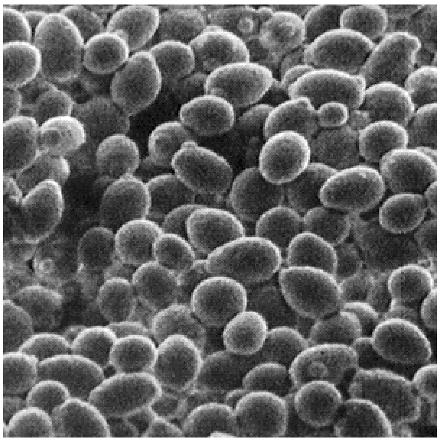
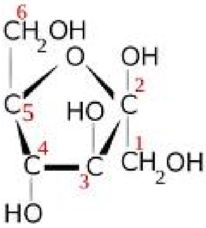
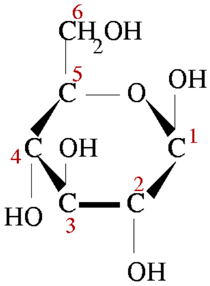


Fermentation

D-glucose





D-fructose

Ontwikkeld voor scholen binnen Bètapartners

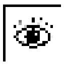
Auteurs: Scheikundenetwerk Onderwijscentrum/VU

Bewerkt door: Lisette van Rens

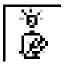
Klas: 5 vwo

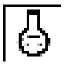
Vak: Scheikunde

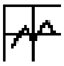
Explanation of the used icons:

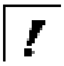
 Observation

 Inquiry Question

 Theory formation

 Experiment

 Execution

 Conclusion

Name:

Cooperated with:

Mark:

Content

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. They 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 the 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. You mail this first report to your teacher. All of the first reports will be published on a website or elo. In this way you can discuss your results with peers in your class, giving and receiving suggestions. You have to use these suggestions to improve your report, when you write your final article.
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 a *chemistry inquiry award*.

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). Then you can start with your own inquiry.

Schedule for the procedure of the 'Fermentation' inquiry project (10-20 hours):

Procedure	Part of the project
Start with the task	*1. Understand aim and nature of the inquiry project*
	2. Understand the research of Slaa, Gnode &Else: • Predict, observe, explain • Conduct guide experiment • Judge accuracy, reliability and validity
Conduct research	*3. Own inquiry in teams*
Write report	*4. Report*
Send first report	*5. Report* to: teacher All reports on a website/*elo*
Peer discussion	*6. Peer discussion* The peer discussion on: • Accuracy in the inquiry plan • Accuracy in performing the inquiry • Reliability of the results • Validity of the conclusions
Process comments	*7. Teamwork*: Processing the comments received, improve report
Send final article	*8. Report* to: teacher All final articles on a website/*elo*
Receive prize	Jury selects the best inquiry

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.

2.1. Prediction

What do you expect to happen in the Erlenmeyer flask?

I expect that

Why?

2.2. Write down your observations, conclusion and explanation.

Observations

Conclusion

Explanation

(i) Do you still agree with your expectations as written under "prediction" [2.1.]?

Yes / No, because

(ii) What causes the change in the flask?

2.3. Browse the Internet for information on fermentation of glucose.

Write down your findings

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.

3.1. Prediction

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

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

(ii) Why?

3.2. 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.

3.3. Results, conclusion and discussion

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

(ii) 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)?

(iii) How would you explain this difference?

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.

(i) What would you do to measure as accurately as possible? Explain

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

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

(iii) When is a series of measurements reliable?

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?'*

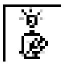
From the demonstration and mind experiment you have learned that carbon dioxide gas and ethanol are produced in an anaerobic or oxygen free fermentation process.

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.

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

Use the part on 'Experimental procedure' in the article of Slaa et al. (2009); see page b.

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

(i) Variables:

(ii) Factors:

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

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

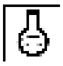
(v) What are the control variables in the experiment done by Slaa et al.?

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

If yes, which one(s)?

(vii) Compare your answers to these of the other groups in your class.

C. 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 article of Slaa et al. (2009) page b.

Discuss and answer in your group the following questions:

C. 1. Decisions regarding the experimental set-up

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

(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.

C.2. Decisions regarding the measuring instrument

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

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

C.3. Decisions regarding the number of measurements

Slaa et al. did at each temperature the experiment in duplicate.

(i) Was this enough times, according to you? Explain.

D. 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.

D.1. Presentation of measurements

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

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.

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

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.

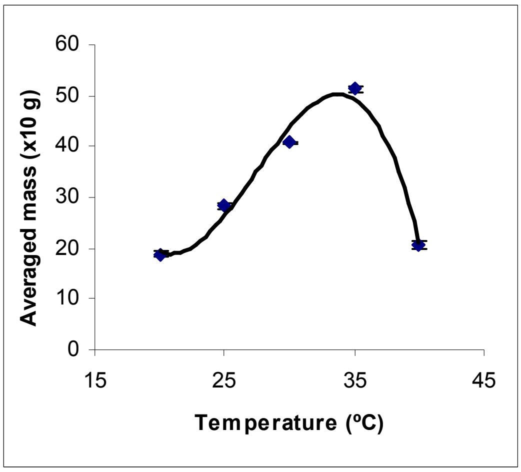


Figure 3: Averaged measured release of CO_2 (g) (in 10x grams) against temperature (^oC).

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

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

D.2. Reliability of measurements

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

Temperature (°C)	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 °C.

When looking at a series of measurements, e.g. at 30 °C, we see:

Temperature (°C)	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 **<u>within</u>** 0.5% of the average result.

Which of the values in Table 1 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.

E. 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 (x 10 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* $35^{\circ}C$*'*.

(i) Do you agree with the conclusions Slaa et al. (2009)? Yes / No Explain.

(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 optimised 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 part in 5.1.

5.1. Formulate your own inquiry question

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.

Write down:

(i) inquiry question:

(ii) our hypothesis:

(iii) based on which theory:

Now write your own inquiry plan as a team.

5.2. Inquiry plan

5.2.1 Variables

Dependent variable

What variable are you going to measure? Explain why.

Independent variable

What variable are you going to change? Explain why.

Control variables

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

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

How to make accurate measurements?

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

(ii) What is the accuracy of the instrument?

(iii) Up to what significant figure can you read your instrument?

(iv) Are repeated measurements needed? Explain.

(v) Which materials do you need? List these materials below.

(vi) Make a drawing of your experimental set-up.

(vii) What results do you expect? Explain why.

(viii) 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

Inquiry dairy

Date	Work done	Remarks / Observations

6. First and final report, peer discussion: 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 article and for your participation in the peer discussion.

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 website/*elo* the 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 website/*elo*. Then a professional jury will compare all articles and nominate the best research for Chemistry inquiry award.

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 **teamnumber_first name_first name**.
- Add **separately the email addresses of all team members.**

6.2. The peer discussion in the Fermentation symposium

To have a meaningful and fruitful discussion with another inquiry team in your class, 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 discussion 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

- 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:

- 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, et al. '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 and collect measurements
- Write a first report as a team (see **Planning**)
- Send your first report **with the right code** to your teacher
- Discuss the report of another team in the Fermentation symposium
- Improve your report and write a final report
- Send your final report **with the right code** to your teacher

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

9. Some words explained

accuracy	nauwkeurigheid
amount	hoeveelheid
average	gemiddelde
control variables	controlevariabelen
dependent variable	afhankelijke variabele
deviation	afwijking
eukaryotes	eukaryoten, cellen met een kern (b.v. 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 lopen
to elucidate	verhelderen, duidelijk maken
to ferment	vergisten