Bioethanol Production from Corn, Pumpkin and Carrot of Bangladesh as Renewable Source using Yeast *Saccharomyces cerevisiae*

M.N. Yesmin[a], M.A.K. Azad[a], M. Kamruzzaman[b], M.N. Uddin[b]

[a] Institute of Environmental Science (IES), University of Rajshahi, Rajshahi-6205,
[b] Tamaltala Agriculture & Technical College, Affiliated by University of Rajshahi, Natore
*Corresponding author email: nilufayesmin3466@yahoo.com

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Abstract:
Bangladesh produces a large amount of corn, pumpkin and carrots every year. To meet its huge energy demand and to lessen dependence on traditional fossil fuel these products are cost effective, renewable and abundant source for bioethanol production. The research was aimed to evaluate Bangladeshi corn, rotten carrot and pumpkin for bioethanol production. About 100 g of substrates was mixed with 300 ml distilled water and blended and sterilized. All the experiment was conducted with a temperature of 35oC, pH 6.0 and 20% sugar concentration. For fermentation, 200 ml yeast (Saccharomyces cerevisiae CCD) was added to make the total volume 500 ml. Addition of small amount of 1750 unit α-amylase enzyme to the substrate solution was found to enhance the fermentation process quicker. After 6- days of incubation, corn produced 63.00 ml of ethanol with 13.33 % (v/v) purity. Bioethanol production capacity of two different local varieties of pumpkin (red and black color) was assessed. Red pumpkin (Cucurbita maxima L.) produces 53 ml of ethanol with purity 6 %v/v and black color pumpkin produces 40 ml of yield with a low purity 4 %v/v. Carrot (Daucus carota L.) produces 73.67 ml of ethanol with 12.66 % (v/v) purity.

Keywords: Corn, Pumpkin, Carrot, Bioethanol, Saccharomyces cerevisiae CCD, Alcoholic fermentation.

1.0. Introduction

Fossil fuels are the major energy sources in the world but for rapid consumption its reserve will be finished in near future. On the other hand, fossil fuel has resulted in the global warming and climate change. Therefore, there is thrust towards replacing fossil fuels with cleaner and renewable fuels such as bioethanol and biodiesel. Bioethanol is considered as the most optimistic biofuel fermented from renewable sources or biomass-based waste materials for fuel or fuel additives [1]. Fuel ethanol production has been fascinated now, because many countries look for reducing oil imports, boosting rural economies and improving air quality [2].

It mainly produced from biological methods with the use of microorganisms and enzymes involving fermentation of biomass based waste materials in a broad spectrum. The final product is a liquid which is flammable. Same type of ethanol is found in alcoholic beverages. Ethanol is one of the most important feedstock and it uses as a chemical and solvent in various industries. From the beginning of the biofuels development, most of bioethanol has been produced from crops, such as corn (North America), sugarcane (South America) or sugar beet (Europe). Bioethanol can be produced from different raw materials classified in three types: sugars, starches and lignocelluloses [3].

Ethanol can be made of renewable feed starch sources such as sugarcane industrial wastes, agricultural low cost fruits, vegetables, starch materials cellulosic materials, agricultural by products of starch industries, such as potato pulp, sweet potato, maize, rice straw, tapioca pulp and papaya fruit pulp. The raw materials that can be used for alcoholic fermentations are sugar crops (sugar cane, sugar beet, sorghum and fruits), starchy crops (corn, wheat and barley) and cellulosic crops (stems, leaves, trunks, branches and husks), the latter needing a pre-treatment to make fermentation possible. They vary in relation to geographic areas: corn is generally used in USA and China, while in tropical areas (India, Brazil and Colombia) sugarcane is more diffused [4].

The first generation of bioethanol production used corn as a substrate, later corn was considered as a feedstock lead to the second generation of production of ethanol which used microorganisms and different wastes as a substrate [5]. Bioethanol can be made from common crops such as sugarcane, potato and corn [6]. There are two primary technologies to make bioethanol fuel on industrial scale. The first option, in wide use today, is to convert the starchy part of foods such as corn into bioethanol through seven steps: starch extraction, liquefaction, saccharification, fermentation, distillation, dehydration and denaturing. When sugarcane is used, only four or five steps are required: milling, pressing, fermentation and distillation; and dehydration only in the case of alcohol blends. Although bioethanol has the advantage of being derived from renewable sources but it is also not environmentally sustainable, especially regarding greenhouse gas emissions [7].

Development of ethanol in Brazil has been running 30 years with from sugar cane as raw material, and in the United States the development of ethanol from corn. Germany used wheat starch and corn fiber as renewable alternative energy sources, while Canada successfully in cultivating grass as an alternative energy source. Indonesia has developed the process production of ethanol from cassava and molasses [8]. Bioethanol is known to be a potential alternative to petroleum-derived fuels and has the potential to meet the increasing demand of energy for industrial processes, heating and transportation. Worldwide production capacity of ethanol in 2005 and 2006 was about 45.42 and 49 billion litres per year, respectively [9]. World ethanol production for transport fuel tripled between 2000 and 2007 from 17 billion to more than 52 billion litres. From 2007 to 2008, the share of ethanol in global gasoline type fuel use increased from 3.7% to 5.4%.

In 2009 worldwide ethanol fuel production reached 19.5 billion gallons (73.9 billion liters) of ethanol were produced worldwide. The U.S. and Brazil are the two major countries and produced 10,600.00 and 6,577.89 million gallon of ethanol in 2009, respectively. In 2011, all over the world bioethanol production increased as showed by the production of 85 billion liters of bioethanol [10,11]. Countries, such as China and Canada, are producing 845 million gallons (3.2 billion L) and 436 million gallons (1.65 billion L) of fuel ethanol, respectively, from various starchy feedstocks, such as corn, cassava, wheat and rice (Table 1), while countries, such as India, France, Germany and Australia are producing about 1 billion L, 1 billion L, 750 million L and 500 million L, respectively, primarily from sugar-rich feedstock, such as sugarcane, molasses, sugar beet, and wheat (RFA, 2017) [12]. Advanced bio-fuels may include ethanol derived from cellulose, sugar or starch, or waste material, including crop residue, other vegetative waste material, animal waste and food waste. Country wise fuel ethanol production is shown in Table 1.

