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Factors affecting organic yield, reaction mechanisms and elemental toxicity (ICP-MS) of bioethanol derivative from dates (fard/khalas cultivars) and century plant (Agave americana)

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***Abstract*— In the production of bioethanol, certain factors influence the formation of undesirable products such as acetaldehyde and lactic acid. This particular area is relatively unexplored. In addition, comparative toxic and trace elemental characterisation of bioethanol from dates (fard/khalas cultivars) and Agave americana (century plant) is uncharted territory in biofuels research. Bioethanol as a fuel additive holds considerable promise, but assay of metal impurities is relatively underexplored. Following standard production protocol our group has successfully derived high-purity bioethanol from dates and century plant on a laboratory scale, and examined the elemental profiles of the biofuel fraction. Moreover, a mechanistic approach was proposed in order to improve the bioethanol production yield as well as to reduce the acetaldehyde, one of the by-products produced during the process. Bioethanol aliquots were diluted in aqueous acid media and subjected to numerical analysis by ICP-MS (Inductively Coupled Plasma Mass Spectrometry). The levels of toxicity of hazardous metals (ppb and sub-ppb range) were compared for the samples of interest. The results showed that concentrations of heavy elements such as Cr, Cu, Zn, Se, Sb, Cd, Ag, Hg, Pb and Bi were comparatively pronounced in the bioethanol fraction of the century plant. It was surmised that these impurities originated from the plant itself, and the potential of phyto-remediation, related to the biomass source, is discussed. The impact of our work on sustainable development is presented.**

***Index Terms*— Bioethanol, century plant, dates, ICP-MS, phyto-remediation, trace/toxic metals.**

I. INTRODUCTION

The basis of this study was to examine bioethanol production and purity from desert plants. As a result we closely explored two key stages in the mechanism of bioethanol formation from glucose. This was accomplished by reviewing the factors that could affect the decarboxylation of pyruvate as well as the reduction of acetaldehyde- the last step in the process. Moreover, we thoroughly investigated the levels of trace and toxic metals in bioethanol fractions derived from dates (khalas/fard) and century plant (Agave Americana). It is well known that bioethanol production has been at the centre of disagreeable land acquisition issues linked to food production. Our group has, therefore, focused largely on biofuel production from non-edible or waste biomass. For example, the plant, Agave americana, is robust and able to survive the harshest arid conditions. It is basically non-edible, and can be cultivated on uninhabitable land and desert, devoid of water and arable soil, and is therefore, suitable for bioethanol production without creating undue competition with food output. On the other hand, dates are edible, but appreciable quantities of dates are damaged, thus bioethanol production from surplus and damaged dates is fast becoming a feasible proposition.

Toxic and heavy metals in biofuel create unwanted problems, and there is, therefore, a growing need for regular assay of metal impurities in bioethanol[1-16]. Abnormal levels of heavy metals could constitute a potential environmental hazard [17-23],and could also damage fuel lines as well as engines [24-28]. Most current methods of analysis [27-30] do not attain detection limits in the sub-µg/L range (sub-ppb). The ICP-MS instrument used in this study is a sophisticated facility built with a quadrupole mass discrimination system that is highly sensitive and can achieve detection limits in the ng/L region. The facility is linear over a wide concentration range and has the unique capacity of superior elemental mass selection for most elements on the periodic table.

The primary objective of this research was to deploy the capabilities of high-performance ICP-MS to examine and compare toxic and trace elemental profiles in bioethanol derived from century plant and dates (fard/khalas cultivars). Research of this nature has not been widely explored and from this perspective represents a contribution to biofuels studies.

II. MATERIALS AND METHODS

A. Sugar extraction/fermentation/distillation

In all samples (date cultivars/century plant) sugar was extracted thermally. Samples were mixed with water in a mass ratio of 1/5 and then placed in an oven for 2 hours at 80 ^{o}C. Centrifugation isolated the solid phase from the extracted date juice. The Brix scale was used to optimize pre-fermentation conditions. Saccharomyces cerevisiae yeast was employed in the fermentation process and was activated prior to use. Activation was achieved by dissolving 5% yeast in 0.5% glucose solution followed by steady stirring for 30 minutes at

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around 35 °C. The activated yeast solution was subsequently added to the date juice samples and fermentation took place at 36 °C for 3 days with constant stirring. After fermentation, samples were centrifuged and subjected to fractional distillation to extract the pure bioethanol. The purity of the bioethanol was established by GC-MS.

