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Article

# Banana-based biofuels for combating climate change: How the composition of enzyme catalyzed solutions affects biofuel yield

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## SUMMARY

**This investigation was inspired by the ever so precarious nature of climate change and its detrimental implications on our planet and society. One alternative form of energy that shows promise amongst others in alleviating the climate crisis, is biofuel. An extensive experiment was conducted to investigate whether amylase or yeast had a more prominent role in determining the bioethanol concentration and bioethanol yield of banana samples. Given that amylase facilitates the breakdown of complex polysaccharides into simple sugars, we hypothesized that amylase would have the most significant impact on the bioethanol yield and concentration of the samples. This was determined to be the case when the fermentation batch samples received enzyme compositions of yeast and amylase independently. However, when added concurrently, the optimal enzyme composition for maximizing the bioethanol concentration and yield was the addition of amylase and yeast in a 3:2 ratio. Thus, while yeast is an essential component for producing bioethanol, the proportion of amylase supplied through a joint amylase-yeast mixture has a more significant impact on the bioethanol yield. This provides some insight into what industrial engineers would need to take into consideration when mass-producing bioethanol on the industrial scale, namely that of the optimal enzyme composition with respect to cost and the bioethanol yield. Moreover, this study provides a greater understanding of the mechanisms and implications involved in enzyme-based biofuel production, specifically of those pertaining to amylase and yeast.**

## INTRODUCTION

Climate change and how to address its detrimental implications have recently become the center of discussion. The Intergovernmental Panel on Climate Change (IPCC) published its most recent report emphasizing the necessity of reforming the way we obtain our energy and the reliance we have on non-renewable fossil fuels (1). The IPCC has stressed the fact that if we do not prevent the global temperature from rising 1.5°C, there will be ravaging ramifications on us and our planet (1). Biofuels derived from biomass — any matter originating from plants or animals — show particular promise for combating climate change. They are advantageous because they emit, on average, up to 65% fewer greenhouse gases than conventional petroleum. Additionally, the crops grown for biofuels absorb $CO_2$ from the surrounding atmosphere and mitigate the carbon footprint created during cultivation, potentially making it carbon neutral (2-3).

Industrially produced biofuel requires two stages, breakdown and fermentation. First, the biomass must be pre-treated either through acid, base, or enzymatic hydrolysis to disrupt the hemicellulose and lignin and facilitate the breakdown of complex sugars to simple sugars (4). Once this process is complete, the biomass is subjected to fermentation, and bioethanol is consequently formed. We chose bananas for this investigation since they are easy to manage as a plantation crop, they readily undergo fermentation and have an average yield of 40-50 tons per hectare (5). In addition to this, 10-15% of the dry mass of bananas consists of simple sugars, which proves especially favourable for fermentation (6). The peels of bananas are commonly discarded and also possess potential for bioethanol production, proving especially advantageous given that banana peels are not relied on as a food source (7).

The biological conversion of biomass to fuels can offer the high yields necessary to be economically viable by lowering economic cost. Extensive literature exists on chemical and physical conditions for optimizing bioethanol yields. For instance, a pH of 4.5-5.5 during enzymatic pre-treatment by amylase increases simple sugar production and a pH of 6.7-7.0 for yeast facilitates fermentation (8). There also exists considerable research into the various methods of pre-treatment for the banana to be broken down into glucose and consequently form bioethanol (9). Biological means of facilitating these processes like enzymatic hydrolysis are particularly attractive. Enzymatic hydrolysis, which promotes the conversion of biomass to fermentable sugars, is arguably the most complex type because the enzymatic interactions and mechanisms are not yet well understood. Therefore, we will investigate these biological processes as they offer greater potential yields, lower energy costs and milder

operation conditions than other chemical processes such as acid-base hydrolysis (10).

This manuscript attempted to provide insight and a better understanding of the mechanisms involved specifically with enzymatic hydrolysis. We tested varying compositions of amylase and yeast to optimize the bioethanol yield through the fermentation of banana samples. We believe that these results will provide important considerations for other fruits and biomaterials which are not used as a food source, such as banana peels or corn husks.

Given that the rate of breakdown and fermentation is dependent on the amount of substrate (glucose) available, greater amounts of both amylase and yeast being supplied to the banana samples should increase the rate. We hypothesize the increase in both amylase and yeast compositions of the batch samples will lead to the proportional increase in glucose concentration post enzymatic hydrolysis.

