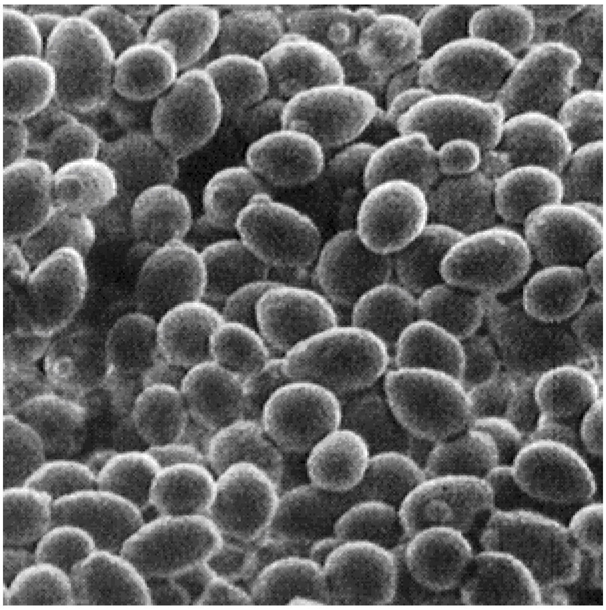
Practical Assignment Chemistry 16-17

FERMENTATION

10 – 20 hours



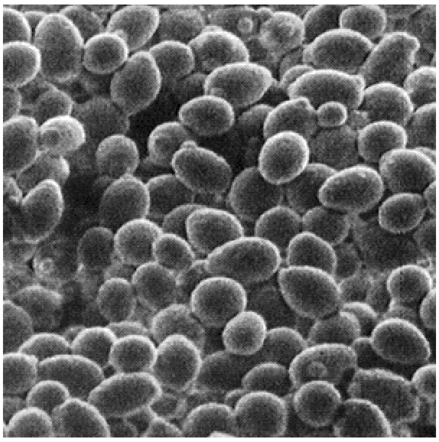
Chemistry Network
Centre for Educational Training, Assessment and Research
Vrije Universiteit Amsterdam The Netherlands, January 2010

Name :

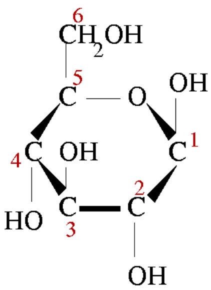
School :

Cooperated with :

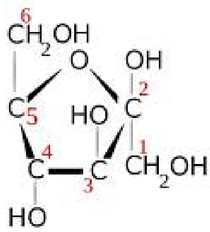
Yeast, *Saccharomyces cerevisiae*, cells



D-glucose

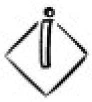


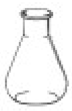
D-fructose



Mark :

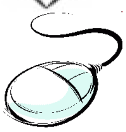
Content


Look for information


Do experiments


Answer questions

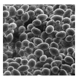

Make observations


Use computer


Write inquiry question
Write inquiry plan


Use model


Keep a record of the inquiry

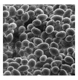


Planning

Below you find a time schedule for the inquiry project, ‘Fermentation’. The first three parts (1-3) are integrated in the chemistry lessons and the others (4-8) will be done outside the chemistry lessons. Your first task is to become familiar with the inquiry. Therefore your teacher will give you a demonstration and you will do a guide experiment. After this you will analyse and judge research done by Slaa, Gnode & Else (2009). These three researchers investigated the optimal temperature to produce bio-ethanol in the fermentation process of D-glucose by yeast cells. How ‘fair’ and accurate is their research? Do you think their research results are trustworthy? Are their conclusions valid? These are questions that you will answer by critically analysing the article written by these three researchers. Following this we expect you – in a team of two – to perform a better inquiry. As a team you will write a first report on your inquiry. All of the first reports will be published on the Internet. In this way you can discuss your results with peers all over the world, giving and receiving suggestions. You have to use these suggestions to improve your report, when you write your final article. All of the articles will compete for the 9th *Natuurwetenschap & Techniek chemistry inquiry award* of 500 Euro.