Table 1: Repeatability study using a multielemental aqueous standard (Fluka 70007) [29]

Measurement	Be	Mg	Co	Ni	In	Ce	Bi
1	10.6	10.6	9.9	9.9	9.5	9.2	9.1
2	10.5	10.3	10.6	10.0	9.6	9.1	9.6
3	10.7	10.6	9.9	10.1	9.6	9.8	9.1
Mean ± RSD	10.6 ± 0.94%	10.5 ± 1.7%	10.1 ± 4.0%	10.0 ± 1.0%	9.6 ± 0.6%	9.4 ± 4.0%	9.3± 3.1%

B. *Hyphenated plasma mass spectrometry*

All samples were diluted in aqueous acid media (1% HNO3) and subjected to numerical analysis in a Perkin Elmer SCIEX DRC-e ICP-MS (Ontario, Canada) equipped with a high-performance mass analyzer (Fig. 1). Liquid samples were aspirated directly into the hot plasma (6000 K), ionized, and conveyed to the mass spectrometer for quantitative elemental analysis. The instrument is fitted with a quadrupole mass selector to attain efficient mass separation. The technique is controlled by an automated sample introduction system and is capable of attaining ultra-low limits of detection in the ng/L range (sub-ppb levels). The analytical process underwent standard correction for background and certified standards [29] were employed to establish repeatability, which occurred within 5% (Table 1). Modern software was deployed for calibration and the elimination of matrix effects.

III. RESULTS AND DISCUSSION

A. *Bioethanol yield/side reaction*

The bioethanol yields for the fard and khalas cultivars and century plant were between 20-25%. Several factors affected this yield and attempts were made to improve the overall yield. Thermal extraction of sugar played an important role, and the bioethanol derivative depended solely on the mass of sugar extracted. This technique, therefore, had to be perfected for maximum sugar extraction. The fermentation process was also carefully monitored and the strain of yeast used was essential for the best results. The optimum fermentation period also contributed to the bioethanol yield. Finally, the distillation process could impede the acquisition of the optimum yield because of undesirable phenomena such as foaming. Suitable anti-foaming reagents were, therefore, employed to improve this process, but the foaming effect tends to linger and it is difficult to remove it completely.

To study the possibility of side reactions and interferences it is necessary to closely examine the breakdown of glucose during the alcoholic fermentation process (Fig. 2). Undesirable interference could occur at any stage. For example, incomplete glycolysis could lead to fractional formation of pyruvates, which results in lower yields. Also, the reduction of pyruvic acid can lead to the production of lactic acid, one of the undesirable products. On the other hand, an incomplete decarboxylation of the α-keto carboxylic acid (the pyruvic acid in this case) could reduce the overall bioethanol yield appreciably, especially if the conversion is not fully optimized. Finally, the oxidation of acetaldehyde, the product of decarboxylation, is a secondary unwanted process as it leads to an additional undesirable by-product, acetic acid, in this case. Several factors could, therefore, affect the bioethanol yield and the process was thus carefully monitored and regulated till the optimum product was obtained.

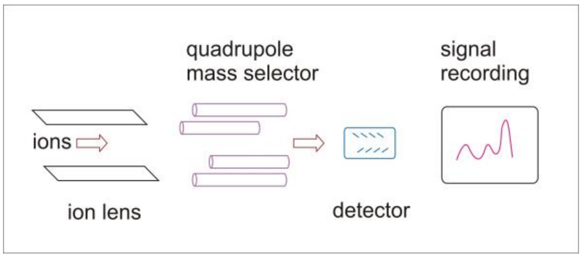


Fig. 1: Schematic of the hyphenated ICP-MS instrument

One suggested mechanism for the decarboxylation step is the unimolecular heterolysis of the anion of the α-keto acid - the pyruvate intermediate and the acyl carbanion in Fig. 3 could be the product of this carboxylation step. Therefore, a small increase of the reaction mixture pH, at the optimal stage e.g. after the glycolysis step, could be one possible way to facilitate the decarboxylation step and a higher ethanol production yield would therefore, be expected.