## RESULTS

The breakdown of the banana batch samples through enzymatic hydrolysis and the consequent fermentation of bioethanol would allow us to determine the glucose and bioethanol concentration of the samples. In order to do so the banana batch samples independently received varying compositions of amylase (A1-A6) and yeast (S1-S6) (**Figure 1A-B**). A combined amylase-yeast batch (C1-C6) was also assembled to allow for greater scrutiny in ascertaining the impact of either amylase or yeast in the bioethanol yield of banana samples (**Figure 1C**). All samples received pre-established amounts (**Figure 1**) of their conjugate solutions to ensure enzymatic hydrolysis and fermentation occurred and also so that differences in glucose and bioethanol concentration could be scrutinized appropriately (**Figure 1**). The samples were subjected to enzymatic pre-treatment by the addition of their respective quantities of amylase for a duration of 15 minutes. Afterwards, we took 1 $cm^3$ aliquots of the samples and measured their absorbance by spectrophotometer. We compared these to a pre-established standardised glucose calibration curve to determine their glucose concentrations. (**Figure 2E**). For the amylase (A1-A6) and yeast (S1-S6) fermentation batches, the greater the proportion of amylase and yeast supplied the higher the consequent glucose formation post-enzymatic hydrolysis (**Figure 2A-B**).

The interaction between the yeast and amylase supplied to the banana fermentation batch samples (the substrate) took place in aqueous solution. Therefore, the breakdown of complex sugars into glucose and the consequent conversion into bioethanol was a result of homogenous catalysis (11). The general reaction scheme of an enzyme-catalysed reaction is as follows:

$$E + S \overset{k_1}{\rightarrow} [ES] \overset{k_2}{\rightarrow} E + P \quad (11).$$

In this investigation, the amylase interacted with the banana samples (substrate) by binding to its active sites to form the enzyme substrate complex *[ES].* This is followed by the decomposition of *[ES]* to regenerate the amylase and to form the new product glucose (*P).* This same process occurs during the fermentation of the banana samples but instead bioethanol (*P*) is formed as the result of yeast catalysis. Enzymatic hydrolysis occurred for fifteen minutes, and we assumed that the correlation between the enzyme composition of the banana samples and the glucose concentration to be akin to that of a first order reaction. This assumption would also hold true for the bioethanol concentration of the banana samples since the more glucose available, the more that could undergo fermentation. Modelling the expected rate equation for the enzymatic hydrolysis of the banana samples and for the bioethanol consequently formed after fermentation can be represented as:

$$r = k[\![Yeast]\!]\,[\![Amylase]\!].$$

This is supported by a variation in the Michaelis-Menten equation which employs the steady-state approximation. It states that for initial enzyme concentrations and low values of substrate (*S*) as employed in this study, the rate of product

**a)**

| Enzyme Batch/Samples | Yeast ($cm^3$) | Water ($cm^3$) |
|---|---|---|
| S1 | 10 | 0 |
| S2 | 8 | 2 |
| S3 | 6 | 4 |
| S4 | 4 | 6 |
| S5 | 2 | 8 |
| S6 | 0 | 10 |

**b)**

| Enzyme Batch/Samples | Amylase ($cm^3$) | Water ($cm^3$) |
|---|---|---|
| A1 | 10 | 0 |
| A2 | 8 | 2 |
| A3 | 6 | 4 |
| A4 | 4 | 6 |
| A5 | 2 | 8 |
| A6 | 0 | 10 |

**c)**

| Enzyme Batch/Samples | Amylase ($cm^3$) | Yeast ($cm^3$) |
|---|---|---|
| C1 | 10 | 0 |
| C2 | 8 | 2 |
| C3 | 6 | 4 |
| C4 | 4 | 6 |
| C5 | 2 | 8 |
| C6 | 0 | 10 |

**Figure 1. The varying enzyme compositions of the fermentation batch samples.** The three enzyme induced fermentation batches were categorized as follows: **(a)** yeast, S1-S6, **(b)** amylase (A1-A6) and **(c)** amylase-yeast combined (C1-C6). To ensure the successful breakdown of complex sugars and fermentation batch samples S1-S6 received 2 $cm^3$ of amylase, A1-A6 received 2 $cm^3$ of yeast and C1-C6 2 $cm^3$ of water. The percentage concentration of both the amylase and yeast solutions were 1%.