At present, ethanol is widely used in Brazil and in the United States, and together both countries were responsible for 89 percent of the world's ethanol fuel production in 2009. The major feedstocks used by these countries are sugarcane and corn. In Europe, ethanol production is based on beet molasses and it is still very sharp due to lack of available feedstocks that can support local ethanol production plants [13]. Most cars on the road today in the U.S. can run on blends of up to 10% ethanol, and the use of 10% ethanol gasoline is mandated in some U.S. states and cities. Since 1976 the Brazilian government has made it mandatory to blend ethanol with gasoline, and since 2007 the legal blend is around 25% ethanol and 75% gasoline (E25). In addition, by 2010 Brazil had a fleet of more than 10 million flexible fuel vehicles regularly using neat ethanol fuel [14]. Brazil is the second largest producer of fuel ethanol in the world with 27% of total global production, which accounts to about 7295 MG of ethanol (Table 1).

Table 1: World Fuel Ethanol Production by Country or Region (Million Gallons)

Country	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
USA	6,521	9,309	10,938	13,298	13,948	13,300	13,300	14,313	14,807	15,329	15,800
Brazil	5,019	6,472	6,578	6,922	5,573	5,577	6,267	6,190	7,093	7,295	7,060
Europea n Union	570	734	1,040	1,209	1,168	1,179	1,371	1,445	1,387	1,377	1,415
China	486	502	542	542	555	555	696	635	813	845	875
Canada	211	238	291	357	462	449	523	510	436	436	450
Rest of World	315	389	914	985	698	752	1,272	1,490	1,147	1,301	1,450
WORLD	**13,123**	**17,644**	**20,303**	**23,311**	**22,404**	**21,812**	**23,429**	**24,583**	**25,683**	**26,583**	**27,050**

Source: Data taken from Renewable Fuels Association

Corn (Zea mays L.) grain contains high amounts of starch, which is readily convertible to monosaccharides upon pretreatment (i.e., cooking in water) and hydrolysis. Glucan is also present in the cob, stalk and leaves, but in a different form, i.e., cellulose, and at lower amounts compared to corn grain. Corn was used as a substrate for the first generation of ethanol production, later corn was considered as a feedstock lead to the second generation of production of ethanol which used microorganisms and different wastes as substrates [15]. Thus, corn being such a widely popular and highly successful agricultural produce worldwide, and with such high content of starch, fiber, protein and oil, it has not only found usage as food, livestock rations and feedstock for renewable fuel ethanol production, but also in many other industrial sectors responsible for production of diverse co-products. A thorough analysis of the chemical composition of a shelled corn reveals that it is composed of starch (72%), fiber (9.5%) and protein (9.5%) [16]. In the USA, starch from maize is used as a feedstock for hydrolysis, where the glucan is converted to glucose by enzyme hydrolysis and fermentation of the glucose to ethanol by yeast. The mash is fermented using natural yeast and bacteria in a process that takes up to 40 hours. The fermented mash is separated into ethanol and residues (for feed production) via distillation. The USA is the world's largest bioethanol producer. The production of fuel ethanol from corn grain is widely carried out in the US, with total current production at 7 billion gallons. This may soon reach10 billion gallons or more (Table 2).

Table 2: Global Corn Production and Yield from 2015–17 (in Million Metric Tons)

	Production (million metric tons)			**Yield (metric tons per hectare)**		
Country/region	**2015/16**	**2016/17**	**2017/18 projected**	**2015/16**	**2016/17**	**2017/18 projected**
World	969.49	1070.51	1036.90	5.43	5.83	5.70
USA	345.51	384.78	362.09	10.57	10.96	10.72
China	224.63	219.55	215.00	5.89	5.97	6.14
Brazil	67.00	98.50	95.00	4.19	5.61	5.37
European Union	58.75	61.14	61.60	6.35	7.11	7.03
Argentina	29.00	41.00	40.00	8.29	8.37	8.16
Mexico	25.97	27.40	25.00	3.60	3.65	3.50
India	22.57	26.00	25.00	2.56	2.71	2.63
Rest of the world	196.06	212.14	213.21	—	—	—

Source: Data taken from Foreign Agricultural Services/USDA Office of Global Analysis. For a more detailed table of data see https://apps.fas.usda.gov/psdonline/circulars/production.pdf.

Ethanol an important biofuel, having high calorific value has the added advantage of being less polluting than most sources of energy that are in use today. Reports available suggest that previous natural substrates for ethanol production via saccharification have included sugarcane bagasse, wheat straw, corn, softwood, etc. S. cerevisiae is usually considered the typical yeast of wine and cider fermentations; among other species of the genus Saccharomyces and S. bayanus characterized by high ethanol tolerance is used for the production of wine, sparkling wines and cider, and it can also be used in industrial applications for bioethanol production [17]. The glucose can then be utilized by organisms of the genera Saccharomyces which can ferment the glucose into fuels such as ethanol [3].

The U.S. Department of Agriculture (USDA) has an active program devoted to the corn ethanol industry. The USA and Brazil are the world's largest producers of fuel ethanol, with outputs of 4.9 and 4.5 billion gallons, respectively, in 2006. US production reached 4.65 billion gallons by September 2007, while demand was 4.85 billion gallons. The demand for fuel ethanol in the USA more than doubled between 2000 and 2004 and has increased to 7 billion gallons in response to the Energy Act of 2005. In comparison, Brazil used about 4 billion gallons of ethanol in 2006 and produced sufficient quantities of ethanol from sugarcane to satisfy its own demand and export 0.43 billion gallons to the USA. In Brazil, the price of ethanol is tied to the price of gasoline. Ethanol production in the USA in 2006 and 2007 increased significantly as new plants started up and increased production by an estimated 2 billion gallons to levels now approaching 7 billion gallons [18].

Corn undergoes many preprocessing steps before it can be considered ready for fermentation for ethanol production. However, as a basic first step postharvest, corn is shelled to remove kernels from the cob, followed by separation from impurities, such as stones and sticks by screeners or scalpers before getting stored in silos. Subsequently, a commercially well-established large-scale biotechnological process is employed via three broad steps: (1) conversion of starchy feedstocks into fermentable sugars via three major sequential unit operations namely milling, liquefaction, and enzyme-based saccharification, followed by (2) fermentation, where yeast metabolically converts these sugars into ethanol, and ultimately (3) purification, where the ethanol, thus generated, is separated out from other byproducts and impurities by distillation before it gets stored or transported to market [19].

