilizes an assortment of bases, incorporating Sodium Hydroxide (NaOH), Calcium Hydroxide ( $Ca(OH)_2$ ), Potassium Hydroxide (KOH), aqueous Ammonia, Ammonia Hydroxide ( $NH_4OH$ ), and Sodium hydroxide (NaOH) in permutation with Hydrogen peroxide ( $H_2O_2$ ). Alkaline pre-treatment also eliminates acetyl and assorted uronic acid replacements on hemicellulose that decrease the openness of hemicellulose and cellulose to enzymes.

**Ammonia fibre/freeze explosion (AFEX)** - AFEX is theoretically analogous to Steam explosion technique. In this phenomenon, the raw material is depicted to hot liquid Ammonia under soaring pressure for a particular time interval, and afterwards the pressure is abruptly discharged.

**Organosolv** - An organic or combination of aqueous and organic solvents in the company of an acid or alkali catalyst is employed to rupture the inner lignin and hemicelluloses attachments.

**Ionic liquids (ILs) pre-treatment**- Ionic liquid pre-treatment can soften huge quantities of cellulose at significant gentle circumstances and probability of recuperating nearly 100% of the utilized ionic liquids to their preliminary purity makes them desirable.

**Sulphite pre-treatment to Overcome Recalcitrance of Lignocellulose (SPORL)**- In this pre-treatment method, raw materials and the pre-treatment solution, beside with sulphite-based chemicals, are mixed together at a elevated temperature. This is followed by a size-reduction process to produce fibrous substrates for successive saccharification and fermentation.

**Wet Oxidation** - This route takes place in the company of oxygen or catalyzed air. Generally Sodium Carbonate ( $Na_2CO_3$ ) is used as catalyst in this process<sup>23</sup>.

**Ozonolysis** - For the humiliation of the lignin and hemicellulose portions from lignocellulosic raw materials, ozone can be exploited.  $O_3$  is a dominant oxidant, can dissolve in water and is voluntarily accessible.

**Oxidative Delignification**- In this course of action, peroxidase enzyme with the company of hydrogen peroxide is used for the lignin humiliation.

**Pyrolysis** - Pyrolysis takes place at elevated temperatures. This progression mortifies cellulose briskly into  $H_2$ , CO, and enduring char. The recuperated solution compiled of glucose obtained after the severance of char, can be ultimately fermented for manufacturing ethanol.

## Material and Methods

For the sake of experiments, the required amount of arum root was collected from the vegetable market of Ambikapur, Chhattisgarh, India. These were selected in the way that they contain basically the solid mass and a very low amount of water content in it which are generally used for the production of new plant. They are given the pre-treatment as follows: i. Peeled off the outer cover of arum root using the domestic vegetable peeler. ii. Cut using knife into smaller pieces for crushing as they are a solid vegetable difficult to crush as it is. iii. Crushed into further smaller pieces using the domestic crusher. iv.

Grinded into a gel type paste with the addition of required amount of distilled water with the help of a mixer grinder.

The general goal of performing the above actions is to increase the surface area of arum root for the exposure of the sugar and carbohydrate molecules present in the raw material. Doing so, the yeast used for production of the bioethanol would get better pathway for doing the reactions and giving a better yield. The paste obtained was now a mixture of all the nutritional ingredients, starch, sugars, and carbohydrates in water. The yeast (*Saccharomyces cerevisiae*) was collected from the Central Scientific Chemical Agency, Nagpur, Maharashtra, India.

