IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 10, Issue 5 Series 1 (Sept. – Oct. 2024), PP 01-08
www.iosrjournals.org

A Comparative Study Of Bioethanol Precursors And Production Methods

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Abstract
The aim of this study was to investigate the bioethanol yield per unit mass of feedstock for the three generations of bioethanol feedstock. The first-generation feedstock has readily available sugars for fermentation whereas the second and third-generation feedstocks are composed of cellulosic material that needs to be broken down into simple sugars before fermentation into ethanol. The hypothesis was that the first-generation feedstock would yield a greater yield for this very reason. The feedstocks used were beetroot juice, sugarcane bagasse, and Callicostella Prabaktiana moss as the first, second, and third-generation feedstocks respectively. The beetroot juice was treated with lime and filtered to remove impurities; the sugarcane bagasse and moss were dried at 57°C and then treated with 1% H_2SO_4, filtered, and centrifuged. The fermentation was carried out for 72 hours in the dark at 30°C, at an agitation rate of 200 RPM, with a pH of 4 and a cell concentration of 10^8 cells/mL. The solution was then distilled to extract the ethanol and analysed using gas chromatography-mass spectrometry. The beetroot juice yielded an ethanol concentration of 494 ppm, the bagasse a concentration of 328 ppm, and finally the moss a concentration of 148 ppm; thus supporting the hypothesis.

Keywords: *bioethanol, generation, feedstock, beetroot, sugarcane bagasse, dilute-acid pretreatment*

Date of Submission: 01-09-2024 Date of Acceptance: 09-09-2024

I. Introduction

Bioethanol is a type of biofuel, which are renewable fuels derived from biomass. Some of the other types of biofuels are biodiesel, biogas, and biobutanol (Cavelius et al., 2023). Fossil fuels account for over 80% of the global energy demand (Johnsson et al., 2018) which is ever increasing, causing a rise in oil prices as well as greenhouse gas emissions, with CO_2 reaching a record high of 37.4 billion tonnes in 2023 (International Energy Agency, 2023). Furthermore, As the Earth's reserves of fossil fuels are finite, the usage of alternative and renewable fuels such as solar, wind, hydrogen, geothermal, hydrogen etc. is imperative. These can potentially satisfy energy demands across multiple sectors but cannot satisfy the fuel demands of transportation, heating, and industrial processes.

Bioethanol is carbon neutral and is produced by fermenting abundant biomass through the use of bacteria or yeast, wherein simple sugars are converted to ethanol and CO_2 (Nilsson & Holmgren, 2017). It is blended with gasoline to varying degrees, from as little as 10 percent in the case of E10 to as much as 83 percent in the case of E85 (U.S. Department of Energy). As ethanol contains 35% oxygen, this blending with gasoline reduces the toxicity of emissions by facilitating the complete combustion of fuel, reducing particulate emissions by 20%, NO_x emissions by 10%, and SO_x emissions by 80% when using E85.

Multiple types of feedstock are used in the production of bioethanol, which are as follows:

1. First-generation bioethanol, which is derived from sugar rich crops such as sugarcane, or starchy crops such as corn or wheat (Mohanty & Swain, 2018).
2. Second-generation bioethanol which is derived from lignocellulosic feedstock such as rice paddy, wheat straw, or sugarcane bagasse (Nilsson & Holmgren, 2017), with wheat straw contributing approximately 354 million tons to global biomass.
3. Third-generation bioethanol which is derived from algal feedstock such as red, green, and brown seaweed (Ramachandra & Hebbale, 2019).