The most well-known and commercially significant yeasts that been primarily used for bioethanol production are the related species and strains of Saccharomyces cerevisiae. These organisms have long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages and in the backing industry. Pumpkin (Cucurbita maxima L.) is a very popular and one of the most important vegetable crops grown extensively throughout the tropical and subtropical countries like Bangladesh. Due to its high nutritional content and lucrative market price, pumpkin may be considered as a high value crop. Both immature and mature vegetables are used as a vital ingredient for several culinary preparations in Bangladesh. Pumpkins are rich in carbohydrate and minerals and cheaper source of vitamins, especially carotenoid pigments, which have a major role in nutrition in the form of pro-vitamin-A, antioxidants, when used at ripening stage. Thus, this vegetable can contribute to improve nutritional status of the people of Bangladesh and besides this it could be a good renewable source for bioethanol production. In Bangladesh, Pumpkins are very versatile in their uses for cooking and have an advantage over other vegetables as the fruit can be stored for up to 6 months before being consumed and hence can play an important role in maintaining nutritional levels during the long dry seasons. The fruit is typically orange or yellow and have many creases running from the stem to the bottom. They have a thick shell on the outside, with seeds and pulp on the inside. The main nutrients are lute in and both alpha and beta carotene, the latter of which generates vitamin A in the body.

The carrot (Daucus carota L.) is available grown vegetable in our country. Most inhabitants of the country are involved directly in agricultural activities for their livelihood. The sector dominates the economy 16.33% of the country's Gross Domestic Product (GDP). In earlier decades, the sector contributed more than 50% of GDP. Due to gradual transformation of the economy from agriculture to industry and service sectors, this sector has fallen from around 50% in the 1970 to 16.33% in recent year 2013-14 but still it is the single largest manpower engaged in sector. Vegetable wastes occur throughout the supply chain and vary widely depending on its processing and globally, more than 30 % of the loss occurs at the retail and consumer levels. A group researchers reported that wastes from vegetables industries including carrot, peas, and tomatoes are a rich source of several nutrients like vitamins, minerals, fibers, etc [20]. So, a detailed study of waste characteristics is essential for deciding its application and determination of economic feasibility of the process. The wastes from fruit and vegetable processing industries being rich in polysaccharides (cellulose, hemi-cellulose and lignin) can be subjected to solid state fermentation for the production of ethanol and butanol, which has several uses, such as a solvent in many industries and also as a liquid fuel supplement. Vegetable waste can be a potential substrate for bioethanol production due to its availability in abundance, high cellulose and starch content, and non-competitiveness with our food chain [21].

Rapid increase of population and exponential growth on industrialization load on fossil fuel resources and the resources are being depleted very fast. Therefore, it is required to discover alternative cheaper sources of fuel for fulfillment of worldwide demand. The main task is to develop easier techniques by using cheaper source for the production of bioethanol so that the common people can also produce it by themselves. At present, sugar and starch based raw materials and cereal grains are used for the production of bioethanol. In Bangladesh, population already reached about 160 million and thus food security is a national priority and hence Bangladesh cannot afford to use cereal grains for ethanol production as is commonly done in other biofuel promoting countries in Europe and USA. Waste is an inheritable consequence of the food industry [14]. So, the available sources are some selected agro-products and rotten fruit wastes which is an abundant and renewable source of energy-rich carbohydrates & sugars which can be efficiently converted by microbes into biofuels. Bangladesh is amongst rapidly expanding large economy and fulfillment the energy demand of growing population has become the most challenging sector for Bangladesh.

Bangladesh needs to generate more energy than the current output. Bangladesh has shown good economic growth, rapid urbanization and industrialization in recent years. As a result, the numbers of bus, truck, taxi and auto-rickshaw have been increasing day by day. These high numbers of automobiles are consuming a huge amount of fuel oil (diesel, petrol and octane) every day. Bangladesh imports most of the oil from Middle East by spending valuable foreign currencies, the amount reached at 453.15 BDT billion in May 2018 which was only 0.57 BDT billion in November 1976 (https://tradingeconomics.co). The huge spend on oil import has created tremendous pressure on Bangladesh's annual budget. However, Bangladesh can easily reduce its oil import with the production of renewable fuel like bioethanol from sugarcane, corn, potato and sweet potato. At the current level of production and consumption, it seems to be highly difficult target to meet. Thus, it is a good option for countries like Bangladesh to invest in to renewable energy sector.

Bangladesh is an agricultural country and produces many agricultural products every year. For huge production and the prevailing cold storage charge is too high, all farmers can not avail the facility of cold storages due to their financial insolvency. As a result, farmers are compelled to sell a major portion of their produce during harvest time relatively at lower prices. The price becomes very low during peak harvesting period while it becomes too high before planting period. The cost of the bioethanol production would be much lower if we use waste agricultural products that are left in the field due to physical damage during harvesting and the highly perishable rotten fruits and vegetables, which are low cost and easy available. Alternate use of these products like bioethanol production and develop industry in our country for bioethanol production from renewable sources, then it would ensure the real price at farmer's level and can reduce the import pressure on fuels. Not only is producing biofuel locally good for getting away from foreign fuels, it also helps the local population. Biofuel actually opens up the possibility of hundreds and thousands of new jobs. It also gives more money to farms who participate in the growing of biofuel crops. The main objective of this study was to determine the bioethanol production efficiency of corn, pumpkin and carrot of Bangladesh.

2.0. Materials and Methods

2.1. Raw Materials Collection

The agri-products (corn, pumpkin and carrot) were collected from the local market of Rajshahi city, Bangladesh.

2.2. Yeast Strain and Culture Media

The research was conducted in the IES Environmental Microbiology laboratory. Yeast strain (Saccharomyces cerevisiae CCD) was collected from the Spirit Section of Carew and Co., Darsana, Bangladesh. For yeast culture, modified YMPD (Yeast-Malt-Peptone- Dextrose) broth culture media was used. The YMPD media was prepared with yeast extract (3.0 g), malt extract (3.0 g), peptone (5.0 g) and dextrose (10.0 g). All of these ingredients were dissolved in 1000 ml of water and adjusted to pH 6.0.

2.3. Bioethanol Production of from different Agri-Products

About 100g of agri-products (corn, pumpkin and carrot) was boiled blended in 300 ml distilled water. About 1750 unit of α-amylase enzyme and 200 ml of 2-days old yeast were added to each treatment and adjusted to pH 6.0, and incubated at 35˚C for 6-days. After incubation period, turbidity of solution and produced ethanol were measured.