**Production of Bioethanol:** For each batch on the laboratory scale, 784 grams of arum root was undergone the above physical pre-treatment and the total amount of distilled water added in the process was 670 ml to produce a thick paste of arum root. The total amount of the paste obtained was 1200 grams. Now for the experimental purpose, the thick paste was divided equally into 6 beakers (500 ml) each containing 200 grams of the arum root paste. Each one of them was then given a heat treatment of 120°C for the gelatinizing process in an air tight condition and then left out over night. The foamed mash was stirred well under an rpm monitored stirrer to completely remove the foam. Dilute acid pre-treatment was the process followed for the chemical pre-treatment process. 2 ml of concentrated sulphuric acid was added into each of the beaker containing 200 grams of the thick paste of arum root forming a mixture of 1% v/w. the mixture was stirred well for the uniform mixing of the acid with the paste with the overview that acid must be exposed to maximum sugars available in the paste. The acid paste mixture was given a heat treatment of 140-165°C in a hot-air oven for half an hour. It was allowed to cool up to 35-40°C. An addition of 5 gram of edible glucose was carried on for the sake of culture of the yeast. *Saccharomyces cerevisiae* is the yeast added for the fermentation process of the treated arum root paste. An amount of 2 grams of yeast was added to each of the beakers and kept for fermentation anaerobically at 35-38°C.

**Separation Methods:** The resulting solution of fermentation was first centrifuged in a centrifugal separator at 3300 rpm for 30 min. The solution obtained was further undergone the vacuum filtration set up comprised of Lab scale normal filter paper and the Whatman filter paper for the maximum reduction of the solid particulates and the suspended particles. The obtained liquid was a liquid solution containing various fermentation products including Bioethanol. To gain high purity fermented products the liquid became a part of the pervaporation unit preinstalled with the poly vinyl alcohol (PVA) membrane to separate ethanol-water solution. The pervaporation unit was run at the optimum condition of 40°C and at a pressure of 55 mmHg for the maximum separation of water from the fermented product. The fermented products were separated from water upto 99%. It was then applied to a distillation setup to access the ethanol present in the final

fermented product to obtain pure ethanol of around 99.9% purity.

**Analytical methods:** For the pre analysis of the presence of ethanol in the final product, 2 ml of the pure alcohol was added to an acidified solution of potassium dichromate. The green colour of the free chromium ions ( $Cr^{3+}$ ) obtained by the addition of acidified  $K_2Cr_2O_7$  into fermentation product showed the presence of ethanol in the final purified liquid obtained after filtration.

For the purpose of quantitative analysis Gas Chromatography coupled with Mass Spectroscopy (GC-MS) system was used. Gas chromatography analysis stipulations were, Gas chromatography (GC-2010, Shimadzu, Japan) with a glass column (3.2 m × 8 mm i.d.) packed with chromosorb 103 (60/80 mesh) functioned with Helium as carrier gas, column temperature 185°C, injector temperature of 220°C, flame ionization detector (FID) at 250°C, and oven temperature programming: 100°C for 1.5 min, 5°C/min to 220°C.

## Results and Discussion

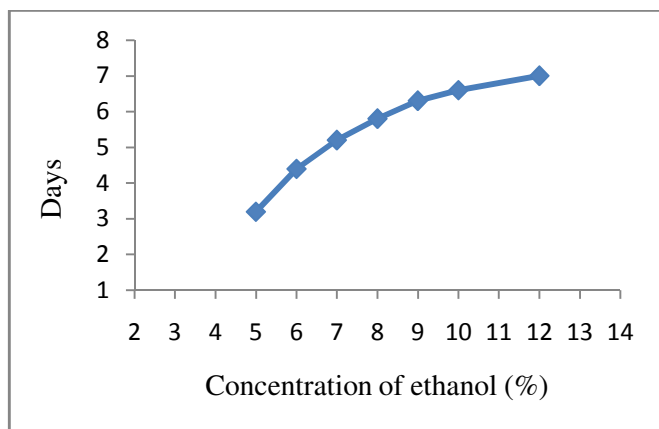
To optimize the production of bioethanol from arum root, the effect of days for the fermentation process, temperature, concentration of acid and the amount of yeast added were the main topics of study under this research paper. For the efficient study the parameters mentioned above in a simpler way, the parameters were arranged as shown in the table 1.