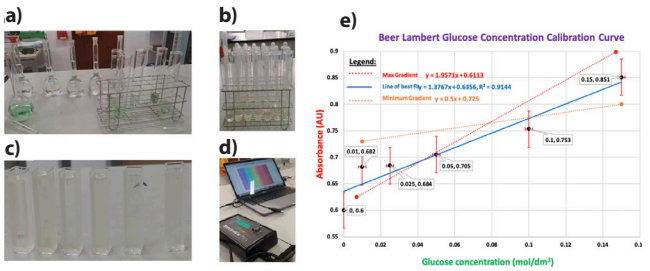
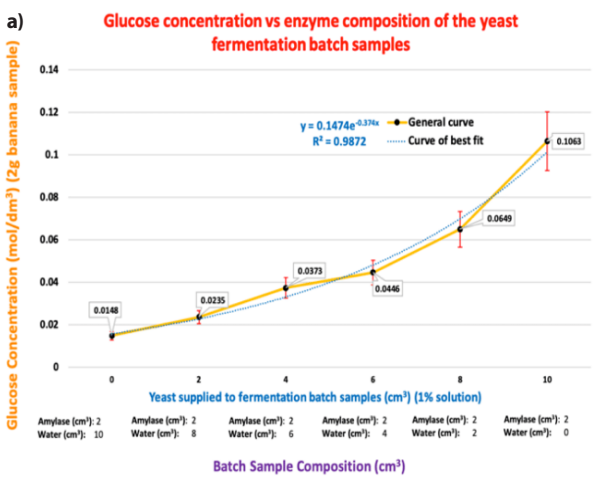
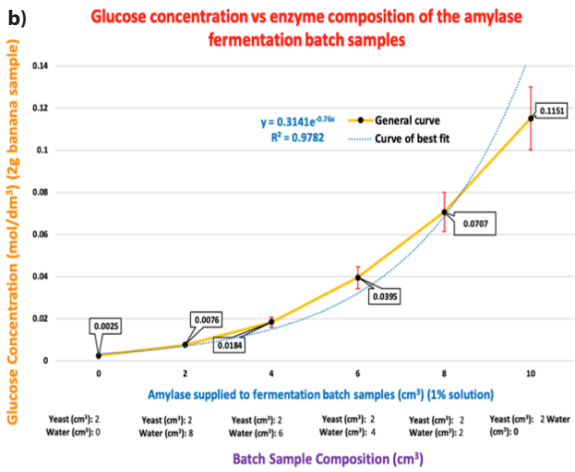



**Figure 2. Establishment of the Beer-Lambert's glucose calibration curve.** Max-Min gradients are extrapolations of the potential discrepancies in the absorbance values as a result of the ± 13% error in the spectrophotometer. **(a)** Preparation of the Quantitative Benedict's Solution (QBS) used for quantitatively determine the glucose concentrations of the varying fermentation batch samples by spectrophotometer. **(b)** 10 $cm^3$ of QBS was mixed with 1 $cm^3$ of solution of the fermentation batch samples. **(c)** The range in the shade of color emerging from the samples indicated the relative presence of glucose. **(d)** These in turn corresponded to different absorbance values recorded by spectrophotometer. **(e)** Standardised Beer-Lambert calibration curve of glucose which was established.




**(a, i)**

| Sample | Yeast ($cm^3$) | Water ($cm^3$) | Glucose concentration ($mol/dm^3$) | % Glucose yield of total banana mass (2g) |
|---|---|---|---|---|
| A1 | 10 | 0 | 0.0446 | 16.07 |
| A2 | 8 | 2 | 0.0395 | 14.23 |
| A3 | 6 | 4 | 0.0381 | 13.73 |
| A4 | 4 | 6 | 0.0373 | 13.44 |
| A5 | 2 | 8 | 0.0235 | 8.47 |
| A6 | 0 | 10 | 0.0184 | 6.63 |

**(b, i)**

| Sample | Yeast ($cm^3$) | Water ($cm^3$) | Glucose concentration ($mol/dm^3$) | % Glucose yield of total banana mass (2g) |
|---|---|---|---|---|
| S1 | 10 | 0 | 0.017 | 6.13 |
| S2 | 8 | 2 | 0.0148 | 5.33 |
| S3 | 6 | 4 | 0.0076 | 2.74 |
| S4 | 4 | 6 | 0.0061 | 2.20 |
| S5 | 2 | 8 | 0.0025 | 0.90 |
| S6 | 0 | 10 | 0.0003 | 0.11 |

**Figure 3. Correlation between the compositions of the amylase and yeast fermentation batches with respect to their glucose concentration and yield. (a, b)** The increase in glucose concentration as a result of the increasing proportion of amylase and yeast in the fermentation batch samples. **(a, i)** The % glucose yields of the yeast samples with respect to the initial banana mass. **(b, i)** The % glucose yields of the amylase samples with respect to the initial banana dry mass.

formation (glucose and bioethanol) is directly proportional to the amount of substrate initially present (*12*). This can be represented as follows:

$$r = \frac{k_2 [E_0][S]}{K_m + [S]}$$

where *[E0].* is the total enzyme concentration and Km the Michaelis constant, a measure of binding affinity which is a way to determine the compatibility of an enzyme with the active site of a given substrate (13).

