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Research Article

## A COMPARATIVE STUDY OF BIOETHANOL PRODUCTION FROM AQUATIC WEEDS

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### Abstract

A greatest challenge for society in the 21<sup>st</sup> century is to meet energy demand, where biomass is subjected for pre-treatment and converted into biofuel (alcohol). Aquatic weeds are potential bio resources which are easily available for biofuel production. Aquatic weeds like *Alternanthera sessilis*, *Typha latifolia*, *Eichhornia crassipes*, *Bacopa monnieri*, *Ipomoea aquatica* and *Pistia stratiotes* are estimated for carbohydrates content. Highest content of reducing sugar was observed in *Alternanthera sessilis* (296.8µg/ml), total sugar in *Ipomoea aquatic* (880.00mg/ml), starch in *Alternanthera sessilis* (57.13mg/ml), cellulose in *Pistia stratiotes* and *Typha latifolia* (280.00mg/ml), hemicellulose in *Typha latifolia* (26.85mg/ml); high cellulosic aquatic weeds were subjected to pre-treatment methods like physical, chemical and enzymatic method.

Meanwhile different yeast strains from the fruits of *Manilkara zapota*, *Cucumis melo*, *Musa paradisiaca*, *Citrullus lanatus*, *Punica granatum* and *Ananas comosus* were isolated yeast of *Citrullus lanatus* shows highest amount of alcohol production (307µg/ml), which is inoculated to pre-treated hydrolysate, where *Alternanthera sessilis* and *Typha latifolia* shows high amount of alcohol in physical method (160.5 and 115.4µg/ml). In chemical method in acid hydrolysis it shows 387.1 and 69.63µg/ml and in alkali hydrolysis 62 and 170µg/ml, so these two weeds were taken for enzymatic method for alcohol production, on seventh day *Alternanthera sessilis* shows highest alcohol production (113.33µg/ml), hence among six weeds *Alternanthera sessilis* and the yeast of *Citrullus lanatus* produces more amount of alcohol than others and it also shows that enzymatic method of pre-treatment is best in hydrolysis of biomass than physical and chemical method. The study revealed the possibility of producing alcohol from locally available fruits using simple, cheap and adaptable technology with biochemically characterized yeast strains.

**Keywords:** bioethanol; aquatic weeds; yeast; hydrolysis.

### Introduction

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized (Hug and Rojas, 2008). Global depletion of energy supply due to the unsustainable consumption and associated environmental problems of fossil fuel utilization have prompted research on alternative energy sources (Bentley, 2002) and an increasing concern for security of oil supply has been evidenced by increasing oil prices (Hahn-Hagerdal *et al.*, 2006). So new sources of alternative energy are being developed which includes solar, wind, geothermal, nuclear, biomass and other forms of renewable energy (Alexander, 2009). Bio energy is renewable energy and is produced by using various biological organisms which is expected to solve global warming problem by decreasing carbon dioxide levels in atmosphere (Mc Kendry, 2009). One of innovative approaches is conversion of biomass into fuel ethanol is being considered as a potential liquid fuel, it is one of the most promising replacements for fossil fuel since

it is renewable and emits 85% less green-house gases compared to gasoline (Mete *et al.*, 2002).

Recently, more research has focused on using non-edible biomass as raw materials including lignocelluloses, celluloses and marine algae rather than first generation biomass such as starch and sugar biomass (Lewandowski *et al.*, 2003). Conversion of biomass to bio fuel can be achieved by thermal, chemical and biochemical methods, it can be converted to usable forms of energy like methane gas or transportation fuels like ethanol provided 2.7 - 3% of world's transport fuel (Turcotte and Schubert, 2002). Recent trends favour the integration of at least three key steps pre-treatment, hydrolysis and fermentation to both improve bio ethanol yields and productivities and to lower capital and operating costs (Girio *et al.*, 2011). Besides terrestrial plants, aquatic plants are also promising renewable resource, they have many advantages as they grow in water bodies without competing with arable lands for grains and vegetables and no data on bio ethanol production from aquatic plants are available except for *Eichhornia crassipes* (Ashish Kumar *et al.*, 2009).