Table-1  
Process generated for this research

Sr.No.	Concentration of acid (% v/v)	Amount of yeast (gms)	Temperature (°C)
1	0.75	1	140
2	1	1	160
3	1.5	1	180
4	2	1	210
5	0.75	2	160
6	1	2	180
7	1.5	2	210
8	2	2	140
9	0.75	3	180
10	1	3	210
11	1.5	3	140
12	2	3	160
13	0.75	4	210
14	1	4	140
15	1.5	4	160
16	2	4	180

**Effect of days:** For the determination of effect of days on the fermentation process, number of batches was set up on the laboratory scale. 784 grams of Arum root was undergone the above physical pre-treatment and the total amount of distilled

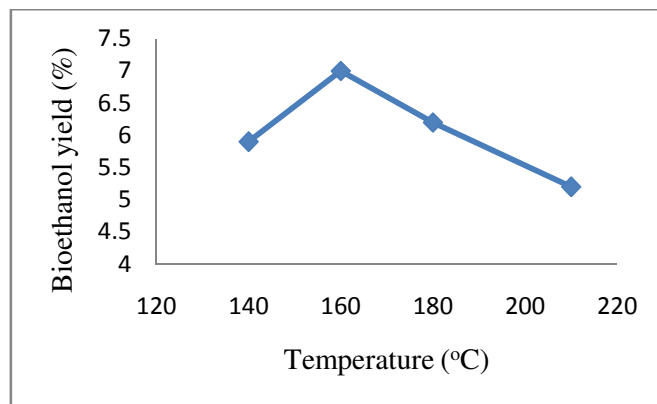
water added in the process was 670 ml to produce a thick paste of Arum root. The total amount of the paste obtained was 1200 grams. Now for the experimental purpose, the thick paste was divided equally into beakers (500 ml) each containing 200 grams of the Arum root paste. Each one of them was then given a heat treatment of 120°C for the gelatinizing process in an air tight condition and then left out over night. 2 ml of concentrated sulphuric acid was added into each of the beaker containing 200 grams of the thick paste of Arum root forming a mixture of 1% v/w. The acid paste mixture was given a heat treatment of 140-165°C in a hot-air oven for half an hour. It was allowed to cool up to 35-40°C. The addition of the glucose and the yeast was done in the similar manner as was done before and kept for fermentation at 30-35°C. The beakers were marked 1-6 in a manner that the beaker with number 1 was withdrawn on the 5<sup>th</sup> day and the last batch was taken out on the 12<sup>th</sup> day.



**Figure-6**  
 Effect of days on concentration of bioethanol

It can be clearly seen from figure-6 that the rate at which the production was obtained was quite higher than the rate of production on the days after the 10<sup>th</sup> day. After 10<sup>th</sup> day the rate almost becomes constant. It is due to the fact that the yeast (*Saccharomyces cerevisiae*) used for the fermentation process cannot withstand higher concentration of ethanol in the fermented products. It may get increased with further increase in the number of days but eventually after a certain period of time the concentration of ethanol in the final product may remain constant or might get decreased. The highest yield obtained as a result of the effect of the days is 7% bioethanol on the 12<sup>th</sup> day of the fermentation.

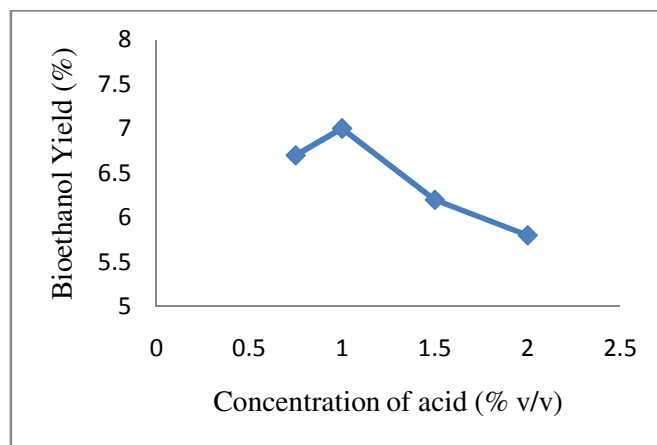
**Effect of temperature of pre-treatment:** The pre-treatment was performed on various temperatures after the addition of acid into the biomass paste. The temperature study was performed on various temperatures ranging from 140°C to 210°C. The temperature range was divided accordingly to 140°C, 160°C, 180°C, and 210°C. Every sample was kept for 20 min after the addition of sulphuric acid of measured quantity as shown in the table. The results obtained are shown in the figure-7.