2.4. Enzymatic Hydrolysis

About 1750 unit α-amylase enzyme used for breaking down complex plant starches into smaller sugar molecules called dextrins and gluco-amylase enzyme responsible for converting dextrin to fermentable sugars for yeast metabolism. The corn kernel is first broken down into its individual components (i.e., germ, fiber, gluten, and starch) and then sent for processing (wet milling) [22]. In a typical dry-milling process, enzymatic hydrolysis of cornstarch to simple fermentable sugars is accomplished via two-steps: (1) liquefaction/cooking and (2) saccharification. Liquefaction is basically a high-temperature cooking step where with time the tightly bound grain starch becomes soft and spongy and becomes amenable to efficient enzymatic digestion. For example, a typical industrial scale liquefaction step would be cooking the ground corn mash either at 165°C for 3–5 min (very high temperature cooking), or 90–105°C for 1–3 h. Alpha-amylase resulting in production of dextrins, subsequently followed by a lower temperature incubation, which is typically at about 55–60°C or even as low as 32°C with an enzyme called glucoamylase to further convert the dextrins into glucose [23]. Adding α-amylases simplify starch processing and reduce chemical costs as well as improve ethanol production.

2.5. Bioethanol Production from Filtering and Non-Filtering Solution

About 100g samples of each were blended with 300 ml distilled water. Then one of them was filtered with a muslin cloth and the other was treated as non-filtered. About 1750 unit of α-amylase enzyme and 200 ml of yeast were added. All the stated experiments were conducted at pH 6.0 and 35°C. After 6-days of fermentation the crude fermented solution was first centrifuged at 12,000 rpm for five minutes to remove the unused starch and yeast cell. Then, the clear solution was taken into rotary evaporator for separation of ethanol at 78.5˚C.

2.6. Fermentation

Fermentation is the process that converts the simple sugars produced by the corn kernel (reactants), carrots, pumpkins and converts the sugar to fuel ethanol and carbon dioxide under anaerobic conditions (without oxygen).

2.7. Estimation of Total Sugar before and after Fermentation

Total sugar content of corn solution was determined by sugar measurement machine named "On Call Plus"(Model: SN 103L1254FDC, Germany). Sugar concentration before and after fermentation was measured.

2.8. Distillation Process

Distillation is a process used in chemistry to separate two liquid substances. The physical property used to separate the different compounds based on boiling point. Distillation was carried out using a distillation apparatus (Witeg, Germany). Heating of fermented materials was carried out at 78.5°C. The condensation product was collected in a flask and used for estimation of ethanol concentration.

2.9. Measurement of Purity of Produced Alcohol

The percent of purity of produced bioethanol from corn was measured by using an alcohol meter (Jiujingnongduji, China). This meter can measure the alcohol purity from 0-100%.

2.10. Data Analysis

Each treatment consists of three replications. All data were expressed as mean values ± SD (Standard Deviation).

3.0. Results and Discussion

3.1. Corn (Zea mays L.)

3.1.1. Bioethanol Production from Corn

Bioethanol production from corn of Bangladesh was assessed. It is found that the sugar content was reduced to 8.23 mmol/L from 5.33 mmol/L after fermentation and 63 ml of ethanol was produced from corn with purity of 13.33% (Figure 1 and Figure 2) as shown in Table 3.

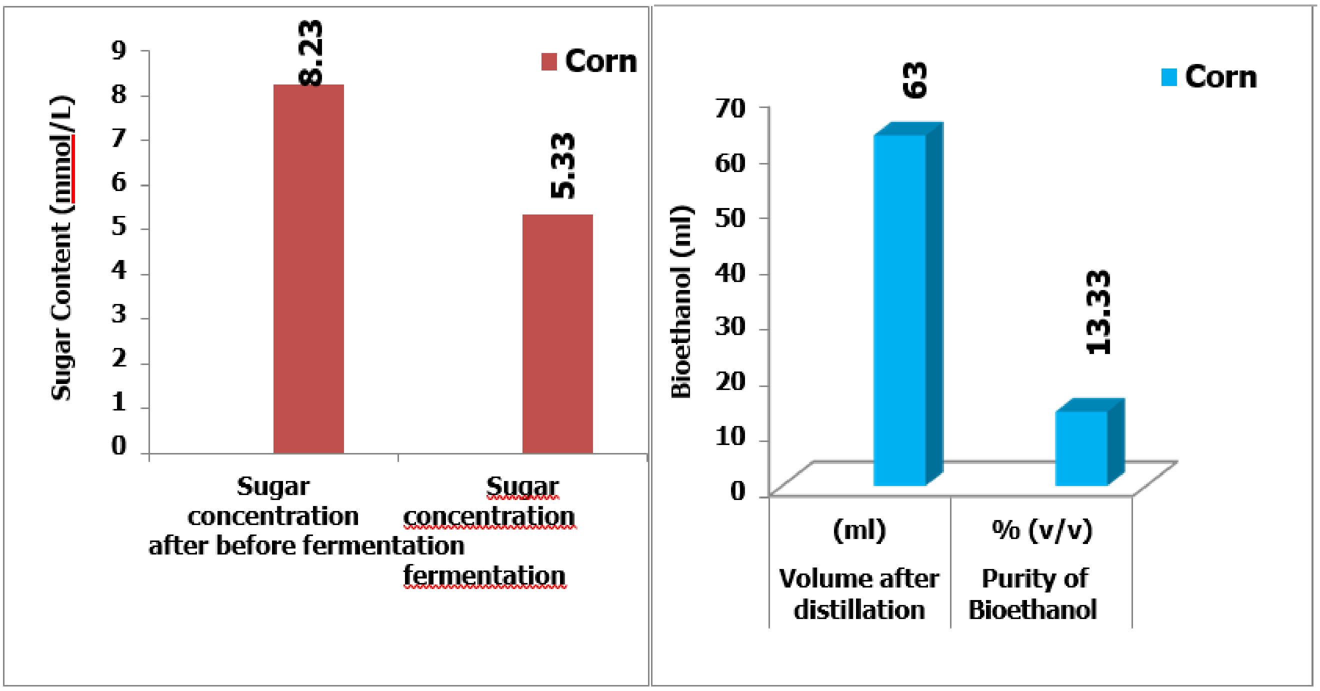

Figure 1: Bioethanol Productions from Corn

Similar work was done and found 6.01% (v/v) bioethanol through (24-120h) fermentation using yeast Saccharomyces cerevisiae [24]. The potential of corn to provide feedstock for alcohol production in excess of 15 billion gallons of ethanol per year in the USA will depend on utilization of cellulose and hemicellulose portions of the corn plant as reported [25]. In other study, researchers used 26 parameters, including corn stover com-positions, solid loadings, operational conditions, conversion efficiencies and material consumption and obtained 11-22% ethanol from the dry solid content of the corn stover and the largest amount of ethanol (19-22%) was produced by using alkaline, solvent or ammonia-based pretreatments technologies, while fungi-based pretreatment produced much less (11%) bioethanol [26]. It is estimated that, one bushel of corn approximately provides 2.8 gal of ethanol along with about 17.5 pounds of DDGs via dry milling process, or 13.5 pounds of gluten feed (with 20% protein), 2.6 pounds of gluten meal (60% protein) and 1.5 pounds of corn oil via wet-milling process (http://www.worldofcorn.com/#one-bushel-of-corn-can-provide).