The acyl carbanion generated is highly reactive because the site of the negative charge cannot undergo delocalization within the side chain. As a consequence, acetaldehyde can still be produced under a relatively high pH since the acyl carbanion is a strong base due to the lack of charge delocalization.

Another interesting prospect to enhance glucose yield is to add a mild reducing agent to the reaction mixture, such as sodium borohydride. It is considered an effective reducing agent and could be potentially useful for the reduction of aldehydes and ketones to alcohols. Sodium borohydride could be added to the reaction mixture at the point of reduction of acetaldehyde to ethanol. The addition of a stronger reducing agent like lithium aluminum hydride is well worth considering because it could be more effective in reducing carboxylic acid as well as aldehydes and ketones to alcohols. However, one possible disadvantage in the deployment of lithium aluminum hydride could be the reduction of other carboxylic acids present in the reaction mixture, e.g lactic acid to propane-1,2-diol, making the purification of the desired alcohol (ethanol) a more challenging process (Fig. 4).

B. *Date cultivars/Toxicity*

The plots in Fig. 5 represent elemental levels in the bioethanol data obtained for the date cultivars and century plant. A point to note is that these are log-plots which tend to make differences between the two cultivars look more compressed than they seem. In discussing these levels emphasis was placed mainly on the elements that are considered to display a degree of environmental toxicity

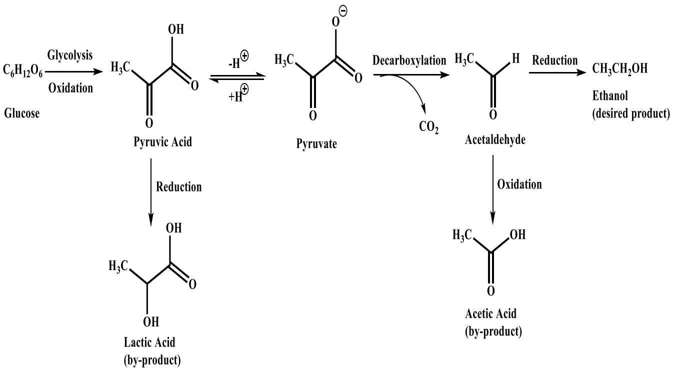


Fig. 2: Production of bioethanol from glucose

The levels of the toxic elements reflect those in the plant itself [29] and originate from the soil, conditioners and water used to improve growth. For the very light elements (Z<10) the khalas cultivar showed a lithium concentration about 20 times higher than for the fard cultivar. It is not clear why the Li level in khalas is so much higher, but it can only be surmised that either the soil or soil-conditioner associated with khalas is rich in Li. On the other hand the aluminium levels (Al) were not widely different, displaying about a 40% disparity between the two cultivars. For some unknown reason, chromium concentrations in both cultivars were unusually high (~2000 ppb) – again a reflection of the agricultural and irrigation conditions associated with these cultivars. Nickel (Ni) displayed a 50% elevation in fard, but no wide variations were observed between copper (Cu), selenium (Se) and lead (Pb) levels. Pb concentrations occurred at <1 ppb, and were not considered to be particularly elevated. Thorium (Th) and bismuth (Bi) levels in the two cultivars showed unexpectedly wide differences. The fard cultivar registered the wider difference: about 6 times higher for Th; and 3 times higher for Bi. Thallium (Tl) results were particularly low, recorded only for the fard cultivar (0.02 ppb), indicating that the environment in which the khalas cultivar is grown contains no detectable thallium. The mercury (Hg) results for both cultivars were about the same, but a marked difference was observed for antimony (Sb), more than 4 times higher for the fard cultivar. Another toxic element is cadmium (Cd), which was about 60% times higher for fard. From previous research, it is known that silver (Ag) is observed in date cultivars and the recorded results revealed that the Ag level for fard was double that for khalas. Overall, the fard cultivar showed wider differences than khalas. This could be due to more effective absorption of metals by the fard cultivar, and is a subject for future review.