Time schedule for the ‘Fermentation’ project (10-20 hours):

Start	**Part of the project**	**Date**	**2010**
February	1. *Understand aim and nature of the inquiry project*	February	**Start with the task**
	2. *Understand the research of Slaa, Gnode &Else:* • Predict, observe, explain • Conduct guide experiment • Judge accuracy, reliability and validity	February	
	3. *Own inquiry in teams*	March	**Conduct research**
March	4. *Write report*		
April	5. *Send report* to l.vanrens@ond.vu.nl	**14 April**	**Send report**
	All reports with photographs on the website http://www.onderwijscentrum.vu.nl/internetsymposium	21 April	
	6. *Peer discussion in Internet symposium*	**21 April**	**Start Internet discussion**
April/May/ June	The gastronomy symposium discussion on: • Accuracy in the inquiry plan • Accuracy in performing the inquiry • Reliability of the results • Validity of the conclusions	12 May	**Check ‘symposium’**
	7. *Teamwork*: Processing the comments received, improve report		
	8. *Send final report* to: l.vanrens@ond.vu.nl	**2 June**	**Final articles should be ready!**
June	All first and final reports will be put on the website: http://www.onderwijscentrum.vu.nl/internetsymposium	9 June	**First reports and final articles on the Internet**
	Independent Jury nominates the five best researches	18 June	**Nomination of the best researches**
	Prize will be announced at the site	**25 June**	**And the winner is …!**
November	Publication of the results of the best inquiry in: Natuurwetenschap & Techniek http://www.nwtonline.nl	November	nwt



1 Introduction

Nowadays chemistry forms an integral part of daily life. Whether it is clothes, food, body care, cars, computers or drugs, everything people produce involves chemistry. Without inventions and chemical research the world would be very different. Chemists conduct research to acquire directly applicable knowledge, but sometimes also to understand things better. Most of the time they build on research done by other chemists. Building on knowledge of others can be advantageous because you do not need to examine things yourself. On the other hand previous research causes problems of its own. When results are not reliable, invalid conclusions may be drawn. Other research can be distorted by invalid information and this may have serious consequences. That's why accurate and reliable research is important.

> One of the aims of this inquiry project is to understand how to measure **'fair'** and **accurate** in your inquiry. This accuracy is necessary to get **reliable results** in your inquiry. The only way to draw **valid conclusions** is to get reliable results.

Only when 'fair' and accurate measurements are taken, research results will be reliable and valid conclusions can be drawn. Research should also be designed in such a way that other researchers can repeat it. This does not mean, however, that knowledge based on research results in itself is justified. Further investigations or other research can yield results that are slightly different or even undermine acquired knowledge. Researchers communicate about their research methods and their conclusions in professional magazines, journals and on the Internet. Another way of informing the public and politicians is by means of papers and television.
The researchers Slaa, Gnode & Else (2009) investigated what happens when yeast cells ferment sugar or D-glucose at different temperatures in an oxygen free environment. We take their article as a starting point in your inquiry project. You analyse and judge their research on whether they designed their experiments in a fair way and measured accurately. Are their results reliable and did they draw valid conclusions?
These questions are to be tackled in small teams. The answers will result in designing your own (team) inquiry that of course will be designed and carried out in an as 'fair' and accurate manner as possible. You will write (as a team) a report about your inquiry that you will send to l.vanrens@ond.vu.nl. Your report will be submitted to fellow researchers at others schools in an Internet symposium. In this so-called "fermentation discussion" you can comment on inquiries of other investigation teams and expect critical comments on your own inquiry as well. When the symposium is finished every team has to correct and improve their report. This will be your final article.
Each inquiry team will send their final article to l.vanrens@ond.vu.nl. A professional jury will judge all incoming final articles and will select the best inquiry. Those students whose inquiry is considered the best will win the 9th *Natuurwetenschap & Techniek* chemistry inquiry award and their results will be published in *Natuurwetenschap & Techniek*, November 2010.

2 Demonstration: sugar and baker's yeast

A 0.5 L Erlenmeyer flask is filled with lukewarm sucrose (or sugar) solution three quarters full. Then a package of dried *Saccharomyces cerevisiae* (baker's yeast) cells are added to the bottle and mixed with the solution.

Prediction

What do you expect to happen in the Erlenmeyer flask?

2A I expect that ……………………….

Why?

Write down your observations, conclusions and explanation.

