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Yeast and fermentation: the optimal temperature

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Summary
In the future oil resources will be exhausted. This knowledge has stimulated interest in one of mankind's oldest chemical processes: the production of bio-ethanol from sugars by fermentation. Yeast is an eukaryotic organism and can ferment D-glucose into ethanol and carbon dioxide. This fermentation occurs in an oxygen free environment and raises the question of what the optimal temperature is in the conversion of glucose to ethanol by yeast cells, also known as *Saccharomyces cerevisiae*, baker's yeast. The fermentation process was observed by measuring the release of carbon dioxide at temperatures of 20, 25, 30, 35 and 40°C. This resulted in an optimal temperature for the fermentation process at 35°C. But it also raised further questions such as how can the amount of ethanol be optimized. We found an amount of carbon dioxide that was less than optimal.

 Fermentation, yeast, temperature, carbon dioxide mass measurements

Introduction

Synthetic ethanol for industrial and laboratory use is chiefly produced by the reaction between ethene (g) and water (g):

$$CH_2{=}CH_2 + H_2O \xrightarrow[\text{acid catalyst}]{300^o,\ 70\text{-}280\ bar} CH_3CH_2OH$$

This method of ethanol production may become a problem as crude oil, the source of ethene, runs out. This has stimulated interest in one of mankind's oldest chemical processes: the production of ethanol from sugars by fermentation. The main source of sugars for fermentation is starch. Americans obtain starch from corn. In Europe starch is obtained from potatoes whereas Asians use their rice as a raw material. Brazil obtains most of its starch from cassava roots, although sugar cane provides much of its ethanol.
The energy derived from (bio)ethanol comes from the sun. The sun's energy is used in the photosynthesis of sugars. Calvin elucidated the pathway of glucose formation from atmospheric carbon dioxide and water:

$$6CO_2(g) + 6H_2O(g) \xrightarrow{\text{sunlight}} C_6H_{12}O_6(s) + 6O_2(g)$$

Mono-sugars like D-glucose (see Figure 1), D-fructose, D-mannose are fermentable to ethanol. D-glucose and D-mannose have the same molecular formula but differ in the way the hydroxyl groups are oriented. These compounds are called stereoisomers.
Disaccharides and polysaccharides need hydrolysis to be converted to fermentable sugars. Sucrose, the disaccharide found in beet sugar and molasses, is hydrolyzed to glucose and fructose (see Figure 2) by invertase, an enzyme found in yeast:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}(s)} + H_2O(l) \xrightarrow{\text{invertase}} \underset{\text{Glucose}}{C_6H_{12}O_6(s)} + \underset{\text{Fructose}}{C_6H_{12}O_6(s)}$$

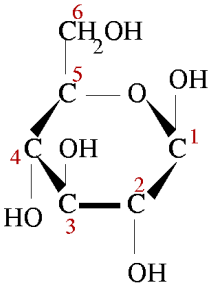

Figure 1: D-glucose

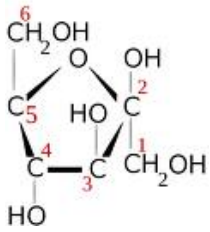

Figure 2: D-fructose

Yeast cells *Saccharomyces cerevisiae* (baker's yeast) are currently used in research to increase the yield of the production of bio-ethanol from sugars. Yeast cells belong to the eukaryotes and are classified as Fungi. Yeasts do not require sunlight to grow, but do use sugars as a source of energy.
S. cerevisiae cells use three major pathways for growth on glucose (1).

First, the fermentation of glucose:
$C_6H_{12}O_6(s) \longrightarrow 2CH_3CH_2OH(l) + 2CO_2(g)$

Second, the oxidation of glucose:
$C_6H_{12}O_6(s) + 6O_2(g) \longrightarrow 6CO_2(g) + 6H_2O(l)$

Third, the oxidation of ethanol:
$CH_3CH_2OH(l) + 3O_2(g) \longrightarrow 2CO_2(g) + 3H_2O(l)$

These three pathways show that *S. cerevisiae* cells can grow in both an oxygen free environment and an oxygen rich setting. Moreover, it shows that growth can occur when glucose becomes very limited or absent and oxygen gas is present.
The first pathway is interesting for our research, because it involves the production of ethanol.
Some research seems to indicate that in the oxygen free process *S. cerevisiae* cells grow best in an environment that is controlled at 20-40°C (2). This raises the question: *what will be the optimal temperature in the conversion of D-glucose to ethanol when S. cerevisiae cells grow in an oxygen free environment?*
Our hypothesis is that the optimal temperature will be closer to 40 °C than to 20 °C, because yeast cells are living organisms. In living organisms enzymes work best at 37 °C. So at that temperature we expect the highest amount of produced carbon dioxide (CO_2) gas at that temperature.