However, the relationship between the batch compositions of the banana samples and their respective glucose concentrations post-enzymatic hydrolysis was not first order and hence not linear (**Figure 3**). In fact, we found a strong exponential correlation between the glucose concentration post enzymatic hydrolysis of the amylase ($R^2$ = 0.9872) and yeast ($R^2$ = 0.9782) fermentation batch samples and their respective compositions. This discrepancy from our hypothesis might be due to the addition of yeast to the samples. Although the yeast is supposed to catalyze fermentation, adding it concurrently with amylase rather than separately may have impacted the concentrations of glucose post enzymatic hydrolysis (**Figure 4A**) The non-linear proportional increase in glucose concentration therefore could be a result of the interaction or interference between the yeast and amylase, also known as enzyme inhibition.

The banana fermentation batch samples were then incubated within water baths at the optimal pH (yeast 4.5-5.5 and amylase 6.7-7.0) and temperature conditions (36.8°C) conducive to fermentation (**Figure 4B**). The samples were then transferred to conical flasks and suspended over dichromate solution to undergo reduction overnight (**Figure 4C**). This was done since alcoholic beverages contain other oxidizable substances that could interfere with the titration (14). The ethanol produced as a result of fermentation oxidized to ethanoic acid by reacting with an excess of potassium dichromate in acid. The unreacted excess was determined by the addition of potassium iodide solution which was oxidized by the potassium dichromate, forming iodine. Then, the iodine was titrated against a standard solution of sodium thiosulfate to calculate the original bioethanol content present in the banana fermentation batch samples (**Figure 4D-Eiii**).

The titrations soon revealed that more amylase and yeast yielded higher bioethanol amounts since more glucose was readily available to undergo fermentation. (**Figure 5A-B)**. The graphs had a strong adherence to the data points plotted and $R^2$ values of 0.984 (amylase) and 0.994 (yeast), which indicated a strong linear correlation between the two variables. Unlike with the glucose, the bioethanol concentration of the amylase and yeast fermentation batch samples was directly proportional to their compositions as stipulated by the Michaelis-Menten equation. The concurrent addition of amylase and yeast in the amylase-yeast fermentation batch samples allowed for the direct gauging

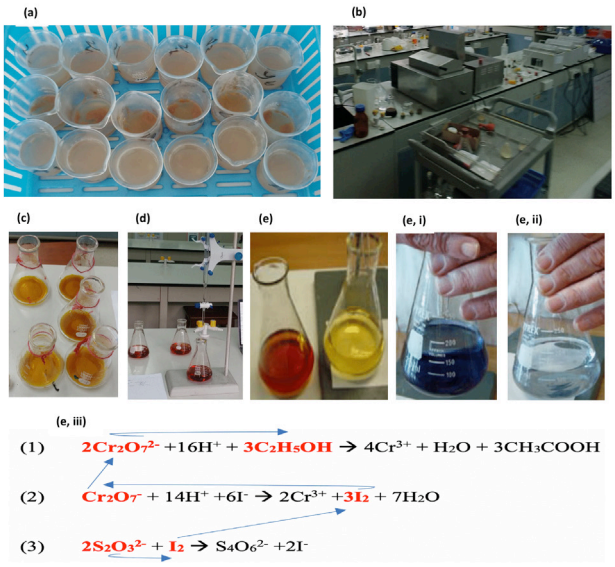



**Figure 4. Enzymatic Pre-treatment, fermentation and bioethanol formation. (a)** Fermentation batch samples post enzymatic pretreatment being transferred for overnight fermentation. **(b)** The incubation of the banana batch samples for optimal fermentation conditions. **(c)** The batch samples were suspended over dichromate solution and left to reduce overnight. Variations in the extent of reduction arose, indicating tangible differences in the bioethanol concentrations of the banana batch samples. **(d)** The extent of ethanol oxidation indicates the initial concentration which can be determined by redox back titration. **(e)** Left flask shows the brown coloured solution resulting from the formation of iodine. The other indicates the color transition which occurs as the iodine is titrated with thiosulfate. **(e, i)** Upon the addition of starch the analyte solution takes becomes blue due to a formation of a starch-iodine complex. **(e, ii)** The endpoint of the titration occurs when the solution becomes colorless. **(e, iii)** Chemical pathway of the redox titration for determining the bioethanol concentration of the banana fermentation batch samples.