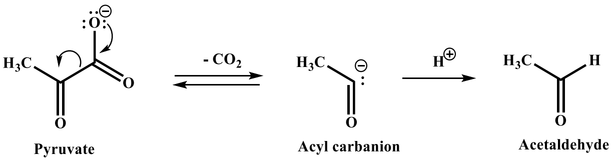


Fig. 3: Decarboxylation of pyruvate to produce acyl carbanion

C. Century plant/Toxicity

In general, century plant (known also as Agave americana) displayed higher elemental levels in the bioethanol extract, when compared with bioethanol concentrations from the date cultivars (Fig. 5). It is possible that growth conditions for the century plant differ widely; or the absorption efficiency of this plant for metals is more marked. For Z<20, no marked differences were observed between the date cultivars and century plant, except for aluminium. The Al level in the century plant was about a factor of 5 higher when compared to the date cultivars. Iron (Fe) was more than 10 times higher. The Cr level (>4000 ppb) was generally higher by a factor of two in the century plant. For some reason the date cultivars of interest and century plant possess strong tendencies to absorb Cr from their surroundings. Chromium in fuel could be deleterious, and should be removed chemically. The Ni level (Agave americana) was about the same for fard, but 50% higher than that for khalas; the Cu concentration exceeded a factor of 500 in general indicating strong absorption for Cu; and Cd and Ag levels surpassed those in the date cultivars by more than about a factor 5. It is interesting to note that zinc (Zn) and manganese (Mn) levels were higher in Agave americana by about a factor of 100 in relation to both cultivars; vanadium (V) and cobalt (Co) concentrations differed in general about a factor of 10; while the Sb level was elevated by roughly 10 for khalas and 3 for fard. The Pb level for century plant was approximately 20 times higher for the date cultivars; while the Se result displayed an overall increase of more than 5 times. Lead in the bioethanol by-product from century plant is about 20 ppb, which could constitute an environmental hazard especially if the bioethanol is to be used for fuel purposes. For strontium (Sr) and Bi century plant showed concentrations more than 40 times higher than fard and khalas. This is unusual, and can only be due to the strong absorption that this plant has for these elements. Bismuth,

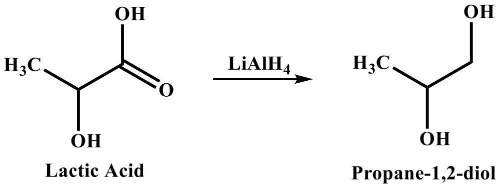
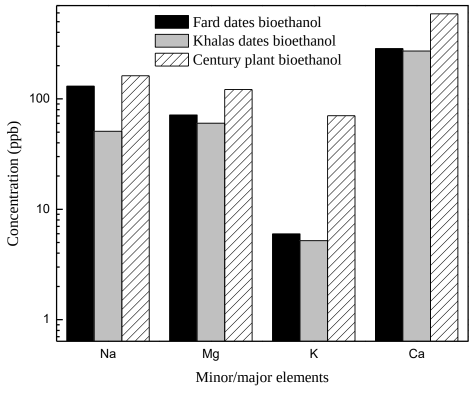