Figure 2: Different steps of Bioethanol Production from Corn

Thus produced corn hydrolysate, then proceeds to the fermentation step where yeast Saccharomyces cerevisiae converts sugars into ethanol [27].

3.1.2. Bioethanol Production from Filtering and Non-Filtering Corn

In each 100 g substrate with 300 ml distilled water was blended and prepared 20% concentration. Then two experiments were done, one was filtered with a moslin cloth and the other was kept without filtered. In non-filtered solution the sugar concentration (8.23 mmol/L) and yield (63 ml) of bioethanol is comparatively high than the filtered solution (7.73 mmol/L and 53.66 ml) as shown in Figure 3.

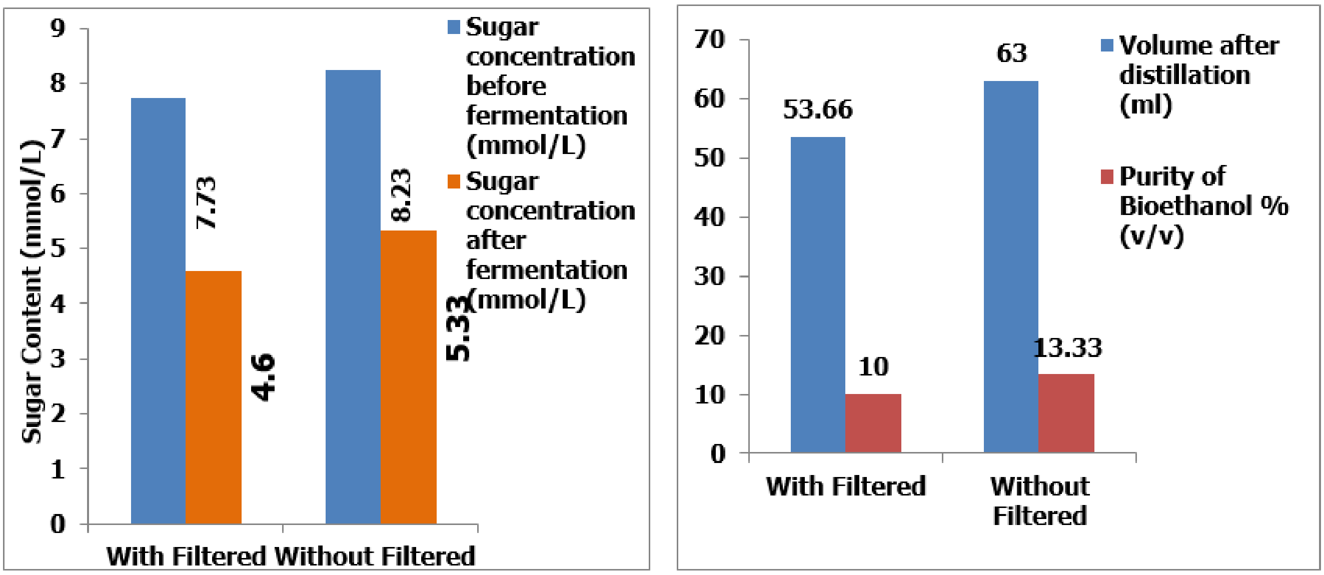

Figure 3: Bioethanol Production from Filtered and Non-Filtered Corn

Starch from starchy crops, such as cereals, to become fermentable needs a pre-treatment composed of three steps: gelatinization, to allow the starch to lose its crystallinity and become an amorphous gel; liquefaction, where starch is hydrolyzed to dextrins by an-amylase and viscosity is reduced; and scarification, where a gluco-amylase is added to convert dextrin's to glucose [28, 29]. Scarification can be managed to be simultaneous to fermentation: this makes the glucose gradually available to microorganism's and reduces contamination risks, process duration and costs. Ziska et al. has been reported that some industrial corn could produce ethanol yields of 2800–3800 L/ha compared to 4500–6500 L/ha for developed sweet potatoes breeding lines [30]. Mohanty and Swain stated that in the year 2016–17, the United States alone used about 28.9% of its total corn harvest, which is about 4.38 billion bushels of corn, to produce over 15 billion gallons of ethanol fuel [31].

3.2. Pumpkin (Cucurbita maxima L.)

3.2.1. Bioethanol Production from Different Varieties of Pumpkin

Two types of pumpkin varieties were collected from local market which is available in our country. These two types (black and red color) pumpkins were tested to find out the effect of varieties on bioethanol production. The yield was comparatively high (53 ml) in red pumpkin than the black pumpkin, 40 ml.