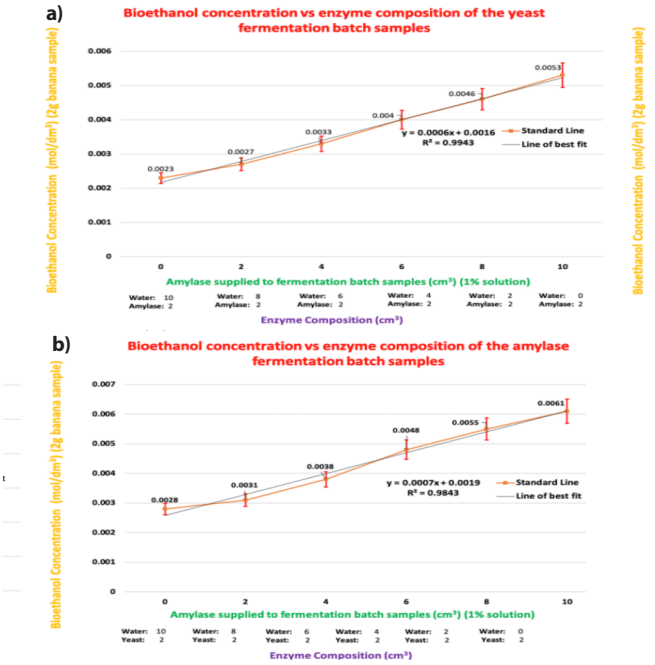
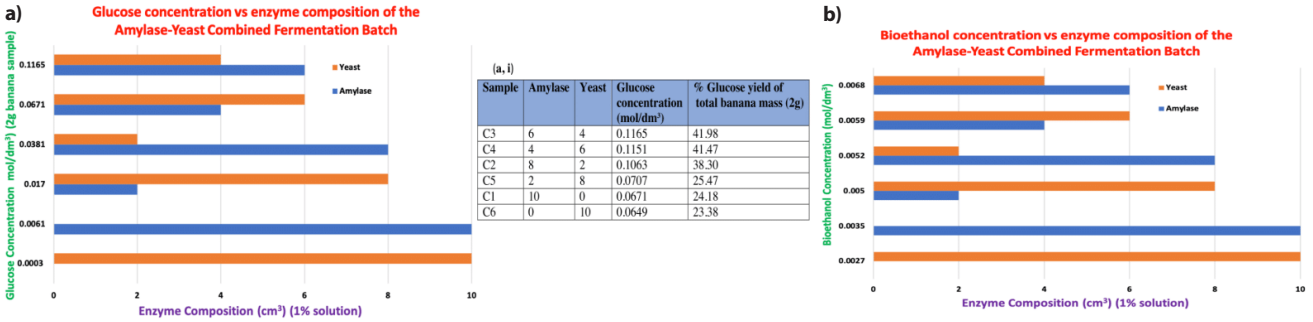



**Figure 5. The correlation between the compositions of the amylase and yeast fermentation batch samples and their bioethanol yields. (a, b)** The increasing prominence of amylase and yeast as they compromise more of the total batch sample composition and the associated linear increase in bioethanol concentration. This is to be expected given the prior increase in glucose concentration. A greater proportion of glucose available allowed the samples to undergo fermentation and form bioethanol more effectively.

of their relative prominence with regards to their glucose and bioethanol yields. The trend for this fermentation batch was more complex. Sample C1 (**Figure 1**), with 10 cm³ of amylase, did not produce the greatest concentration of glucose, but rather sample C3, with a composition of 6 cm³ of amylase and 4 cm³ of yeast followed by samples C4, C5, C1, and C6 (**Figure 6A**). Not only did this combination outperform the other samples of its fermentation batch with 0.1165 mol/dm³, but also that of the amylase and yeast batch samples (**Figure 3**). Consequently, the same compositions of amylase and yeast produced the greatest bioethanol concentrations. It is clear that for the combined amylase-yeast fermentation batch samples, a greater percentage of amylase yielded greater glucose concentrations after enzymatic hydrolysis and ultimately bioethanol post fermentation (**Figure 5A-B**).

## DISCUSSION

To better understand the implications of the results pertaining to the combined fermentation batch samples, it is worthwhile to compare the enzyme composition of samples C3 and C5 in particular. Sample C5 is the conjugate in terms of composition, of sample C3 with 6 cm³ of yeast and 4 cm³ of amylase. Regardless, C3 still yielded a greater glucose concentration after pre-treatment, as well as a higher bioethanol concentration. This is most likely the case because the amylase acts as the 'solvent' and breaks down the complex sugars — disaccharides and polysaccharides — into simple sugars like glucose of our substrate, which is our varying banana fermentation batch samples. This process is known as glycolysis and is an essential step in the production of bioethanol from organic matter (15). A greater amylase concentration would ensure a more efficient glycolysis process resulting in a greater amount of complex sugars being broken down, resulting in a greater amount of glucose readily available to undergo fermentation. (**Figure 7A iii**). Essentially, amylase facilitates the glycolysis process, the most important step in the lead up to ethanol formation. Since amylase determines the amount of substrate that will be available for the conversion to ethanol to take place, compositions favouring yeast like that of C5 and C6 would certainly facilitate the oxidation of NADH to NAD+ allowing for further glycolysis to occur (15). In addition to this, amylase enhances the efficiency of the conversion of acetaldehyde to ethanol. However, given the limited quantity of the substrate in the first place, the formation of ethanol would be curtailed, and the re-initiation of glycolysis would not remain feasible for long. Given these direct comparisons between the varying