Observations

Conclusion

Explanation

2B (i) Do you still agree with your expectations as written under “prediction” [2A]?
Yes / No, because

2B (ii) What causes the change in the flask?

Browse and use information from ’the fermentation-tracker’ on www.onderwijscentrum.vu.nl/internetsymposium (2009-2010), click ‘organisation’ and then ‘inquiry’ to find support to your answers.

3 Thought experiment: how much carbon dioxide?

The researcher Slaa et al. (2009) let the *S. cerevisiae* (yeast) cells grow in a 18% D-glucose solution in an oxygen free environment.

Prediction

What do you expect about the amount of carbon dioxide that can be produced?

3A I expect,
that the amount of the produced carbon dioxide gas (in grams) will be smaller/equal/larger than the amount of D-glucose.

Why?

Observations

Slaa et al. (2009) found, in one of their experiments in which they used a 18% D-glucose solution, that 5.17 g of carbon dioxide gas was produced.

Results, conclusion and discussion

3B Calculate the maximum amount of carbon dioxide (in grams) that can be produced by *S. cerevisiae* (yeast cells) in a 18% D-glucose solution.

3C What is the conclusion about this amount compared to the amount of carbon dioxide gas (in grams) that was found by the researchers Slaa et al. (2009)?

3D How would you explain this difference?

Browse and use information from 'the fermentation-tracker' on www.onderwijscentrum.vu.nl/internetsymposium (2009-2010), click 'organisation' then 'inquiry' to find support to your answers.

4 Judging the research of Slaa, Gnode, & Else

‘Yeast and fermentation’

Before analyzing Slaa et al.‘s research on ‘fairness’, accuracy, reliability and validity you will first answer some questions concerning accurate and reliable measurements.

§4.1 Orientation on accurate and reliable measurements

Suppose that you would like to compare the mass of carbon dioxide (CO_2) gas produced by *S. cerevisiae* (yeast cells) in a sucrose or sugar solution at four different temperatures.

What would you do to measure as accurately as possible? Explain

4A

Assume that the recorded mass of CO_2 (g) at a certain temperature equals 2.50 grams.

What would you do to find out if this measurement is reliable? Explain.

4B

When is a series of measurements reliable?

4C

§4.2 The research of Slaa, Gnode, & Else: accuracy, reliability, validity

The research question of Slaa et al. (2009) was: *'what will be the optimal temperature in the conversion of D-glucose to ethanol when S. cerevisiae cells grow in an oxygen free environment?'*
From the demonstration and mind experiment you have learned that carbon dioxide gas and ethanol are produced in an anaerobic or oxygen free fermentation process.
In paragraph 4.1 you have thought about reliability of measurements. In order to be capable of measuring accurately, experiments need to be designed in a 'fair' way. You need accurate measurements to come to reliable results. Only when results are reliable can one draw the most valid conclusions. So the question is how to design your experiments as 'fair' as possible to measure most accurately.
To achieve accurate measurements researchers have to follow certain procedures. You will practice this procedure using the article of Slaa et al. (2009):

> How 'fair' is the design of the research of Slaa et al.?
> How accurate are the measurements of Slaa et al.?
> How reliable are the measurements or results of Slaa et al.?
> How valid is the conclusion drawn by Slaa et al.?

By practicing these steps you will be able to critically judge other research and be capable of doing an accurate inquiry yourself.

A. How 'fair' is the design of the research of Slaa et al.?
To judge a research design, you need to identify all of the variables that play a role in the experiment. To take accurate measurements researchers want to know which variable they will measure. Variables are quantities (e.g. temperature), which can be measured as a number. Usually variables also have a unit (e.g. degree Celsius). Researchers should also carefully take into account other factors (e.g. when measuring the height of a person the floor on which the person stands should be straight), which can interfere with the variable to be measured. When taking variables into account:

1. **List** all of the variables;
2. Choose **one** of the variables
3. **Change** this variable;
4. **Measure the effect** of this change; and at the same time
5. Keep all other variables and factors **constant**.