Experimental procedure and approach

We prepared 8 L. of a 18% D-glucose solution in distilled water. Then 10 plastic 0.5 L mineral water bottles were filled with the D-glucose solution three-quarters full. Two bottles were labelled with 20 °C, two bottles with 25 °C and so on. Then one package of dried *S. cerevisiae* (baker's yeast) was added to each plastic bottle. Each bottle was shaken for a few minutes. After that all bottles were filled right up to the top with the D-glucose solution. Then a deflated and labelled balloon, of which the mass was measured beforehand, was fit over the neck of a bottle.
Next the sets of the labelled bottles together with their balloons were placed in 5 different water bathes with temperatures of 20, 25, 30, 35 and 40 °C respectively. Each culture was left in the water bath at a constant temperature for two days. Then the bottles were taken out two by two and dried with clothes. The balloons were carefully tied off and reweighed.
The difference in masses within each set of balloons after and before fermentation were calculated. For each temperature the masses of released CO_2 gas were averaged and the deviation determined and graphically presented.

Results

Within a day we observed that all the balloons started to puff up.
Table 1 presents, in duplicate, the mass (in grams) of the released CO_2 gas at 20, 25, 30, 35 and 40°C. Moreover, it presents the averaged masses of the released CO_2 (in grams) and its deviations at the various temperatures.

Temperature (°C)	Mass CO_2 (g)	Averaged Mass CO_2 (g)
20	1.82 1.94	1.88 ± 0.06
25	2.91 2.75	2.83 ± 0.08
30	4.10 4.06	4.08 ± 0.02
35	5.17 4.97	5.12 ± 0.05
40	2.23 1.91	2.07 ± 0.16

Table 1: Release of CO_2 (in grams) and averaged release of CO_2 (in grams) at 20, 25, 30, 35 and 40 °C.

Figure 3 shows the averaged measured release of carbon dioxide (g) when *S. cerevisiae* cells grow at the temperatures of 20, 25, 30, 35 and 40 °C.

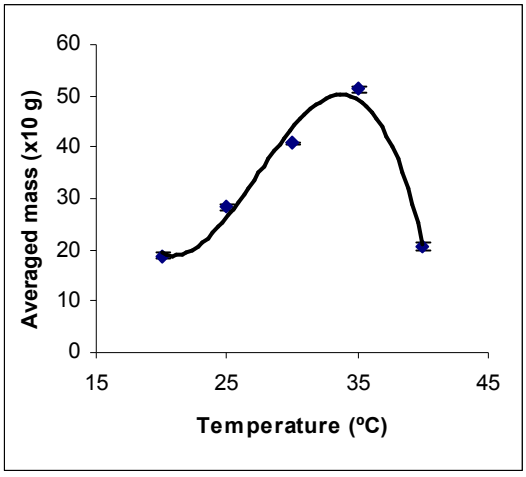

Figure 3: Averaged measured release of CO_2 (g) (in 10x grams) versus temperature (°C).

Data analysis

As presented in Table 1, the average maximum amount of released CO_2 (g) in the fermentation process is 5.12g. This is equal to 5.12g/44.01u = 0.13 mol CO_2. The first equation shows that in theory 1.00 moles of CO_2 gas can be produced.

Interpreting the graph in Figure 3 we see that the highest amount of CO_2 gas is produced at a temperature that is close to 35 °C.

Conclusion and discussion

The observation that all balloons puff up after one day indicates that in all bottles the *S. cerevisiae* culture was growing and produced CO_2 gas.

As is shown in Table 1, the CO_2 gas production was highest at a temperature that was just below 35 °C (Figure 3).

However, in our data analysis we found that the 5.12g or 0.13 mol of CO_2 gas produced is less than the 0.2 moles CO_2 gas that can theoretically be produced. This shows that the temperature close to 35 °C is most probably the optimal temperature for the working of the enzymes in the yeast cells, but it also shows that possibly other factors influence the growth of *S. cerevisiae* cells.

Looking critically at our experimental procedure and approach we see that in all sets of experiments we considered the same independent and dependent variables and we kept the same variables constant.

So, perhaps the problem lies in the possibility that we have overlooked some of the control variables.

Is it necessary to regulate the acidity of the sugar solutions in which the *S. cerevisiae* cells grow as was found for other yeasts (3)?

Or perhaps, in a closed system, the produced ethanol it self creates a stress factor on the growth of the yeast cells and thus the amount of produced bio-ethanol will be less.

This raises a further question for inquiry: how can the yield of bio-ethanol be optimized in an oxygen free environment?

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