The aquatic weeds are fast growing, widely distributed tropical plants cause infestations over large areas of water causing considerable socioeconomic problems affect biodiversity as well as water quality and have become a source of concerns to ecologists and fishermen, the biomass in aquatic plants was proven to be suitable for bio-ethanol production, in general it is considered to be rich in hemicellulose and with very less lignin content (Aswathy *et al.*, 2009). More recently, attention has been focused on potentials and constrains of using these biomass for variety of applications, the low cellulose and hemicellulose could be easily converted to fermentable sugars resulting enormous amount of utilizable biomass for biofuel industry (Masami *et al.*, 2008).

The aim of this paper therefore is to assess the sugar content and evaluate the bioethanol potentials of aquatic weeds like *Alternanthera sessilis*, *Typha latifolia*, *Eichhornia crassipes*, *Baccopa monnieri*, *Ipomoea aquatica* and *Pistia stratiotes* in different pre-treatment methods and yeast strains.

## Materials and Methods

### Collection of Biomass

In the present investigation different aquatic weeds like *Alternanthera sessilis*, *Typha latifolia*, *Pistia stratiotes*, *Baccopa monnieri*, *Ipomoea aquatica* and *Eichhornia crassipes* were selected for bioethanol production, which are collected from Yelahanka and Batrahalli lakes of Bengaluru, Karnataka. In laboratory weeds are chapped one kg of wet weight sample was dried and powdered to find water content, followed by other laboratory studies, all experiments were conducted in triplicates.

### Isolation of Yeast

Yeast strains were isolated from the fruits of Manilkara zapota, Cucumis melo, Musa paradisiaca, Citrullus lanatus, Punica granatum and Ananas comosus, for comparative studies pure cultures of *Pichia jadinii* (MTCC-185) and *Saccharomyces cerevisiae* (MTCC-170) were procured from MTTC. Pure cultures were obtained by quadrant streaking method on YEPDA medium and grown on YEPDA broth and glycerol stocks were made for further studies (Obire *et al.*, 2008) and further tested for their alcohol production.

The dried powdered samples were estimated for following carbohydrate contents.

### Estimation of Reducing Sugar

Reducing sugar was estimated by DNS method (Miller, 1972)

### Estimation of Total Sugar

Total sugar was estimated by phenol-H<sub>2</sub>SO<sub>4</sub> method (Dubois *et al.*, 1956)

### Estimation of Starch

Starch estimation was performed by anthrone method (Hodge and Hofreiter, 1962)

### Estimation of Cellulose

Cellulose was estimated following the protocol of Updegraff, (1969)

### Estimation of Hemicellulose

Hemicellulose was estimated by Neutral detergent fibre and Acid detergent fibre (Goering and Vansoest, 1975)

### Estimation of Alcohol

Alcohol was estimated by potassium dichromate method (Caputi *et al.*, 1968)

### Pre-treatment methods

Collected six aquatic weeds were dried at 45<sup>0</sup> C in a hot air oven, powdered in a grinder (dry milling) and sieved to obtain fine particle, subjected for three pre-treatment methods like Physical method, Chemical methods (Acid and alkali Hydrolysis) and Enzymatic hydrolysis for saccharification of substrate (Sun and Cheng 2002).

### Fermentation

Pre-treated samples were subjected for fermentation by the protocol of Sun and Cheng (2002).

### Statistical Analysis

Statistical values were calculated using statistical software (SPSS). Values are the means  $\pm$  SD of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P<0.05).

## Results and Discussion

### Collection of Biomass

The dry weight of *Alternanthera sessilis*, *Typha latifolia*, *Pistia stratiotes*, *Baccopa monnieri*, *Ipomoea aquatica* and *Eichhornia crassipes* was 274, 147,150,164, 148 and 183 grams/kg respectively, where the results are supported with Randive *et al.*, 2015.