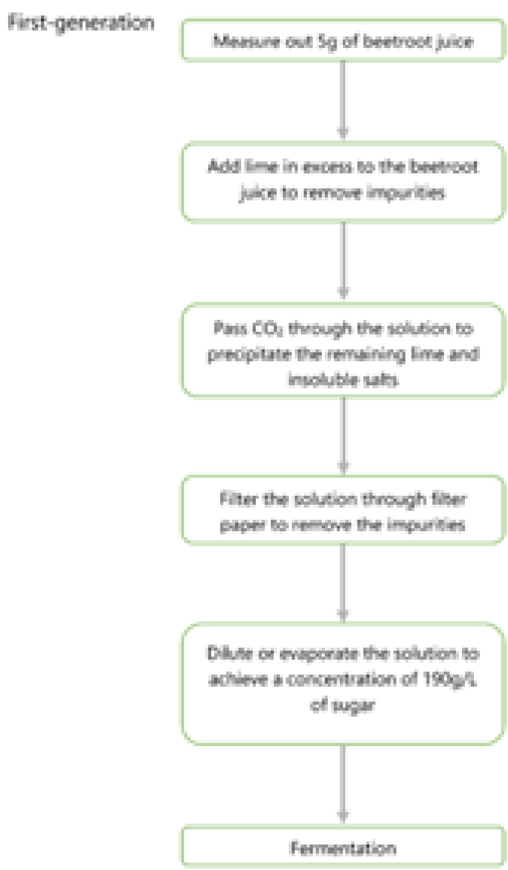
First-generation feedstock such as sugarcane, sugar beets, wheat, corn, molasses etc. has readily available sugars or starches which require minimal processing and is significantly more cost effective to produce as compared to the later generations. It requires only enzymatic hydrolysis and saccharification which is a cheaper process than those required for the later generation alternatives, especially when paired with the fact that the energy demand can also lower, such as in the case of sugarcane, wherein the bagasse produced are used to produce energy for the production of the bioethanol (Senatore et al., 2019). However, first-generation bioethanol raises the question of "food vs fuel", as considerable amounts of land are needed for the production of feedstock, which could instead be used for food. Additionally, the estimated 130,000 to 210,000 hectares of deforestation for palm oil production has had a significant effect on biodiversity and soil quality (Cavelius et al., 2023).

Second-generation feedstock such as rice paddy, sugarcane bagasse, wheat straw, corn stalks, sorghum straw etc. does not have readily available sugars and instead consists of cellulose, hemicellulose, and lignin. It possesses the advantage of not requiring large amounts of land to produce as it is considered waste material that would be discarded or potentially even burnt.

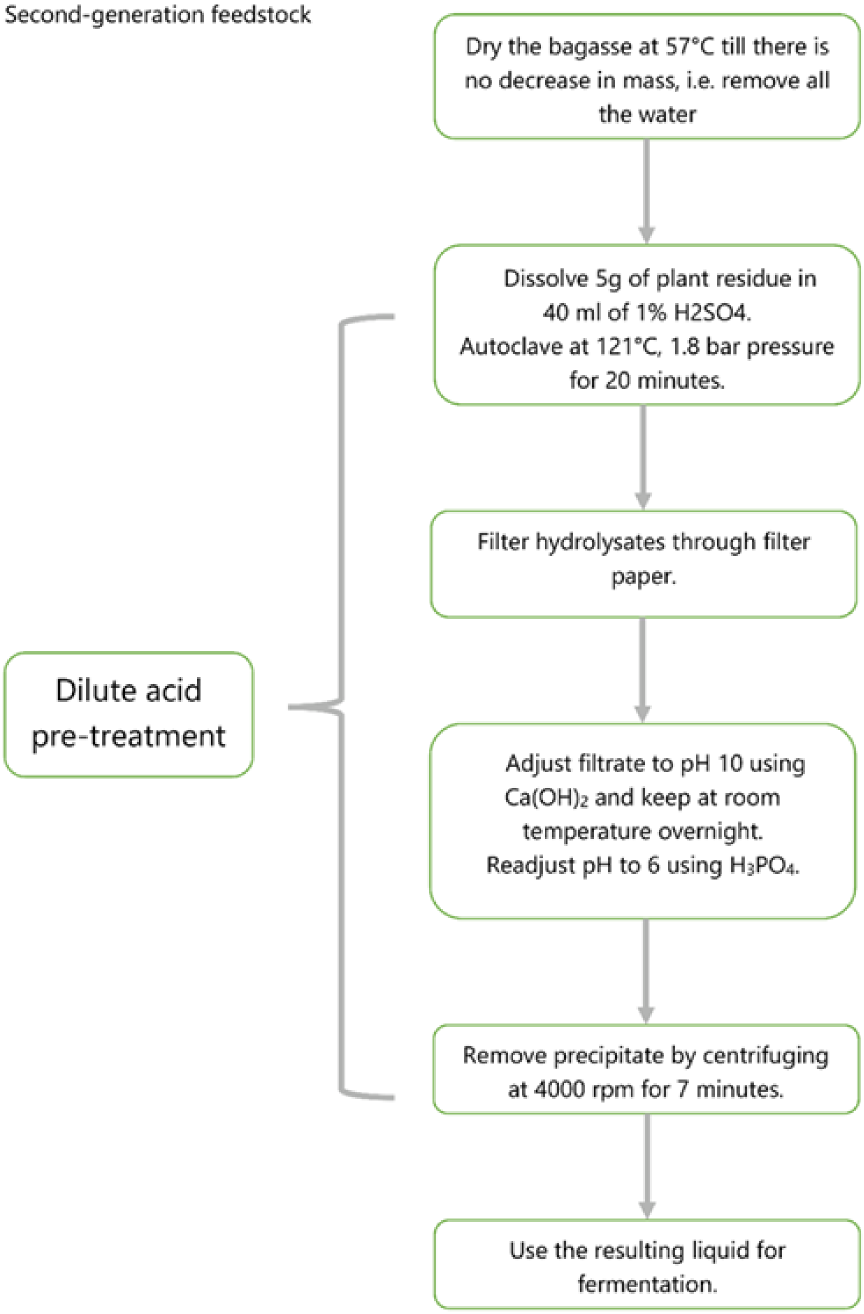
Third-generation feedstock is derived from macro/microalgae and cyanobacteria. The cultivation of macroalgae in Korea has resulted in a CO_2 uptake of ~10 tonnes per hectare when populated with the brown alga *Ecklonia* (Chung et al., 2010). They also consist of cellulose, hemicellulose, and lignin. A significant advantage of third generation is that the feedstock can grow all throughout the year, ensuring a constant supply of different types of algae for the various different types of biofuel production, which is not necessarily limited to bioethanol.