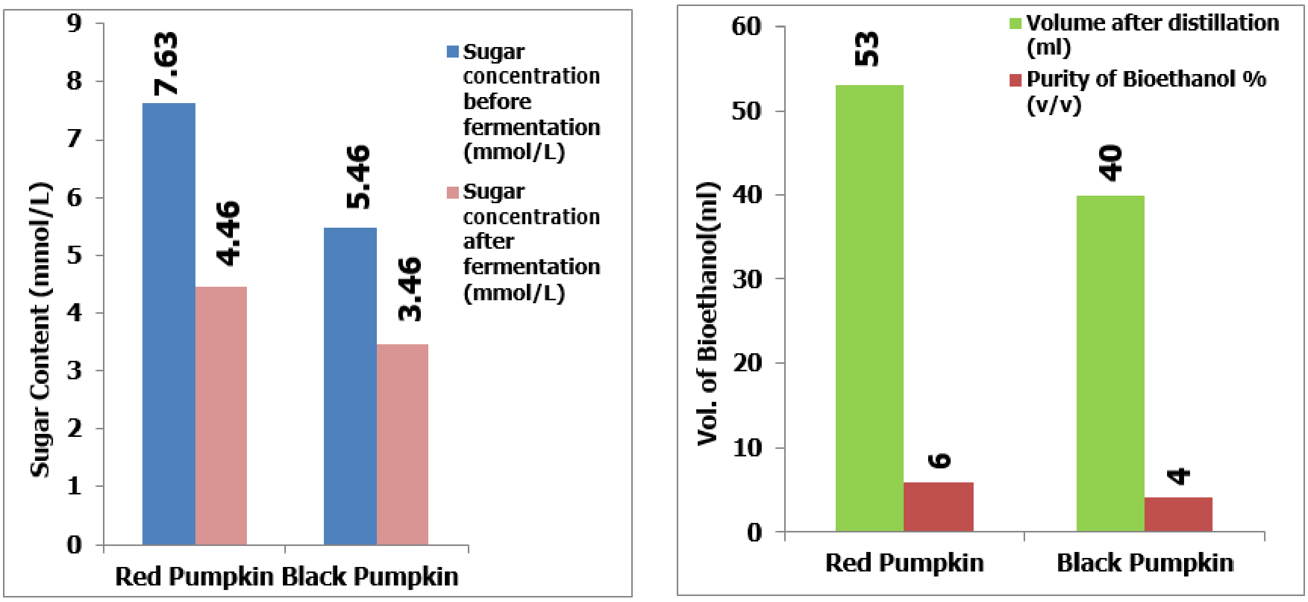

Figure 4: Bioethanol Productions from Two Different Varieties of Pumpkin

Figure 4 shows that the sugar content 7.63 mmol/L in red pumpkin is comparatively higher than the black pumpkin 5.46 mmol/L and the purity of produced bioethanol is also high 6% v/v in red color than the black pumpkin 4% v/v (Figure 5). Agricultural wastes are rich source in fermentable sugars that can be good substrate for ethanol production, a potential energy source for crude oil [32]. The fermentation duration must also be chosen to obtain an adequate microbial growth and ethanol yield, taking into account that the shorter the duration is, the lower the costs are. To remove the remaining water, special processes are required to reach anhydrous ethanol, that include: chemical dehydration process, dehydration by vacuum distillation process, azeotropic distillation process, extractive distillation processes, membrane processes, adsorption processes, and diffusion distillation process. The evaluation of the energy balance of bioethanol production reveals that most of the energy is required for the distillation, also because of the low concentration of ethanol in the fermented broth [33]. This observation is consistent with the report of Akin-Osanaiye et al., which indicated that the amount of yeast influenced ethanol production in agro wastes [1]. The current observations are in good agreement with similar results reported by Pramanik and Rao for grape waste [34].

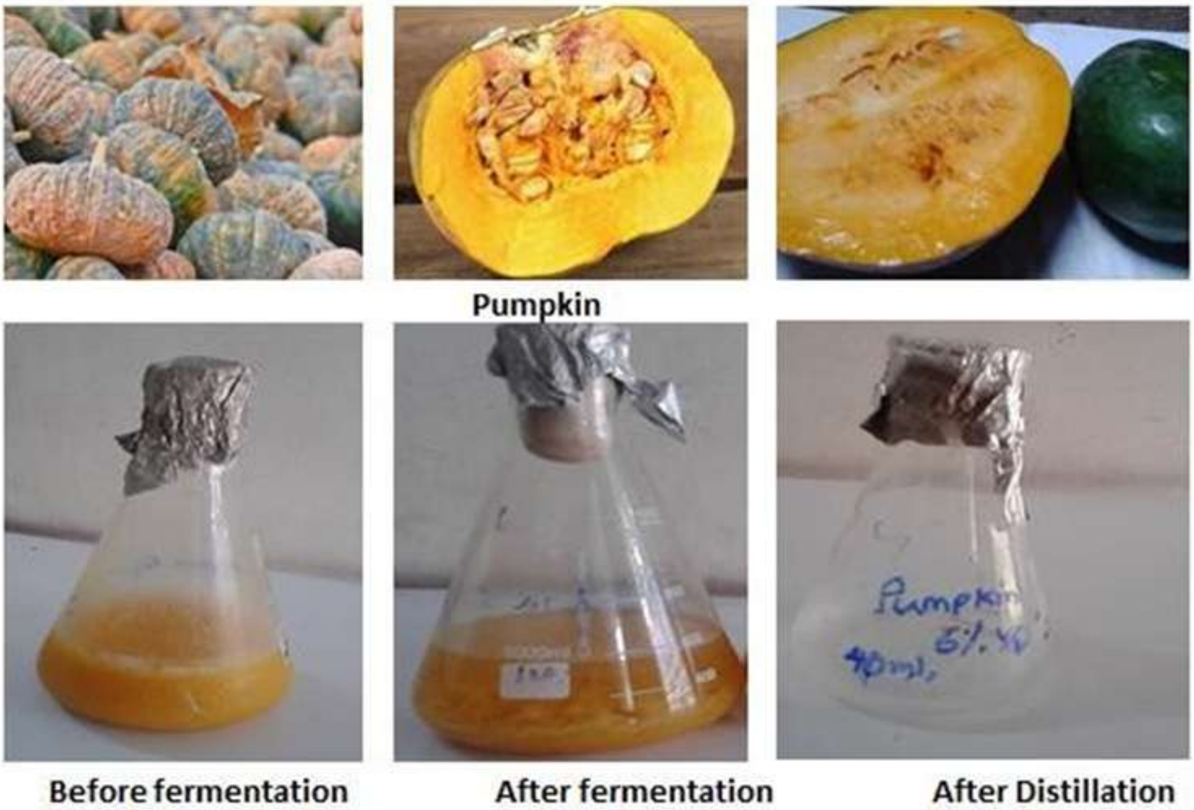

Figure 5: Different Steps of Bioethanol Production from Pumpkin

3.3. Carrot (Daucus carota L.)

3.3.1. Bioethanol Production from Carrot

Bioethanol production from carrot of Bangladesh was assessed. It is shown in Figure 6 that the sugar content was reduced to 4.06 mmol/L from 7.96 mmol/L after fermentation. After 6-days of fermentation 73.67 ml of ethanol was produced from carrot (Figure 7).