Researchers distinguish three types of variables:

Independent variable	This is the variable to be **changed**
Dependent variable	This is the variable to be **measured**
Control variables	These are the variables to be kept **constant**

By using distinct variables it is easier for researchers (and other interested people) to understand the research and follow its progress. 'Fair' handling of variables is a difficult aspect of research design. For researchers it is difficult both to recognize 'all' of the variables and to exclude those variables and factors that they do not want to measure or to change. In

other words: to keep all interfering variables and factors constant.
Now it's up to you (in groups) to recognize the different variables in the experimental procedure of the research of Slaa et al. (2009) and to find out whether they handled the variables carefully.
Use the part on 'Experimental procedure' in the article of Slaa et al. (2009); see appendix page b or surf to www.onderwijscentrum.vu.nl/internetsymposium (2009-2010), click 'organization' then 'inquiry' till you reach the article (next to the fermentation-tracker).

Recognizing variables in the research of Slaa et al. (2009):

List all variables and factors that influence the measurements in the experiment as done by Slaa et al. (2009).

4D (i) Variables:

4D (ii) Factors:

What is the independent variable in the experiment done by Slaa et al.?

4E

What is the dependent variable in the experiment done by Slaa et al.?

4F

What are the control variables in experiment done by Slaa et al.?

4G

Did Slaa et al. (2009) forget any control variables?

4H (i) If yes, which one(s)?

4H (ii) Compare your answers to these of the other groups in your class.

B. How accurately did Slaa et al. measure?

When the variables – related to the question under research – are known, the next step is to think about the design and set-up of the experiments. It is important to decide carefully, in advance how to conduct the actual experiment, both the set-up and the measurements. Slaa et al. had to make decisions about:

i. How much D-glucose to use?
ii. How many different temperatures to use and in what range?
iii. How many *S. cerevisiae cells* (yeast) cells to use?
iv. How long should the yeast cells grow in the solution?
v. How often should each experiment be repeated?
vi. What instrument should be used to measure the mass of carbon dioxide?
vii. To what significant figure can the measuring instrument be read off?

With a well-developed research you will be less likely to encounter unpleasant surprises while the experiment is being conducted.
To find out whether Slaa et al. (2009) did collect accurate measurements, you judge the decisions made by them in their experimental procedure, see appendix, page b. Discuss and answer in your group the following questions.

Decisions regarding the experimental set-up

4I (i) Did Slaa et al. choose a suitable D-glucose solution? Explain.

4I (ii) Did Slaa et al. choose appropriate temperatures and range of temperature to be able to measure the released amount of carbon dioxide gas by the yeast cells? Explain.

Decisions regarding the measuring instrument

4J (i) Is the measuring instrument used by Slaa et al. accurate enough? Explain.

4J (ii) Did they read off the mass to a correct significant figure? Explain.

Decisions regarding the number of measurements

4K Slaa et al. did at each temperature the experiment in duplicate.
Was this enough times, according to you? Explain.

C. How reliable are the measurements or results of the research of Slaa et al.?

Before collecting measurements researchers think about how to collect their observations and data, how to present and analyze their results. Collected measurements are presented in tables and graphs. Furthermore, researchers always need to check whether their results are reliable. When measurements show too much deviation, they need to be repeated. Repetition of measurements enhances the reliability.
You are now to judge whether Slaa et al. presented their measurements in a correct manner and whether their measurements are reliable.

Presentation of measurements

4L (i) Slaa et al. presented the masses of CO_2 (g) and the averaged mass in Table 1 as:

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

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

Did they present the measurements in a correct manner? Explain.

4L (ii) Slaa et al. used a graph (see Figure 3) to find the optimal temperature (^oC) at which *S. cerevisiae* cells grow in an oxygen free, anaerobic, environment.

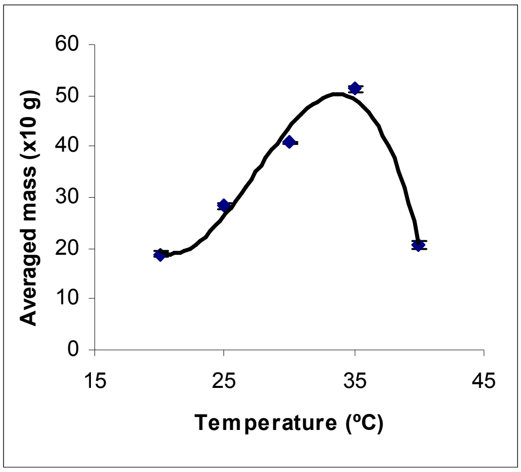


Figure 3: Averaged measured release of CO_2 (g) (in 10x grams) against temperature (^oC).