(a, i)

| Sample | Amylase | Yeast | Glucose concentration (mol/dm³) | % Glucose yield of total banana mass (2g) |
|---|---|---|---|---|
| C3 | 6 | 4 | 0.1165 | 41.98 |
| C4 | 4 | 6 | 0.1151 | 41.47 |
| C2 | 8 | 2 | 0.1063 | 38.30 |
| C5 | 2 | 8 | 0.0707 | 25.47 |
| C1 | 10 | 0 | 0.0671 | 24.18 |
| C6 | 0 | 10 | 0.0649 | 23.38 |

**Figure 6. Correlation between the ratio of amylase to yeast enzyme in sample composition and their glucose/bioethanol yields. (a)** The increase in glucose concentration as a result of the changing proportions of amylase and yeast in the fermentation batch samples. **(b)** The % glucose yields of the amylase-yeast combined samples with respect to the initial banana dry mass. **(c)** Sample C3 with 4 cm³ of yeast and 6 cm³ of amylase supplied formed the greatest glucose concentration of 0.1165 mol/dm³ post enzymatic pre-treatment. Naturally, it yielded the greatest bioethanol concentration given that a greater proportion of glucose was available to undergo fermentation and form bioethanol.

compositions of the amylase-yeast combined fermentation batch, we soon realised that amylase in conjunction with yeast, even when in lower concentrations, takes precedence in determining the concentration and yield of bioethanol for a given fermentation batch sample. Moreover, the optimal batch composition consists of a greater proportion of amylase to yeast to ensure the sufficient breakdown of complex sugars and conversion of acetaldehyde to ethanol, resulting in greater bioethanol yields. In this experiment, the optimal composition was revealed to be C3 consisting of 6 cm³ of amylase and 4 cm³ of yeast, which is analogous to a 3:2 ratio. Lastly, the glucose yields with respect to theoretical presence of glucose in the 2 g banana for the varying samples was impressive with some yielding over 30%, twice that of the most optimistic yield predicted (**Figure 6B**).

Given the extensive experimental procedure and use of equipment for the investigation, there is room for potential error. We used the redox-titration to determine the ethanol concentration of the fermentation batch samples and the standardized Beer-Lambert calibration curve to measure the glucose concentrations. During the fermentation process, Parafilm strips were used to cover the flasks allowing for slight expansion due to an increase in $CO_2$ produced as a result of anaerobic respiration. Because of the expansive nature of the parafilm, some oxygen could have seeped into the flasks reducing the efficacy of the fermentation process, especially given its anaerobic nature. Additionally, due to time constraints, the fermentation batch samples only underwent fermentation for 24 hours as opposed to the optimal 72 hours. This reduced the confidence in the results as not all fermentation parameters were optimized, therefore the optimal enzyme composition may have differed slightly.

The preliminary redox titration was conducted with beer that contained 5% ethanol. There was a discrepancy between the calculated ethanol concentration and the actual of 13% which was not too disconcerting. However, the potential error in employing this method for the fermentation batch samples would be far greater given the smaller ethanol concentrations of the banana samples. For the redox titrations of the samples, the final colour transition from blue to colourless did not occur at all. The addition of $H^+$ ions (by concentrated $H_2SO_4$) to the dichromate solutions had no bearing on this, and the nature of the occurrence remained elusive. However, the penultimate color transition remained mostly consistent, which was reassuring (**Figure 8A**). Lastly, some of the aliquots taken from the fermentation samples contained particle contamination (**Figure 8B**), which influenced the recorded absorbance values compared to the standardized glucose calibration curve that was established.