The second and third generations differ from the first as they do not have readily available sugars which results in a cost-intensive delignification process which causes difficulty in scaling the process (Ramachandra & Hebbale, 2019). Different pretreatment methods are employed to break the cellulose into simple sugars for fermentation, such as steam explosion pretreatment, dilute acid pretreatment, biological pretreatment etc. with the most popular being dilute acid pretreatment.

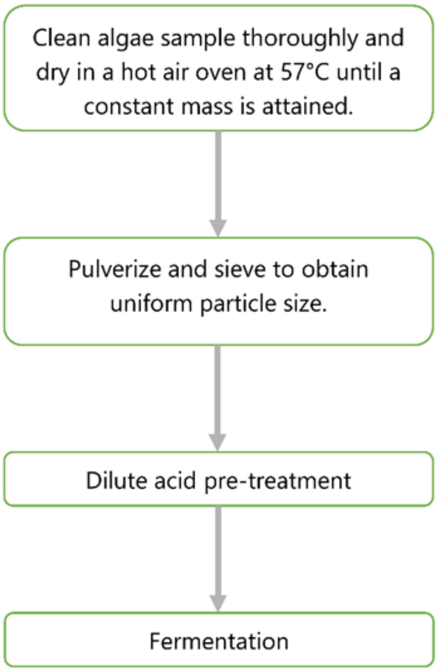
II. Methodology



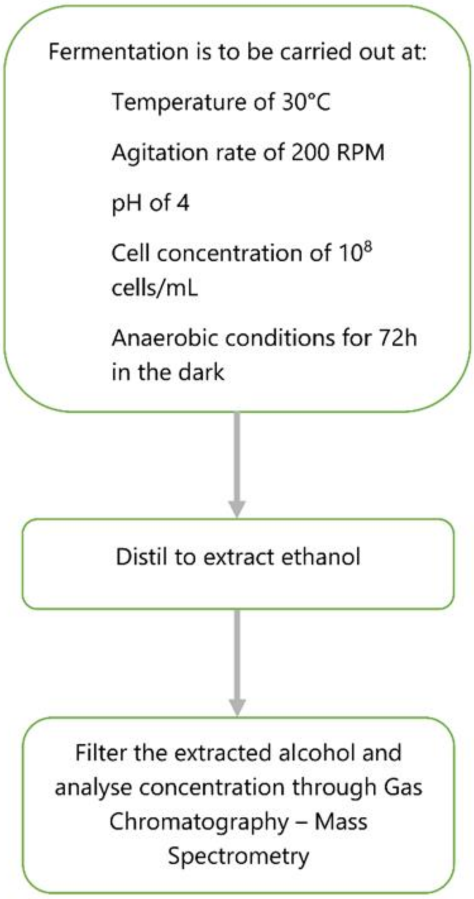
Second-generation feedstock



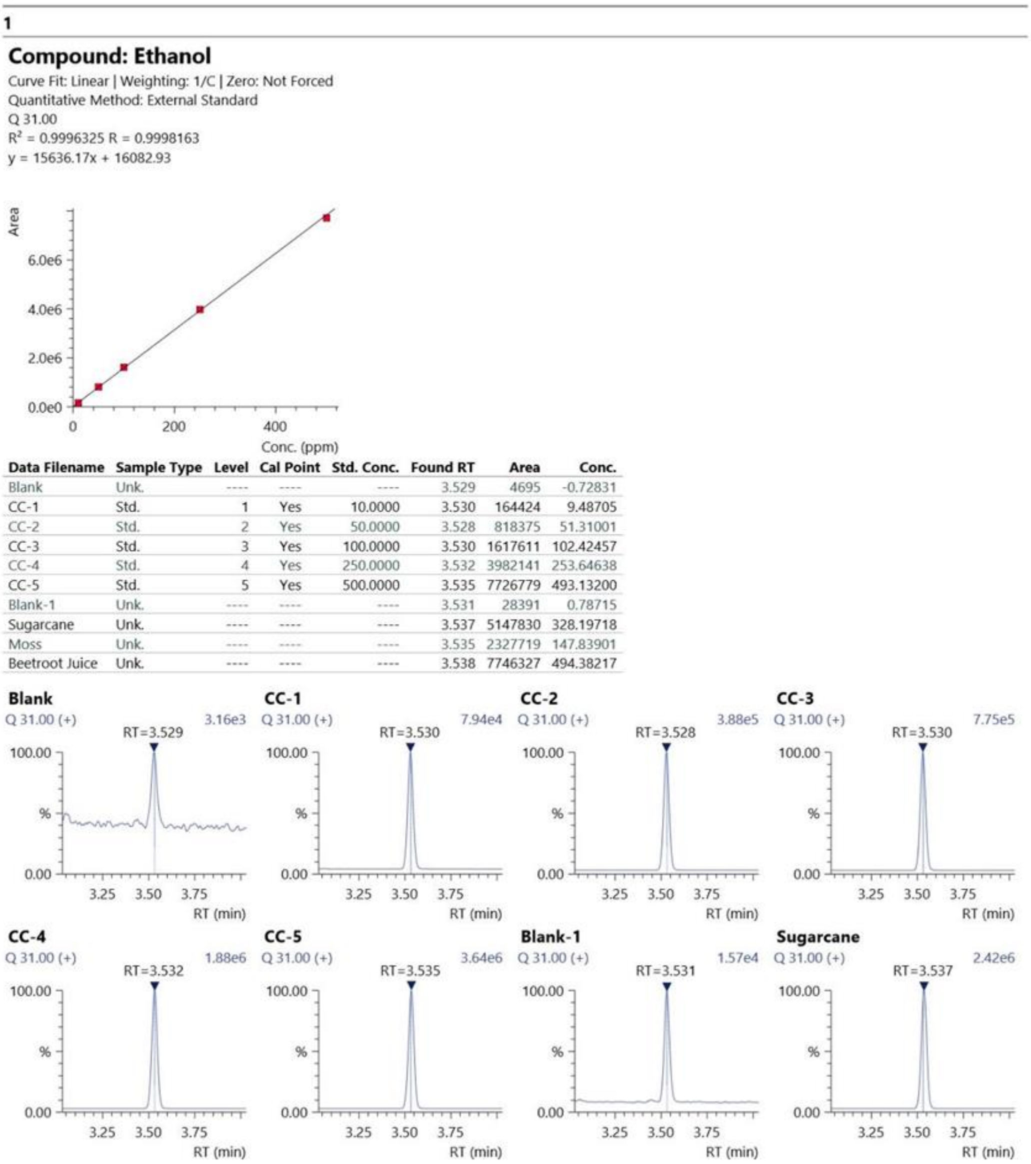