### Isolation of Yeast

Isolated yeast strains were inoculated in 20% YEPD broth, after 7 days of incubation, the amount of alcohol was estimated, It was found that yeast isolated from *Citrullus lanatus* yields highest amount of alcohol (307 $\mu$ g/ml) followed by the yeast of *Ananas comosus* (175 $\mu$ g/ml), *Musa paradisiacal* (80 $\mu$ g/ml), *Punica granatum* (4 $\mu$ g/ml), *Cucumis melo* (2 $\mu$ g/ml) and *Manilkara zapota* (0.14  $\mu$ g/ml) respectively. Similarly Rao *et al.*, 2008 isolated a total of 374 yeasts strains from a variety of rotten fruits and barks of trees, out of these, 27 yeast strains were able to assimilate xylose and produce ethanol. Maragatham and Panneerselvam, (2011) isolated 64 yeast strains and identified from seven varieties of rotten papaya, later the ability of different yeast strain to produce ethanol was investigated such as *Saccharomyces cerevisiae*, *S. bayanus*, *S. uvarum*, *S. italicus*, *S. pasteurianus*, *Schizo*

*saccharomyces pombe* and *Zygo saccharomyces*, their ability for wine production was tested by using sugar and ethanol tolerance tests. The best biochemically active strain is *S. cerevisiae* to produce wine. After fermentation for one month the highest (11.59%) alcohol concentration with corresponding residual sugar concentration of (1.87) were produced.

The estimated carbohydrate contents shows following results

#### Reducing Sugar

*Alternanthera sessilis* showed highest concentration of reducing sugar (296.80mg/ml) followed by *Ipomoea aquatica* (224.00mg/ml), *Pistia stratiotes* (168.00mg/ml), *Baccopa monnieri* (89.6mg/ml), *Typha latifolia* (72.80mg/ml) and *Eichhornia crassipes* (50.40mg/ml) (Table 1), where similar observations were made by Ganguly *et al.*, 2013.

#### Total Sugar

Highest total sugar was observed in *Ipomoea aquatica* (880.00mg/ml) followed by *Eichhornia crassipes* and *Pistia stratiotes* (680.00mg/ml), *Alternanthera sessilis* (7.80mg/ml), *Typha latifolia* (1.27mg/ml) and *Baccopa monnieri* (1.25mg/ml) (Table 1), where similar studies were made by Shweta and Nirali, 2015.

#### Starch

In *Alternanthera sessilis* the amount of starch was (57.13mg/ml), followed by *Typha latifolia*-(21.60mg/ml), *Eichhornia crassipes* (18.71mg/ml), *Pistia stratiotes* (16.00mg/ml), *Ipomea aquatic* (7.39mg/ml) and *Baccopa monnieri* (0.73mg/ml) (Table 1), where similar observation were also made by Maripi, *et al.*, 2014 which supports the results.

#### Cellulose

In *Pistia stratiotes* and *Typha latifolia* the amount of cellulose was (280.00mg/ml), followed by *Alternanthera sessilis* (260.00mg/ml), *Baccopa monnieri* (140.00mg/ml), *Eichhornia crassipes* (100.00mg/ml) and *Ipomea aquatic* (80.00mg/ml) (Table 1). In accordance with these studies, water-hyacinth cellulose acid hydrolysate has been utilized as a substrate for ethanol production using *Pichia stipitis* (Nigam, 2002).

#### Hemicellulose

Total amount of hemicellulose in *Typha latifolia* was (26.85mg/ml), followed by *Pistia stratiotes* (15.70mg/ml), *Alternanthera sessilis* (15.35mg/ml), *Baccopa monnieri* (8.70mg/ml), *Eichhornia crassipes* (7.55 mg/ml) and *Ipomea aquatica* (1.59mg/ml) (Table.1), similar observations were reported by Van Zyl *et al.*, 1991 and Amartey and Jeffries (1996), during neutralization of sugar cane bagasse and corn cob.

#### Pre-Treatment Methods

In this method *Typha latifolia*, *Pistia stratiotes* and *Alternanthera sessilis* samples shows good amount of cellulose and hemicellulose along with reducing sugar and total sugar, hence these samples were subjected to several pre-treatment methods for hydrolysis of cellulose and hemicelluloses for bioethanol production. The production of fuel ethanol from biomass involves prehydrolysis, fermentation and distillation. The hydrolysate resulting after prehydrolysis and hydrolysis, contain varying amounts of monosaccharides, both pentoses and hexoses and broad range of substances either derived from raw material or resulting as reaction products from sugar and lignin degradation. Many of these substances may have an inhibitory effect on microorganisms in subsequent fermentation steps (Van Zyl *et al.*, 1991).