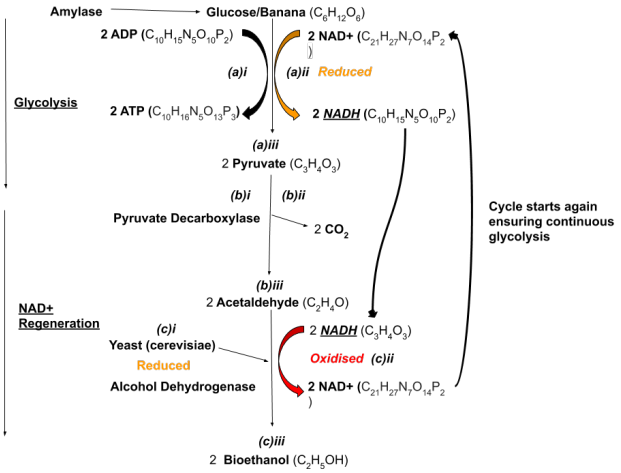



**Figure 7. Chemical pathway of ethanol fermentation.** Glycolysis of the substrate occurs, in this case the banana samples. **(a, i)** Two molecules of ATP are formed. **(a, ii)** NAD+ is reduced to NADH by the addition of a hydride ion. **(a, iii)** Two molecules of Pyruvate are formed. **(b-c)** NAD+ regeneration. **(b, i)** Pyruvate loses its carboxyl group, facilitated by the enzyme pyruvate decarboxylase. **(b, ii)** $CO_2$ is released due to carboxyl group leaving. **(b, iii)** Results in the formation of two molecules of acetaldehyde. **(c, i)** Addition of yeast catalyses the reduction of acetaldehyde to bioethanol. **(c, ii)** The acetaldehyde is reduced to ethanol, in this case bioethanol by the addition of a hydride ion. This conversion is facilitated by the enzyme alcohol dehydrogenase. **(c, iii)** by the addition of a hydride ion. This conversion is facilitated by the enzyme alcohol dehydrogenase. **(c, iii)** Whilst this occurs the NADH from earlier is becoming oxidised this ensures a plentiful regeneration of further NAD+ which allows glycolysis to re-initiate and the further formation of ethanol.

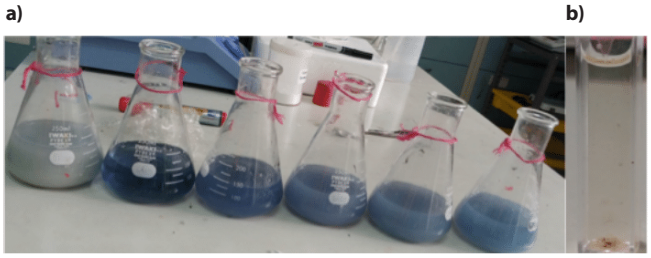



**Figure 8. Discrepancy in color transition of redox titration and particle contamination during colorimetry. (a)** Samples turned navy blue during the penultimate transition however, shortly afterwards different shades of blue began to emerge. **(b)** Cuvette sample after it was mixed with QBS which turned slightly orangered indicating a relatively strong presence of glucose. The sample contained some particle contaminate potentially stemming from banana remnants which were not completely broken down as a result of enzymatic pre-treatment.

Interestingly, it was observed that the particle contamination was more prominent for fermentation samples that were diluted in terms of enzyme composition (concentration) for both the amylase and yeast fermentation batches. In the combined batch samples, particle contamination became increasingly prevalent the further it deviated from the optimal enzyme composition observed in sample C3 (6 $cm^3$ of amylase and 4 $cm^3$ of yeast). If time had allowed, the fermentation batch samples could have been filtered to reduce the particle contamination, providing more accurate absorbance readings. The spectrophotometer used had an error deviation of 13%, which was unfavourable. More accurate equipment would have lowered the error deviation and re-affirmed confidence in the results obtained. Overall, these occurrences contributed the greatest to the systematic error pertaining to the bioethanol produced.

To better understand the use of banana-based biofuel, the mechanisms of glycolysis and fermentation which are catalyzed by amylase and yeast, respectively, need to be analysed and substantiated. Ascertaining the Michaelis-Menten constant of both reaction processes would determine the binding affinity between the substrate's active sites and that of the amylase and yeast used in this investigation. This could provide further insight into their 'compatibility.'

Food waste is the largest greenhouse gas contributor after China and the United States. Our experiments were conducted with fresh bananas but repeating these experiments with a decomposing banana would be worthwhile given the significant potential greenhouse gas savings in preventing fruit from decomposing and releasing methane in landfills (16). It is likely that there would be differences in the bioethanol and glucose concentrations of the fermentation samples depending on the extent of decomposition of the banana. Moreover, determining whether the efficacy or efficiency of fermentation changes based on the extent of said decomposition would be noteworthy. Given the potential benefits of algae-based biofuels, specifically its high energy content and $CO_2$ absorption, it would be worthwhile to investigate the optimal enzyme composition for the ethanol fermentation of algae. (17-18) Testing various strains of algae and investigating which strain produced the greatest amount of bioethanol in combination with the optimal enzyme composition would be valuable.