Third-generation feedstock



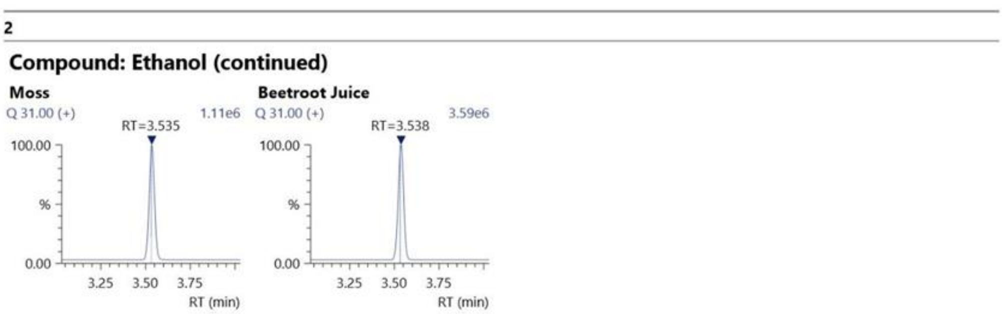
Fermentation and distillation



III. Results



Data Filename	Sample Type	Level	Cal Point	Std. Conc.	Found RT	Area	Conc.
Blank	Unk.	----	----	----	3.529	4695	-0.72831
CC-1	Std.	1	Yes	10.0000	3.530	164424	9.48705
CC-2	Std.	2	Yes	50.0000	3.528	818375	51.31001
CC-3	Std.	3	Yes	100.0000	3.530	1617611	102.42457
CC-4	Std.	4	Yes	250.0000	3.532	3982141	253.64638
CC-5	Std.	5	Yes	500.0000	3.535	7726779	493.13200
Blank-1	Unk.	----	----	----	3.531	28391	0.78715
Sugarcane	Unk.	----	----	----	3.537	5147830	328.19718
Moss	Unk.	----	----	----	3.535	2327719	147.83901
Beetroot Juice	Unk.	----	----	----	3.538	7746327	494.38217