Did they use the correct variables on the x-axis and y-axis? Explain.

Is the graph in Figure 3 a good representation of the measurements as shown in Table 1? Explain.

Reliability of measurements

4M (i) Slaa et al. (2009) presented their measurements in Table 1 as:

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

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

When looking at a series of measurements, e.g. at 30 ^oC, we see:

Temperature (^oC)	Mass CO_2 (g)	Averaged Mass CO_2 (g)
30	4.10	4.08 ± 0.02
	4.06	

With 4.08 ± 0.02 Slaa et al. state that the measured mass values lie between 4.10 and 4.06 gram.
Their measurements deviate 0.02 from the average mass which is 4.08 gram.
Suppose that the mass values are allowed to deviate **within** 0.5% of the average result.

4M (ii) Which of the values in Table 2 are accurate enough to be reliable?
Encircle them in the Table.
What possible causes of inaccuracy in Slaa et al.'s measurements occur:

(1) Low significance of the mass values. Yes / No Explain.

(2) Low number of measurements. Yes / No Explain.

(3) Lack of keeping control variables and factors constant. Yes / No Explain.

(4) Other causes. Yes / No Explain.

D. How valid is the conclusion of Slaa et al.?

A conclusion can be considered as valid when experiments are accurately designed and carefully executed. Of course, experiments should be designed in such a way that answering the research question is possible. Slaa et al. (2009) research question was: *'what will be the optimal temperature in the conversion of D-glucose to ethanol when S. cerevisiae cells grow in an oxygen free environment?'*. To answer this question first a stock solution of 18% D-glucose was made. Then 5 sets of each two labeled plastic bottles of 0.5L were filled up with this solution and a package of dried yeast cells. Then deflated balloons were fit on the neck of the 10 bottles. The mass of each balloon was determined, after which the bottles were two by two put in a water bath with different, but constant, temperatures. After two days the balloons were carefully tied off and reweighed. For each temperature the averaged masses and the deviation were determined and plotted in a – averaged mass (x10 grams) against temperature (T in ^{o}C) – graph.
The results were presented in Table 1 and Figure 3. From the results as presented in Table 1 and Figure 3 Slaa et al. concluded that *'the CO_2 gas production was highest at a temperature that is close to 35 ^{o}C'* .

	Validity of the conclusion drawn by Slaa et al.
4N (i)	Do you agree with the conclusions Slaa et al. (2009)? Yes / No Explain.
4N (ii)	Is the experimental design of Slaa et al. suitable or valid to find an answer on the research question as stated in their article. Yes / No. Explain.

5 Inquiry in teams

The research question of Slaa et al. (2009) was *'what will be the optimal temperature in the conversion of D-glucose to ethanol when S. cerevisiae cells grow in an oxygen free environment?'* Out of their discussion further questions arise e.g.:

> "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?"

To answer one of these questions, or your own question, you design and conduct your own inquiry. You will do an inquiry and write a report in a 'fair', accurate, reliable and a step-by-step manner.
It is all up to you! Before starting your own inquiry answer the following questions.

An inquiry question can be investigated when this question has an independent (what are you going to change?) and a dependent (what are you going to measure?) variable.

5.1 Formulate your own inquiry question

5A

5B Write down:
(i) your hypothesis

(ii) based on which theory

Now write your own inquiry plan as a team.

5.2 Inquiry plan

A copy of this inquiry plan document can be found at: www.onderwijscentrum.vu.nl/internetsymposium (2009-2010), click ‘organisation’, ‘inquiry’ and then ‘inquiry plan’, next to the fermentation-tracker.

A. Variables

Dependent variable

5C What variable are you going to measure? Explain why.

Independent variable

5D What variable are you going to change? Explain why.

Control variables

5E Which variables and factors do you need to control - keep constant - in your experiment? Explain why.

B. Decisions on the experiment, the experimental set-up and the measurements

How to make accurate measurements?

What instrument for measurement are you going to use? Explain.

5F

What is the accuracy of the instrument?

Up to what significant figure can you read your instrument?
Are repeated measurements needed? Explain.