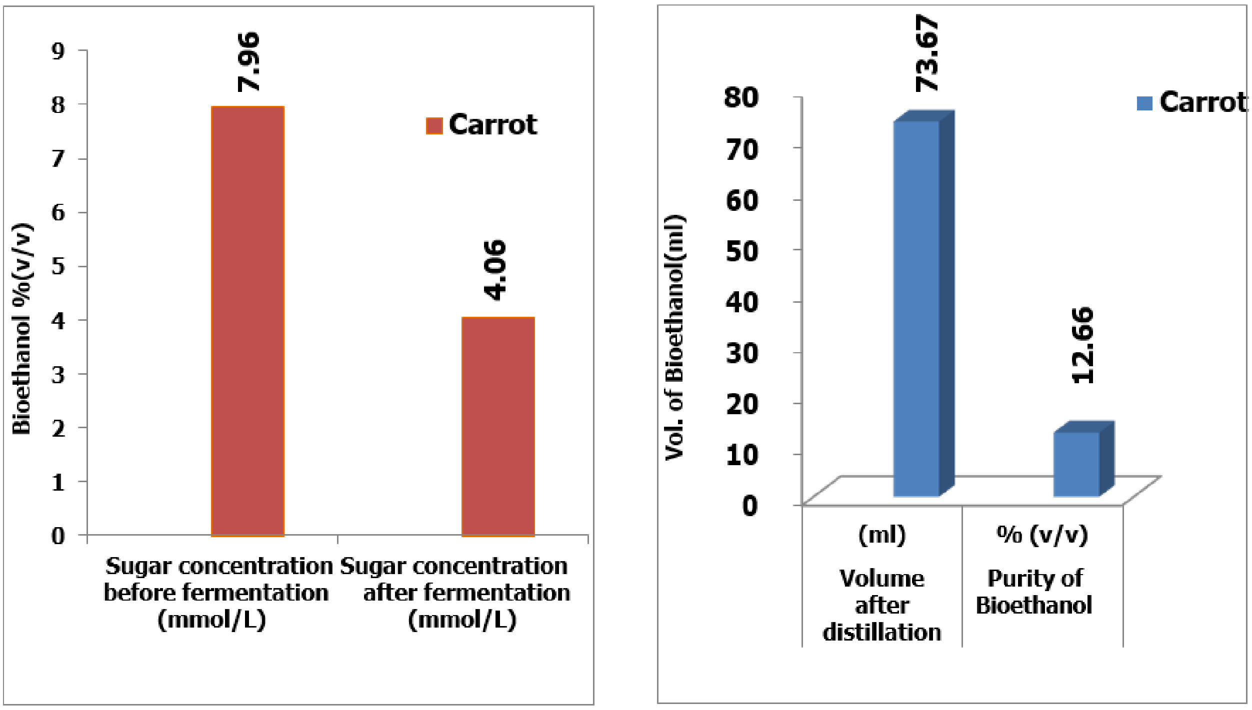

Figure 6: Bioethanol Productions from Carrot

There are no direct land-use costs with the corn cultivation. So, corn can be considered as one of the most potent feedstock sources in the bioethanol production industry. Agricultural practices for the corn are quite well established with its wide range of advantages over other energy crops and its high starch content provides suitable substrates and makes the process easier to produce ethanol.

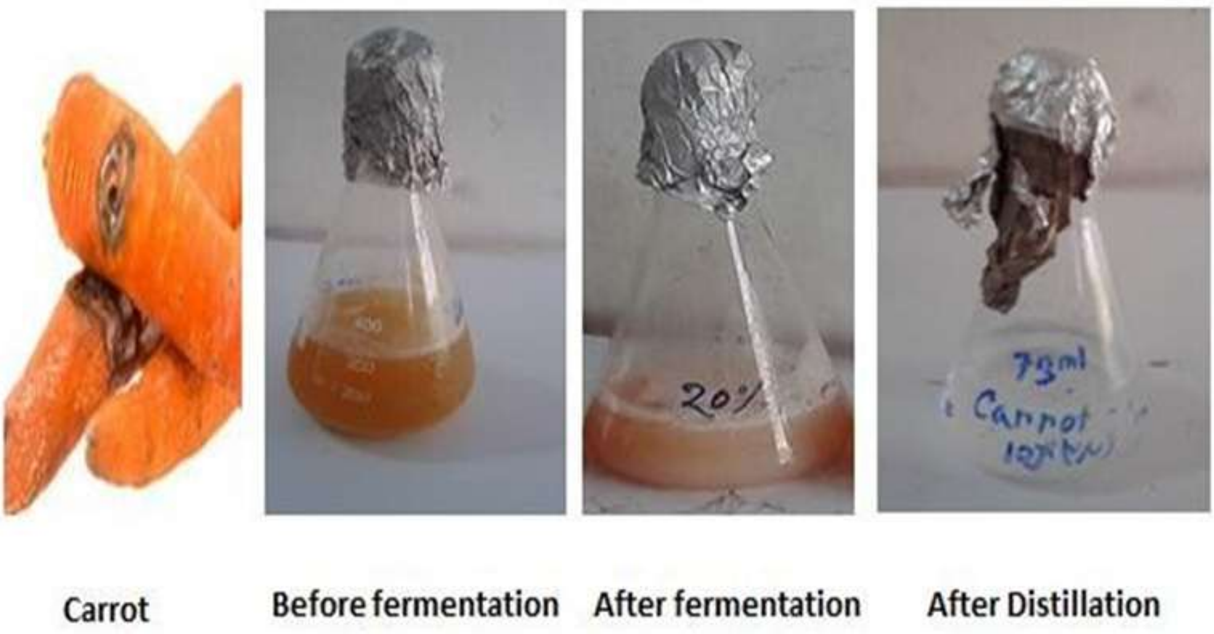

Figure 7: Different Steps of Bioethanol Production from Carrot

The fermentation of carrot using S. cerevisiae (distillery strain) under optimized conditions i.e. pH 6, sugar concentration 20% and temperature 35ºC revealed an increase in ethanol production with purity 12.66 %v/v (Figure 6). With the increase in sugar concentration, the ethanol production increased significantly. Because the sugar concentration was decreased after fermentation as most of the sugar converted into ethanol after fermentation.

3.3.2. Bioethanol Production from Filtered and Non-Filtered Carrot solution

In 100 g sample with 300 ml distilled water was added and blended and prepared 20% concentration. Then two experiments were done, one was filtered with a moslin cloth and the other was kept without filtered. The Figure 8 shows that in non-filtered solution the sugar concentration (7.93 mmol/L) and purity 12.33% v/v of bioethanol is comparatively higher than the filtered solution (6.96 mmol/L and10.66 % v/v purity).