Fig. 4: Reduction of lactic acid using $LiAlH_4$



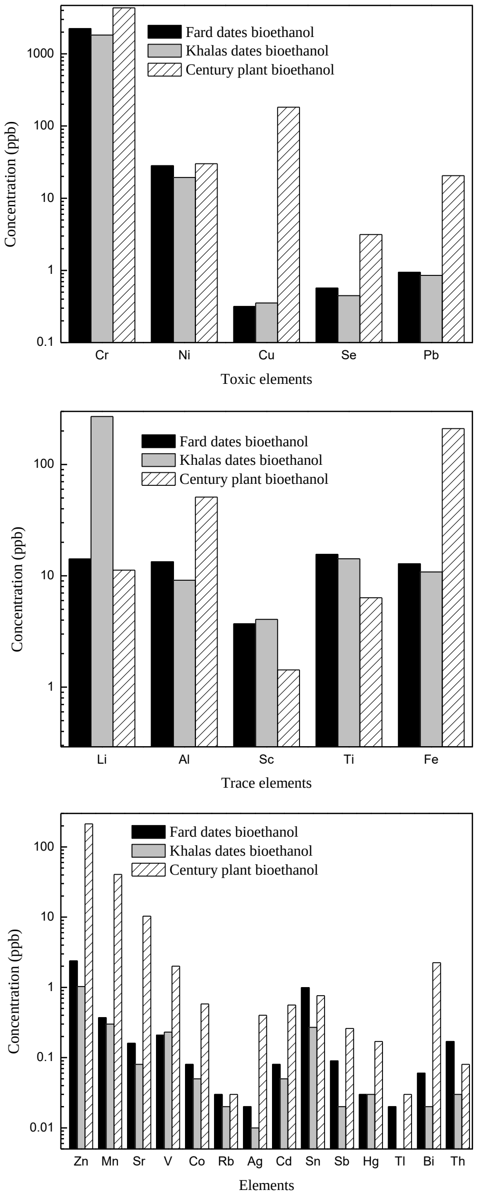


Fig. 5: Levels of trace and toxic elements in dates and century plant

especially, is a rare element and it is not common in most plants. The Th concentration in century plant was about twice as high for khalas; but fard exceeds thorium concentrations in the century plant by a factor of two. On the other hand, Hg is roughly 5 times higher when compared with Hg concentrations for both cultivars. Again, this pronounced difference for Hg is unexpected. These huge differences proclaim that bioethanol from century plant is comparatively impure in terms of metal contaminants and should be suitably de-metallized prior to use.

IV. IMPACT OF THE STUDY/PHYTO-REMEDIATION

Phyto-accumulation is an essential feature of phyto-remediation, where plants absorb toxic metals from the soil and ground water thus de-contaminating the environment around them. Clearly, if the bioethanol data for century plant reflects its absorption capacity, then the century plant has an unusually strong capacity to absorb Al, Fe, Cr, Mn, Cu, Zn, Sr, V, Ag, Sb, Hg and Bi. This is a novel aspect that has emerged from this study and should be considered for future review. As mentioned earlier, the century plant is a desert plant and usually grows under stringent conditions. It requires sandy soil and lots of sunlight. It is highly suitable to be cultivated on land that grows date cultivars to remove toxic metals from the soils so that the dates themselves are devoid of these metals. According to this study the century plant has the power to de-metallize soil and ground water used for irrigation. This is a unique characteristic and the strong potential of century plant as a metal remover would not have come to light had it not been for this study.

In previous reports we discussed the possible medical disorders that could arise from high concentrations of toxic metals in biofuel. We shall not repeat them here, but, it should be underscored that biofuel with appreciable levels of Cr, Cd, Hg and Pb should not be used for fuel until it is appropriately de-metallized. Toxic metals of this nature can be disseminated into the atmosphere through exhaust fumes and create an environmental hazard. It should be pointed out that very few studies have been undertaken on elemental profiles of biofuel, and more studies of this nature should be conducted to establish metal impurities in both biodiesel and bioethanol. It is well known that lead causes neuro disorders in children. Abnormal chromium, cadmium and mercury levels could lead to renal, respiratory and neuro malfunctions. Some of these elements are carcinogenic and, therefore, in the interest of sustainable development [31,32] suitable measures should be taken to protect our environment.

V. CONCLUSION

Our work has demonstrated that assay of metal impurities in biofuel is essential and should be undertaken on a routine basis in the interest of maintaining sustainability. The results of this study showed that trace toxic heavy metals can occur at elevated levels in bioethanol and such occurrence could necessitate de-metallisation prior to use as a fuel additive. We also extrapolated that the source of these toxins is the actual plant or biomass itself and the potential for such plants to play a role in phyto-remediation exists. Our work forms a definite contribution to sustainable development and environmental science.

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