5G

Which materials do you need? List these materials below.

5H

Make a drawing of your experimental set-up.

5I

What results do you expect?
Explain why.

5J

5K Check whether your inquiry plan is really answering your inquiry question.
If not, change your question into a question that fits to your plan.

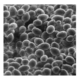
Discuss your plan with your teacher. If she/ he agrees, you can start your experiments.
Good luck!

5.3 Keep a record of the inquiry

A copy of this inquiry plan document can be found at: www.onderwijscentrum.vu.nl/internetsymposium (2009-2010), click ‘organisation’, ‘inquiry’ and then ‘keep a record of the inquiry’, next to the fermentation-tracker.

Inquiry dairy

Date	Work done	Remarks / Observations



6 First and final report, Internet symposium: guidelines

This booklet needs to be handed in to the teacher. As a team you will get a mark for your inquiry plan, your final report and for your participation in the peer discussion in the Internet symposium.

§6.1 Writing a report: guidelines

The layout of a report depends on the journal you are writing for. A report will be published when it satisfies criteria posed by the journal. This will also be the case for your article. After publishing the reports on the Internet the Fermentation symposium or peer discussion starts. You can use the comments to improve your first report as you write a final article. These articles will also be published on the Internet. Then a professional jury will compare all articles and nominate the five best research teams for the 9th *Natuurwetenschap & Techniek* Chemistry inquiry award of 500 euro.
Take Slaa et al.'s article as an example. Your report should contain the following:

- **Snappy but relevant title**
- **Names of the authors and submission date**
- **Summary of the inquiry**
- **Introduction** with the reason of or problem in the inquiry guided by theory on the problem, with the **inquiry question** and with a **hypothesis** and the **theoretical assumptions** concerning the answer on the inquiry question.
- **Experimental design** with a description of the method of investigation, of the way of handling the different **variables** and of the way of handling the **accuracy** in the experimental set-up and the measuring itself.
- **Results** with a description of the **relevant observations**/ **measurements** that are correctly put into **tables and graphs**.
- **Discussion and conclusion** with a critical interpretation of your results and with a valid answer to your inquiry question.
- **Evaluation** with a critical description of the experimental set-up, with suggestions for improvements and further inquiry questions.
- **Bibliography** with relevant resources like textbooks, websites, magazines, articles.

Further guidelines:

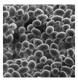
- Use correct **English** and use a layout in **2 columns**.
- Enclose a **picture** or **drawing** of the experimental set-up (max. **100 kb**).
- The report should not exceed **1500 words** (max. **500 kb**).
- **Label** your document with **schoolcode teamnumber_first name_first name**. E.g. ART1_Jens_Sophie
- Add **separately the email addresses of all team members.**
- Add a digital picture of your team (max. **100 kb**) with **schoolcode teamnumber_first name_first name**. E.g. ART1_Jens_Sophie

§6.2 The peer discussion in the Internet Fermentation symposium

To have a meaningful and fruitful discussion with another inquiry team at a school elsewhere, you first need to read their report. Then judge their inquiry report by using the following questions.

- Are the dependent and independent variable visible in their inquiry question?
- Are their assumptions and theory about their hypothesis relevant?
- Did they manage the control variables well?
- Did they measure accurately?
- Are their results well presented?
- Did they track the reliability of their results?
- Can you approve of their discussion and conclusions?
- Did they write a critical evaluation?
- Did they come up with relevant bibliography?

Halfway the Internet symposium you will be checked to see how well you have participated in the symposium. This will be part of the jury's judgement.



