

## **Fermentation**

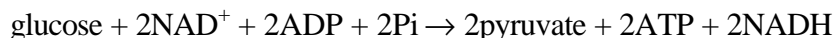
### **Milos Babic**

#### **Abstract**

Fermentation is the process many living organisms use in absence of oxygen, in order to replenish  $\text{NAD}^+$  expended during glycolysis. In one type of fermentation, this is accomplished through conversion of pyruvic acid into  $\text{CO}_2$  and ethanol. We have examined the process by allowing yeast to ferment glucose, and analyzing the products. Using Benedict's reagent and  $\text{Ba}(\text{OH})_2$  test for  $\text{CO}_2$ , we show that the levels of glucose fall during fermentation, and that carbon dioxide is released. We also show that a flammable substance, consistent with ethanol, is present in the final solution.

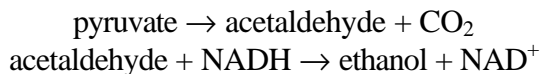
#### **Introduction**

In most living organisms, various sources of energy are converted to glucose, and glucose itself is used as the direct source for production of ATP. This process, glycolysis, breaks a molecule of glucose into two molecules of pyruvate, produces two molecules of ATP, and reduces two molecules of coenzyme  $\text{NAD}^+$  to NADH (1). The overall formula of the reaction is:



In order for more glucose molecules to be utilized, the NADH product of the reaction has to be oxidized back into its active form,  $\text{NAD}^+$ . In aerobic organisms, this is performed in the process of oxidative phosphorylation, and the process is used to actually produce additional ATPs. In anaerobic circumstances, however,  $\text{NAD}^+$  is regenerated through another process, fermentation.

Fermentation usually follows one of two main pathways. In one, pyruvate is converted to lactic acid, and the process is used to oxidize NADH. In the other, pyruvate is decarboxylated (with release of gaseous  $\text{CO}_2$ ) to acetaldehyde, which is then converted into ethanol. This second step is used to recover  $\text{NAD}^+$ .



We have examined this process by allowing yeast to ferment glucose in an anaerobic environment. Benedict's test was utilized to measure the concentration of glucose before and after the fermentation, demonstrating that the glucose levels are indeed falling during fermentation. The presence of  $\text{CO}_2$  was demonstrated with  $\text{Ba}(\text{OH})_2$  test, while the presence of ethanol was supported through a flammability test on a distillate of the final product.

## Materials and Methods

*Fermentation of glucose.* Approximately 100mL of 25% glucose solution was added to a flask, along with a spoonful of yeast. The flask was stoppered by a special airtight plug fitted with a tube, which was placed in a vial of  $\text{Ba}(\text{OH})_2$ . The flask was placed at  $37^\circ\text{C}$  for one hour for fermentation to take place.

*Benedict's test.* Since Benedict's test is sensitive only in the 0.1%-1% glucose concentration range, a dilution was required. We took 1mL sample of the initial mixture of glucose and yeast, and diluted that into 4 mL of distilled water. Benedict's test was performed by mixing eight drops of Benedict's reagent with 1mL of diluted mixture in a test tube, and the tube was heated in a water bath for five minutes. The resulting color change was observed.

Also, after the fermentation period of one hour, we took another 1mL sample from the flask, and diluted it as before. The Benedict's test was performed again identically, and the resulting color was observed again.

*Barium hydroxide test.* Gas passing out from the flask was forced to go through the tube in the stopper, and bubble through barium hydroxide solution. Insoluble precipitate formation was observed.

*Alcohol flammability test.* After the fermentation period, the flask was stoppered with a plug containing a rubber hose. The hose was placed into a test tube, and the flask was placed onto a hot plate and heated. First few drops of resulting vapor were collected, and an attempt was made to light them with a match.

## Results

When the sample taken before fermentation was tested, Benedict's reagent turned dark red. The sample taken after fermentation produced a light orange color.

Bubbling the gas collected in the balloon through a  $\text{Ba}(\text{OH})_2$  solution produced a white precipitate (presumably barium carbonate).

The few drops of clear liquid collected in the flammability test produced a pale blue flame.

## Discussion

The experiment performed produced results that are highly consistent with our expectations. The change in color of Benedict's test, from dark red to light orange, shows that the concentration of glucose has indeed been reduced during the process.

Barium carbonate precipitated during the  $\text{Ba}(\text{OH})_2$  test, indicating that carbon dioxide was released in significant quantity. Finally, the pale blue flame observed in the flammability test is consistent with ethanol.

Unfortunately, we failed to perform a control test for presence of alcohol before fermentation, and our test for it could have been more specific. However, given the limited time and resources, we feel that our experiments have successfully examined the broad picture of ethanol fermentation.