IV. Discussion

The aim of this study was to compare the variations in yield and efficiency of bioethanol feedstock. The results show us that the beetroot juice produced the greatest yield of ethanol per unit mass, followed by sugarcane bagasse and finally *Callicostella Prabaktiana* moss, which supports the hypothesis that the first-generation feedstock would produce the greatest amount of ethanol due to the amount of readily available sugars for fermentation.

The sugar content in the beetroot juice was 4.8% and was negligible in the bagasse and moss when calculated through the method of titration. The moss yielded an ethanol content of 148 ppm, the sugarcane 328 ppm, and the beetroot 494 ppm. As the compound analysed was ethanol, the retention time is roughly the same with the area determining the concentration of ethanol.

The findings of this study are consistent with some of the past literature such as the bioethanol yield for sugar beets and algal biomass which are at a conservative estimate of 70 and 32 L/t respectively (Ramachandra & Hebbale, 2019), or the ethanol concentration produced by sugar beet juice and sugarcane bagasse which are 85 g/L and 25 g/L respectively (Zabed et al., 2017). Likewise, the yield has also been stated to be 0.50 and 0.28 litres of ethanol per kilogram of dry biomass for sugarcane juice and bagasse respectively (Kim & Dale, 2003). However some research contradicts these figures stating production figures of 110 L/ton for sugar beets, 318-500 L/ton for sugarcane bagasse, and 72-608 L/ton for green macroalgae (Tse et al., 2021) or 110 L/ton for sugar beet and 280 L/ton for sugarcane bagasse (Balat & Öz, 2008).

These results have implications for the later generations of bioethanol, displaying that their relative efficiency is lower, which further reduces their cost-efficacy (Alonso et al., 2010) when paired with the difficulty of converting the cellulosic matter into fermentable sugars. Further disincentivizing the production of first-generation bioethanol is of paramount importance considering the ecological impact that it can have, along with immense carbon uptake potential of algae (Parikh, 2021) which can be on the order of 8-10 tonnes per hectare (Kraan, 2010). Moreover, the production of first-generation ethanol can compete with food production which can raise the question of food vs fuel (Wang et al., 2016) (Yun et al., 2015).

This paper provides information on the sugar and ethanol content of the feedstock. However, this is limited in its description of the production of bioethanol as there are more factors at play when comparing the generations for industrial production. These include the energy requirement and carbon footprint per unit ethanol, the cost per unit ethanol, the waste products produced etc.

Future research can account for these factors along with testing multiple feedstocks across each generation. Additionally, other factors can also be tested to provide a comprehensive overview of the different permutations of bioethanol production such as different species of fungi, fermentation times, agitation rates, pretreatment methods etc., due to the inherent variations in feedstock.

To conclude, this study displays that first-generation feedstocks are more efficient for bioethanol production given the presence of readily available sugars which results in a greater ethanol yield and lower cost per unit mass as compared to cellulosic feedstocks such as bagasse and algae.

V. Conclusion

The increased usage of fossil fuels has led to their depletion and increased carbon dioxide emissions. Bioethanol is a renewable and environmentally friendly alternative to fossil fuels. The feedstocks for bioethanol are broadly categorised into three generations. First-generation (1G) feedstock such as sugarcane juice, molasses, or beetroot juice have readily available sugars and require little treatment before fermentation. Second-generation (2G) feedstock such as paddy straw, sugarcane bagasse, or switchgrass are also called lignocellulosic biomass and contain cellulose and hemicellulose that must be broken down into simple sugars to be fermented. Finally, third-generation (3G) feedstock such as macro- and microalgae contain starch or cellulose in their cell walls that must also be broken down into simple sugars to produce ethanol through fermentation. The beetroot juice was treated with lime and filtered to remove impurities; the sugarcane bagasse and *Callicostella Prabaktiana* moss were dried and treated with dilute acid to break apart the cellulose into simple sugars. The three solutions were then fermented, distilled, and analysed through GC-MS. The beetroot juice yielded the greatest concentration of bioethanol, followed by the sugarcane bagasse, and finally the moss, in line with the hypothesis.

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