7 Study guide

Before	**Understand what your inquiry project is about:** - Read the planning - Choose your inquiry partner
Lesson 1	**Understand what Slaa, Gnode & Else (2009) investigated:** - Read the introduction - Follow the demonstration: Predict, observe and explain - Find information in the fermentation-tracker **Homework:** - **Look at the website, browse the site and the fermentation-tracker** - **Read the article of Slaa, Gnode & Else (2009)**
Lesson 2	**Judge the research of Slaa, Gnode & Else:** - Conduct the mind experiment: how much carbon dioxide? - Orientation on accurate and reliable measurements - Read about variables - Judge Slaa, Gnode & Else's article on handling variables - Judge Slaa, Gnode & Else's article on accuracy
Lesson 3	**Judge the accuracy and reliability in Slaa, Gnode & Else 's research:** - Judge Slaa, Gnode & Else's experimental set-up - Judge the reliability of Slaa, Gnode & Else's measurements - Judge the presentation of Slaa, Gnode & Else's results - Judge the validity of Slaa, Gnode & Else's conclusion
Lesson 4	**Your own inquiry project: question and plan** - Formulate an inquiry question - Design an inquiry plan - Hand in your inquiry plan to the teacher
Lesson 5/6	**Your own inquiry project:** - Conduct your planned experiments - Collect measurements
	Your own inquiry project: - Write a first report as a team (see **Planning**) - Send your first report **with the right code** to: l.vanrens@ond.vu.nl - Discuss the report of another team in the Fermentation Internet symposium - Improve your report and write a final report - Send your final report **with the right code** to: l.vanrens@ond.vu.nl The jury only **judges final reports of teams that participated in the symposium**.

8 List of concepts

Complete the list of concepts. Work gradually on this list as the project proceeds.
Write the definition of:

FERMENTATION

ANAEROBIC FERMENTATION

AEROBIC FERMENTATION

ENZYME

SUCROSE

INDEPENDENT VARIABLE

DEPENDENT VARIABLE

CONTROL VARIABLES

ACCURACY

RELIABILITY

VALIDITY

OVIDUS

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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 will be in the conversion of glucose to ethanol by yeast cells, *Saccharomyces cerevisiae*, baker’s yeast. The fermentation process was followed at temperatures of 20, 25, 30, 35 and 40°C by measuring the release of carbon dioxide. This resulted in an optimal temperature for the fermentation process at 35°C. But it also raised further questions like 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):

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

This method of ethanol production may become a problem as crude oil, the source of ethene, will run short. This knowledge 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 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 is 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 must be converted to fermentable sugars by hydrolysis. 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:

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

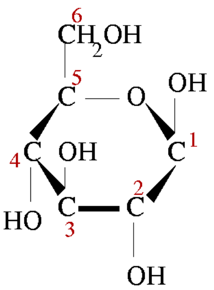

Figure 1: D-glucose

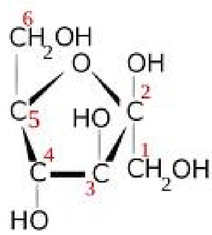

Figure 2: D-fructose

Yeast cells of *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 in the kingdom of 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.

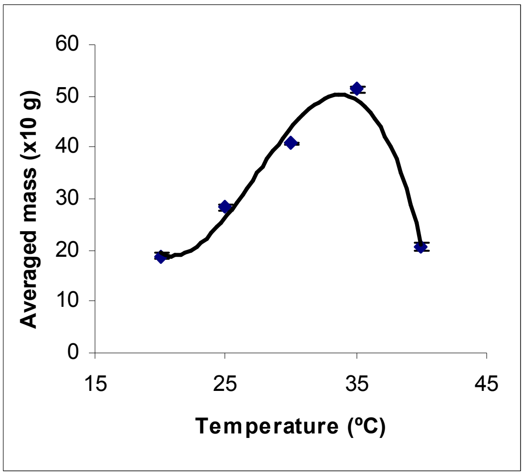

Figure 3: Averaged measured release of CO_2 (g) (in 10x grams) versus temperature (°C).

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?

Bibliography

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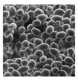

Some words explained.

accuracy	nauwkeurigheid
amount	hoeveelheid
average	gemiddelde
control variables	controlevariabelen
dependent	afhankelijk
dependent variable	afhankelijke variabele
deviation	afwijking
elucidate	toelichten, ophelderen
eukaryotes	eukaryoten, cellen met een kern (bijvoorbeeld gistcellen)
exhausted	uitgeput
Fungi	schimmels
independent variable	onafhankelijke variabele
inquiry	onderzoek
indigestible	onverteerbaar
nature	aard
permeable	doorlaatbaar
reliable	betrouwbaar
reliability	betrouwbaarheid
snappy	pakkend
starch	zetmeel
to deflate	leeglaten
to elucidate	verhelderen
to ferment	vergisten
starch	zetmeel
to submit	insturen
validity	geldigheid