**Figure-7**  
 Effect of temperature on bioethanol yield

It is clearly seen from figure-7 that maximum yield of the required product bioethanol was obtained at the optimum temperature of 160°C for the pre-treatment with sulphuric acid when kept for 20 min. The concentration of the final product increases from the temperature range of 140-160°C and then decreases on the further increment of temperature of pre-treatment. The maximum yield obtained was 7%.

**Effect of the concentration of Acid:** The effect of concentration of acid plays a major role on the fermentation process especially when whole of the pre-treatment procedure is performed using the dilute acid pre-treatment process. Here in this paper the concentration of the sulphuric acid was varied from 1.5 ml per 200ml to 4ml per 200ml making variation of 0.75 to 2 % v/v ratio of the acid to the volume of the biomass slurry. The variations made in the concentration of sulphuric acid are 0.75%, 1.0%, 1.5%, 2.0%

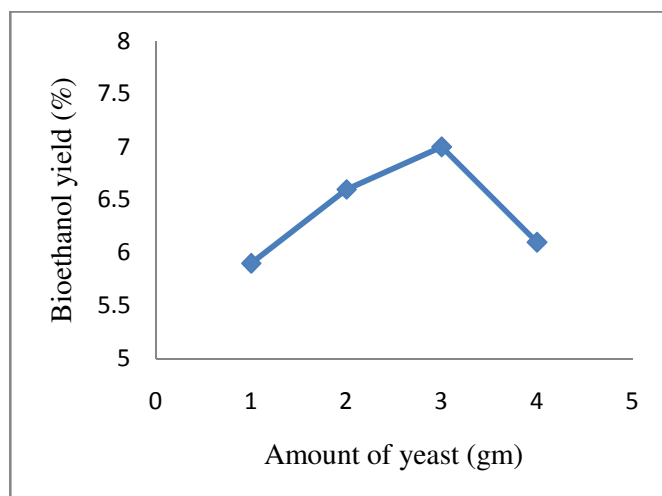


**Figure-8**  
 Effect of concentration of acid on bioethanol yield

Figure-8 clearly shows that as the concentration of the sulphuric acid used for the dilute acid pre-treatment process increases, the yield of bioethanol decreases. But till the v/v ratio of acid to biomass is 1, it increased and after that the production rate

decreases as the acidic environment increases which may make it difficult for the yeast to survive under those conditions.

**Effect of the amount of dry yeast:** The effect of amount of yeast also affects the fermentation process to a certain extent. Hence to report the effect of the yeast concentration, the production of the required product bioethanol was analysed by four different amounts which were 1, 2, 3 and 4 grams of *S.cerevisiae* added to the sample of 200 grams of arum root paste.



**Figure-9**  
**Effect of amount of yeast on bioethanol yield**

It is clearly shown in figure-9 that the yield of bioethanol increases as the amount of dry yeast increases from 1gm/200gms to 3gm/200gm that is 0.05% to 1.5% w/w ratio of dry yeast to the arum root paste but as the amount of yeast is further increased the production rate decreases or may remain constant. It shows that the optimum operating amount for the optimum production of the required fermentation product bioethanol is 1.5% w/w ratio of dry yeast to the arum root paste.

## Conclusion

The feasibility of the production of bioethanol from arum root is justified. From our study the highest concentration of the required product (bioethanol) was obtained as 7%. There can be a lot of scope for the production of bioethanol from arum root by various other pre-treatment techniques which can lead to a better yield and may be economically more viable. Thus from our study the optimum conditions for the production of bioethanol from arum root can be concluded briefly as physical pre-treatment process, 1% v/v ratio of sulphuric acid to biomass paste, heat treatment of 160°C for 20 min, addition of 1.5 % w/w ratio of dry yeast to the biomass paste and kept for fermentation at 30-35°C.

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