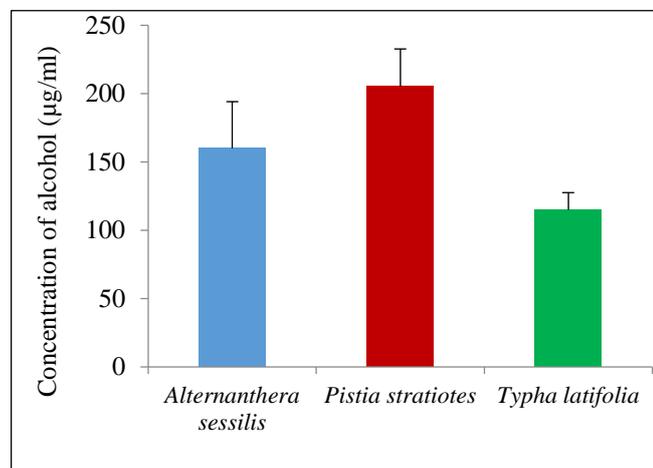
Table 1: Carbohydrates content in aquatic weeds (mg/ml)

S.N.	Plant name	Reducing sugar	Total sugar	Starch	Cellulose	Hemi cellulose (%)
1	<i>Alternanthera sessilis</i>	296.80	007.80	57.13	260.00	15.35
2	<i>Ipomoea aquatica</i>	224.00	880.00	07.39	80.00	01.59
3	<i>Pistia stratiotes</i>	168.00	680.00	16.00	280.00	15.70
4	<i>.Baccopa monnieri</i>	089.60	001.25	00.73	140.00	08.70
5	<i>Typha latifolia</i>	072.80	001.27	21.60	280.00	26.85
6	<i>Eichhornia crassipes</i>	050.40	680.00	18.71	100.00	07.55

Values are the means of three replicates data are subjected to analysis of varians and compared for significance according to DMRT (p<0.05)

### Physical Method

In this method *Pistia stratiotes* was found to have more amount of alcohol (205.9 $\mu\text{g/ml}$ ), than *Alternanthera sessilis* (160.5 $\mu\text{g/ml}$ ) and *Typha latifolia* (115.4 $\mu\text{g/ml}$ ) (Fig. 1), similar to the present studies Sun and Cheng (2002) in their experiments reduce the substrate to 0.2-2mm size by milling and grinding for improving the digestibility of biomass.



**Fig. 1:** Alcohol present in different samples (Physical method).

### Chemical Methods

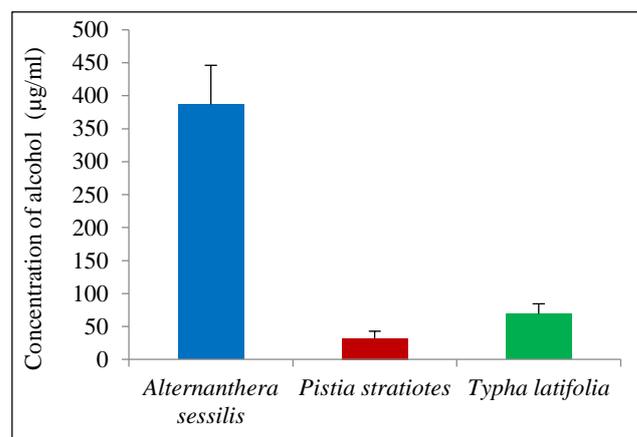
#### Acid Hydrolysis

In this method  $\text{H}_2\text{SO}_4$  is used, among three samples *Alternanthera sessilis* shows 387.1  $\mu\text{g/ml}$  of alcohol followed by *Typha latifolia* (69.63  $\mu\text{g/ml}$ ) and *Pistia stratiotes* (32.14  $\mu\text{g/ml}$ ) (Fig. 2). According to Ayodele *et al.*, (2015) water hyacinth yield a maximum value of between 40 to 100% of fermentable sugars after hydrolysis, there was significant difference in sugar yield, the highest in hydrolysed with 3% KOH (567.11 $\pm$  10.83mg/g) and lowest hydrolysed with 3% w/v oxalic acid (66.43 $\pm$  0.00mg/g). Both water hyacinth and sugar cane leaves could be used as alternative sources of bioethanol especially water hyacinth, this would create a panacea to the problem that water hyacinth constitutes in water ways and also create wealth and reduce demand on sugarcane, maize and other staple food used as sources of bioethanol, similar observations are also made by Singh and Trivedi (2013). According to Li *et al.*, 2010 dilute acid hydrolysis and dissolution in an ionic liquid, are compared in terms of delignification, saccharification efficiency and saccharide yields with switch grass serving as a model bioenergy crop.