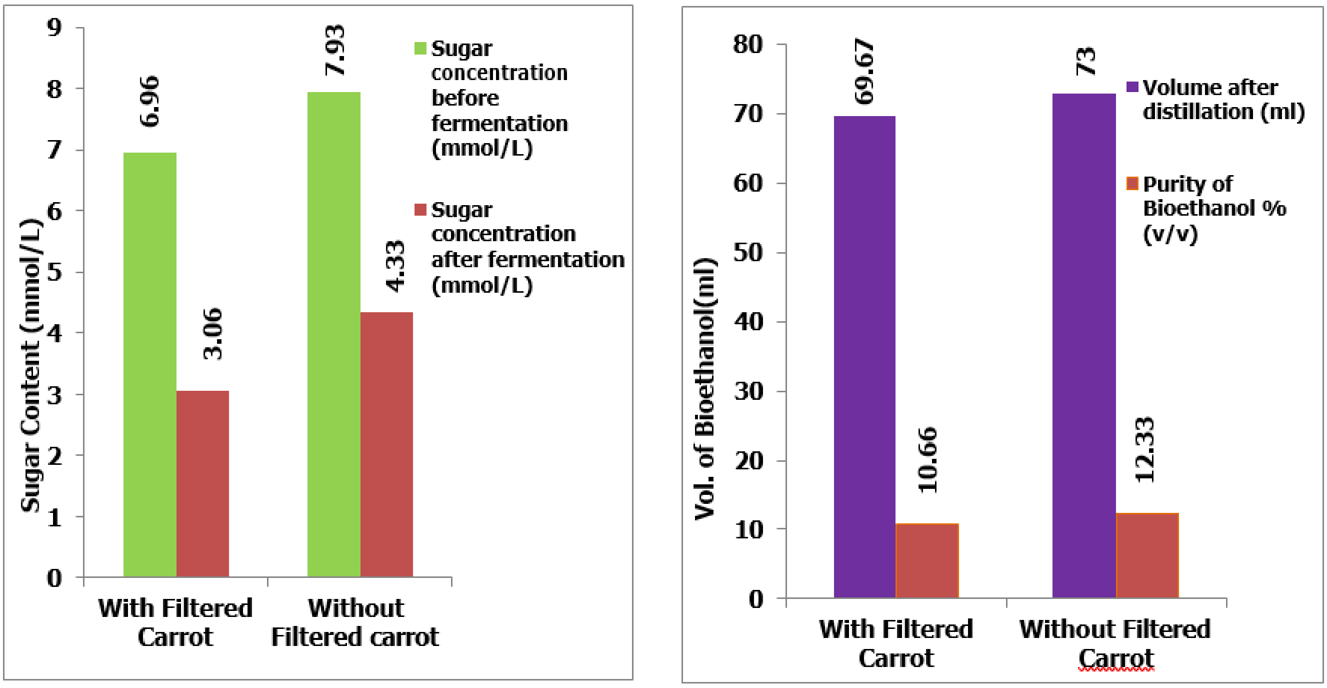

Figure 8: Bioethanol Production from Filtered and Non-filtered Carrot

This result is similar to other researchers, the world over have used such approaches for increasing bioethanol generation. Aimaretti et al. used evalorization of discarded carrots as substrate for the production of second-generation ethanol is proposed [35]. In order to increase the fermentable sugar concentration of the musts two strategies were studied: Strategy 1 consisted in the enzymatic hydrolysis of bagasse must and Strategy 2 by which carrots were milled, dropped into distilled water and hydrolyzed with different enzymes prior to compressing and filtering to obtain carrot must. By applying Strategy 2 using 0.05 % (v/v) of the enzyme Optimase CX255 at 70 °C and pH 5.5 during 2.5 h, the fermentable sugars extracted increased 3.5 times. In this way, the production of 77.5 L of ethanol for each ton of discarded carrots was achieved.

Clementz et al., reported that non-enriched, non-sterile carrot under pH of 5.5 was fermented through immobilized yeasts and found values of ethanol concentration (29.9 g/L), Y p/s (0.409 g/g), and productivity (7.45 g/L/h) [36]. In agreement with these results, Khandaker et al. found the bioethanol yield at pineapple waste showed the highest with 5.371% followed by insignificant between tomato, 5.067% and orange 4.452%, cabbage 4.033% and the lowest is potato 0.929% [37]. Corn produced 63.00 ml of bioethanol with 13.33 % v/v purity. Red pumpkin produced 53 ml of ethanol with purity 6 % v/v and black color pumpkin produced 40 ml of yield with a low purity 4 % v/v. Carrot produced 73.67 ml of ethanol with 12.66 % v/v purity. Ethanol production from spoiled starch rich vegetables by sequential batch fermentation was studied using sweet potato as raw material for the production of ethanol and the production was only 0.15% [38,39]. This vast variation may due to the larger amounts of fermentable sugars are present in agri-products. The maximum yield of ethanol 5.2% was obtained from the red potato variety in Nepal [40].

The present study obtained 13.33% ethanol from corn which is higher than the study which reported 10% alcohol from the fermented mash of corn [7]. They observed that that a plant produces 7300 liter of bioethanol from 160 ton of corn grain; therefore, bioethanol yield is equal to 0.46 liter/kg of corn grain. Removal of water from ethanol is the main challenge for bioethanol production from corn and other agricultural products. Some researchers described a method for making anhydrous ethanol from 10% raw bioethanol of corn [7]. For removal of water, fermented mash is pumped through a multi-column distillation system where the alcohol is removed from the solids and water. The alcohol leaves the top of the final column at about 96% strength and residue mash, called stillage, is transferred from the base of the column to the co-product processing area, where is centrifuged and evaporated to obtain animal feed and condensate, that is recycled in the steeping phase. The alcohol again passes through a dehydration system, with zeolite molecular sieves, where the remaining water is removed. The alcohol at this stage is called anhydrous (pure, without water) ethanol and is about 99.3% strength. Finally, ethanol used for fuel is denatured with a small amount (5%) of gasoline to make it unfit for human consumption. However, due to lack of facility, the present study could not produce the anhydrous ethanol from studied agricultural products. However, fermentation efficiency decreases after the optimum fermentation time. This might be due to substrate limitations or product inhibition. S. cerevisiae reportedly showed decrease of growth with increase in ethanol concentration in the medium. Thus, production of bioethanol by utilizing rotten vegetable is a clean method for converting waste to energy and addressing the issues of sustainability, environmental emissions and energy security.

4.0. Conclusion

From this study we conclude that corn, pumpkin and carrots are an attractive and cheaper sources for bioethanol production. These sources can be used for small- and large-scale production of bioethanol because they are available everywhere in Bangladesh. The production of ethanol from the corn, pumpkin and carrots can be improved further by using genetically engineered yeast strains that are capable of converting multiple sugars into ethanol, and using of high-grade distillation process is required for the preparation of anhydrous bioethanol to be used as fuel in vehicle.

5.0. References

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