The high costs of amylase and yeast would make it important to determine the optimal enzyme composition based on the efficiency of ethanol fermentation with respect to cost, on an industrial scale. While the variations in enzyme compositions of this investigation were rather limited, they could be extended or scaled up to simulate fermentation on the industrial scale. Lastly, it would be worthwhile to ascertain why the titrations of the fermentation batch samples did not transition to colorless. If this were resolved, greater confidence in the experimental results and conclusion would be established.

## METHODS

### Banana sample preparation

A fresh set of bananas (from a local grocery store) was procured and the peel was removed. Bananas were pulverized using a standard kitchen blender at maximum speed for one minute and then medium speed for an additional minute. Once this was complete, eighteen 100 $cm^3$ beakers were collected and zeroed on analytical balances. The beakers were pre-emptively labelled according to the fermentation batch they belonged to and with regards to their compositions of amylase and yeast. Then, 2 g of blended banana was measured into each of the beakers.

### Redox titration

Blank titrations (those without any beer) were prepared by adding 10 $cm^3$ of acid dichromate solution to a 250 $cm^3$ conical flask. This was followed by the addition of 100 $cm^3$ of water and 1 $cm^3$ of potassium iodide solution. The resulting solution was thoroughly mixed. The solutions were then titrated against sodium thiosulfate. As the solution faded to yellow, 1 $cm^3$ of starch solution was added, resulting in the solution turning dark blue. This solution was further titrated against the sodium thiosulfate until the solution became colorless. The titre values were recorded, and the same process was repeated with acidified dichromate solution, which had been reduced overnight by malt beer containing 5% ethanol. The difference in titres between the blank titrations and those of the fermentation batch samples in conjunction with the redox equations previously mentioned were used to determine the ethanol concentration of the malt beer (14).The experimental procedure was repeated with all fermentation batch samples.

### Establishment of the standardized glucose calibration curve

A range of known glucose concentrations was prepared to establish a standardized calibration curve. The following concentrations were prepared: 0.15 mol/$dm^3$, 0.1 mol/$dm^3$, 0.05 mol/$dm^3$, 0.025 mol/$dm^3$, and 0.01 mol/$dm^3$. The 0.15

mol/dm³ concentration of glucose as a percentage mass of 100 g would be commensurate to 15%, 0.1 mol/dm³ to 10%, 0.025 mol/dm³ to 2.5%, and that of 0.01 mol to 1%. This range would account for the most optimist glucose concentration (around 0.15 mol/dm³) obtained from the banana samples to a more pessimistic 1% (around 0.01 mol/dm³). 1 cm³ aliquots of the glucose samples were mixed with 10 cm³ of Quantitative Benedict's Solution (QBS) and then were placed in boiling tubes within a water bath at 80°C for 5 minutes. The solutions were then placed within cuvettes and their respective absorbances recorded by spectrophotometer and substantiated against a negative control of QBS (0.6 AU). These values were then used to establish a Beer-Lambert curve of the known glucose concentrations vs. their absorbance values.

### Fermentation and determining bioethanol concentration

The previously mentioned banana sample solutions were then transferred to their respective conical flasks contingent on their amylase and yeast compositions. As much as possible of the remaining solution was transferred using a miniature spatula. The conical flasks were lightly sealed with paraffin strips to allow for any expansion that could occur due to the increase in $CO_2$ as a result of fermentation. The conical flasks were then categorically submerged based on the fermentation batch and sample composition within three water baths set to 36.8°C. The samples were left to ferment for 24 hours and were then removed and brought to room temperature. Eighteen 250 cm³ volumetric flasks with modified bungs to make use of sample holders were prepared. Then, 1 cm³ from each of the fermentation batch samples were transferred to the sample holders followed by the addition of 10 cm³ of potassium dichromate (0.1 mol) to each of the conical flasks. The samples were then suspended over the dichromate solution and the conical flasks were sealed by the modified bungs and left overnight in water baths at 36.8°C for the dichromate to reduce. The following morning, the conical flasks were brought to room temperature and the samples were removed. The dichromate solution was then titrated against sodium thiosulfate (0.3 mol) to determine the ethanol concentration of the fermentation batch samples (14).

### Enzymatic hydrolysis and determining glucose concentration

Fermentation batch samples received 10 cm³ of pH 7 buffer solution followed by the addition of the respective amylase amounts for 15 minutes. Then, 10 cm³ of pH 4 buffer was supplied to all the samples, as well as their respective amounts of yeast and water. All samples were then shaken and stirred to ensure the contents of the solution that developed were mixed thoroughly. Next, 1 cm3 of aliquots were extracted from each of the samples and mixed with 10 cm3 of QBS and transferred to corresponding boiling tubes. The boiling tubes were then submerged within a water bath at 80°C for 5 minutes. The samples were then removed and their absorbance values were standardized against the negative control of the absorbance value of the QBS and the pre-established standardized glucose calibration curve.

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