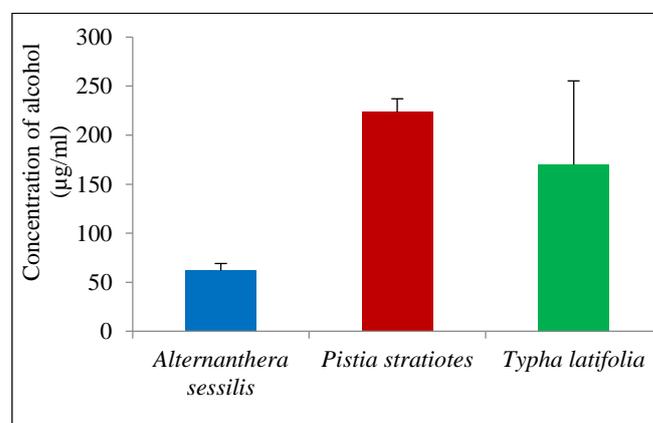
#### Alkali Hydrolysis

In this method NaOH is used, *Pistia stratiotes* was found to be high amount of alcohol (223.5  $\mu\text{g/ml}$ ) followed by *Typha latifolia* (170  $\mu\text{g/ml}$ ) and *Alternanthera sessilis* (62  $\mu\text{g/ml}$ ) (Fig. 3). According to Sun and Cheng (2002) lignocellulosic biomass can be utilized to produce ethanol a promising alternative energy source for limited crude oil. There are mainly two processes involved in conversion:

hydrolysis of cellulose in lignocellulosic biomass to produce reducing sugars and fermentation of sugars to ethanol, similar observation made by Singh and Trivedi (2013) also.



**Fig. 2:** Alcohol present in different samples (Chemical method - acid hydrolysis).

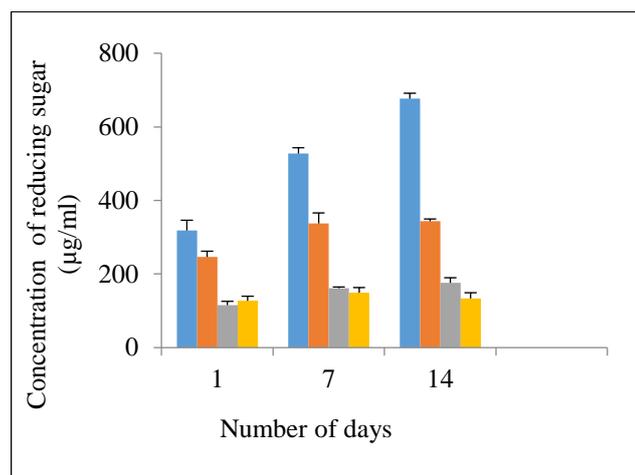


**Fig. 3:** Alcohol present in different samples (Chemical method - alkali hydrolysis).

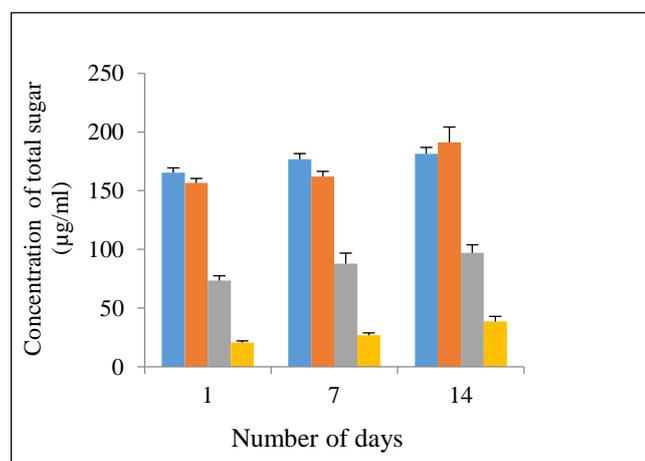
#### Enzymatic Hydrolysis

In this method *Cellulomonas fumi* is used, samples were estimated for reducing and total sugar on first, seventh and fourteenth day, on fourteenth day results shows that, the reducing sugar was (343.33 and 677.3  $\mu\text{g/ml}$ ) in *Alternanthera sessilis* and in *Typha latifolia* it was (134 and 179.66  $\mu\text{g/ml}$ ) with and without autoclave respectively (Fig. 4). The total sugar was (191.16 and 181.33 $\mu\text{g/ml}$ ) in *Alternanthera sessilis* and in *Typha latifolia* it was (38.6 and 97 $\mu\text{g/ml}$ ) with and without autoclave respectively (Fig. 5), enzymatic hydrolysis of corncob and ethanol fermentation from cellulosic hydrolysate were investigated by Chen *et al.*, 2007 after corncob was pretreated by 1%  $\text{H}_2\text{SO}_4$  at 108 $^\circ\text{C}$  for 3 h, cellulosic residue was hydrolyzed by cellulase from *Trichoderma reesei* and the hydrolysis yield was 67.5%. Poor cellobiase activity in *T. reesei* cellulase restricted the conversion of cellobiose to glucose, further fermentation of cellulosic hydrolysate containing 95.3  $\text{gl}^{-1}$  glucose was performed using *Saccharomyces cerevisiae* 316 and 45.7  $\text{gl}^{-1}$  ethanol was obtained within 18h, the

research results are meaningful in fuel ethanol production from agricultural residue instead of grain starch.



**Fig. 4:** Reducing sugar on different days after enzymatic hydrolysis.

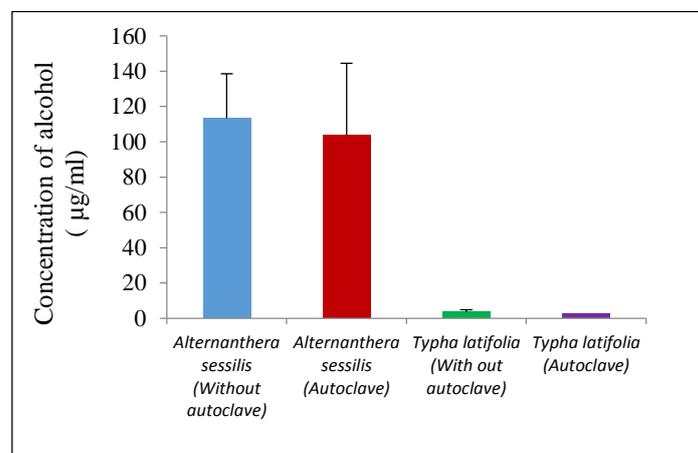


**Fig. 5:** Total sugar on different days after enzymatic hydrolysis.

#### Ethanol Produced after Fermentation (7<sup>th</sup> Day)

Enzymatically hydrolysed sample inoculated with high amount of alcohol producing isolated yeast strain of *Citrullus lanatus*. After 7<sup>th</sup> day alcohol produced was estimated by potassium dichromate method. The results shows that *Alternanthera sessilis* produced highest ethanol (103.66 and 113.33 µg/ml) in with and without autoclave respectively. The result shows that there is no significant difference between ethanol yield by *Alternanthera sessilis* and *Typha latifolia* (Fig. 6), similarly according to Narayanan *et al.*, 2013, in saccharification stage pretreated water hyacinth was added to the growth media and inoculated with saccharification micro-organisms *Trichoderma reesei* and *Aspergillus niger*, the optimum saccharification period for both micro-organisms was found to be 72 hours. On testing, it was found that both organisms were capable of producing glucose, with *Trichoderma reesei* (275 µg/ml) giving a better yield than *Aspergillus niger* (175 µg/ml). Among six aquatic weeds *Alternanthera sessilis* has highest amount of cellulose which leads high bioethanol yield, among six isolated yeast strains yeast of

*Citrullus lanatus* has highest alcohol production capacity and in different pre-treatment methods enzymatic method is the best method for alcohol production, hence *Alternanthera sessilis*, yeast of *Citrullus lanatus* and enzymatic method pre-treatment are more suitable for more bioethanol production.



**Fig. 6:** Amount of alcohol produced on seventh day after enzymatic hydrolysis.

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