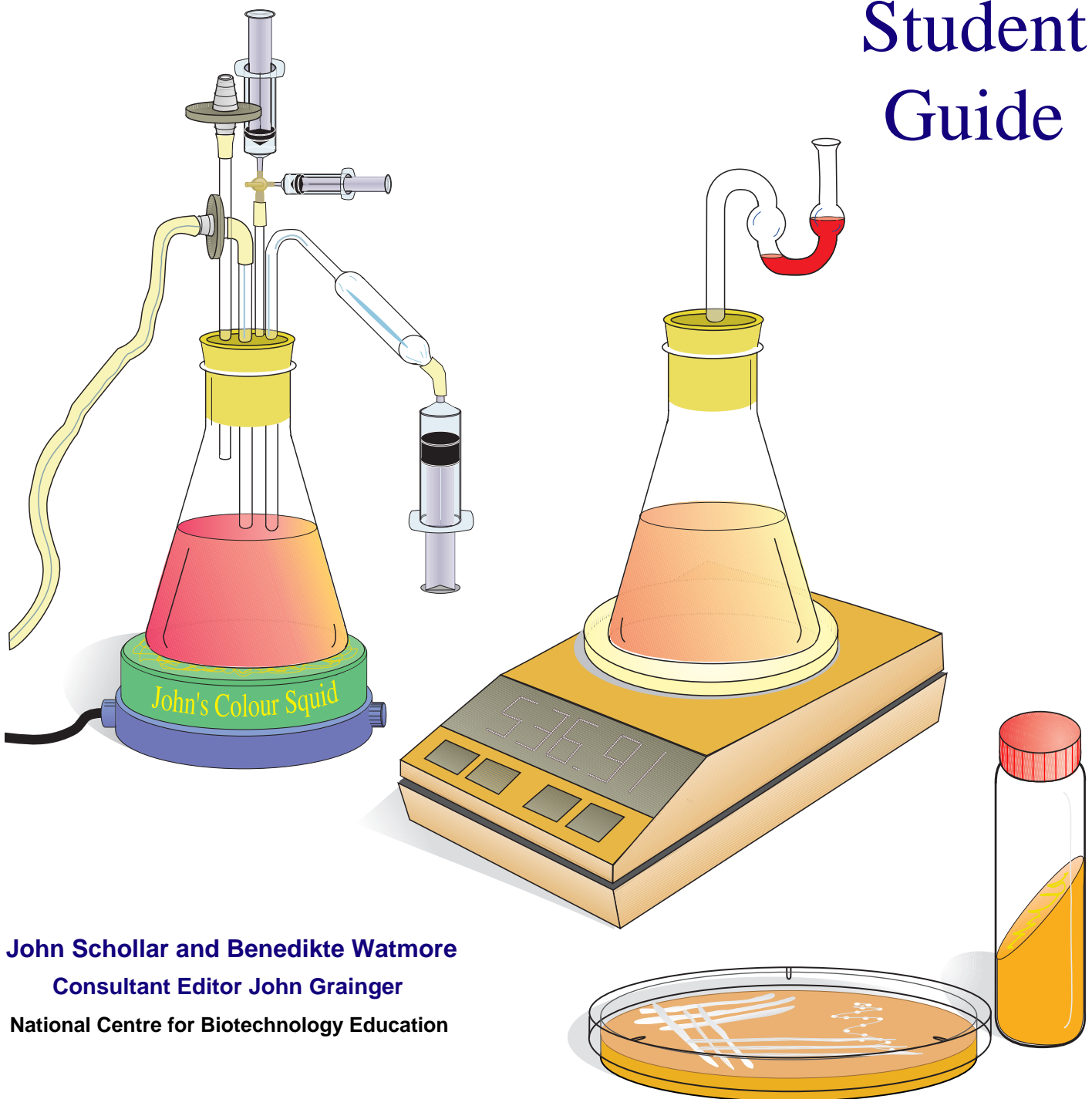




PRACTICAL FERMENTATION

a guide for schools and colleges

Student
Guide



John Schollar and Benedikte Watmore

Consultant Editor John Grainger

National Centre for Biotechnology Education

Project sponsored by The Society for General Microbiology

Contents:

Investigation 1	Sauerkraut – a natural traditional fermentation	3
Investigation 2	Two or three sugar substrate	4
Investigation 3	Balancing the loss of carbon dioxide	5
Investigation 4	Yeast cells and enzyme – together they can do it	6
Investigation 5	A sugary choice	7
Investigation 6	How do they like it? – alcohol levels and pH	9
Investigation 7	Deep purple! – a dark secret	9
Investigation 8	Nothing's for free – you gain some, you lose some!	10
Investigation 9	Ester production – a fragrant or smelly fermentation?	11
Investigation 10	Dextran production – a sticky fermentation	12
Investigation 11	Some sticky investigations – by gum!	13
Investigation 12	Probably the best yeast in the world	14
Investigation 13	Probably the best pigment in the world	15
Investigation 14	<i>Vibrio natriegens</i> – for a speedy growth curve	16
Information 1	The bubble logger	17
Information 2	Principles of a bioreactor	18
Background reading		
Good Laboratory Practice – GLP for all!		

Safety:

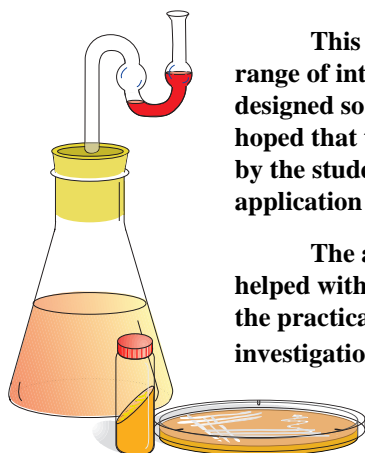
All investigations should be carried out using good laboratory practice. It is essential to read the section on the outside back cover before starting work. Chemicals and procedures requiring special care are marked with a warning symbol in the text.

Introduction:

Practical Fermentation is written for students who are following an advanced course in biology, particularly those taking an option in microbiology and biotechnology. It is also intended to be of value to those students who are studying science courses which contain a fermentation unit.

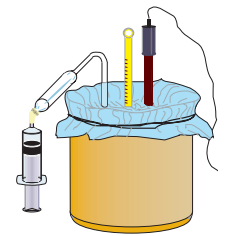
This resource pack is a collection of practical activities aimed at introducing the user to a range of interesting and thought provoking fermentations. The investigations have been designed so that on completion they will give the user a new insight into fermentation. It is also hoped that the extension activities will lead on to other more demanding investigations designed by the students themselves. Many of the extension activities focus on activities that allow the application of statistical analysis.

The authors would like to thank the many students, teachers and colleagues who have helped with comments and suggestions during the development of the activities. We hope that the practicals will not only form the basis for class activities but also the stimulus for individual investigations into fermentation.





Investigation One

Sauerkraut - a natural traditional fermentation



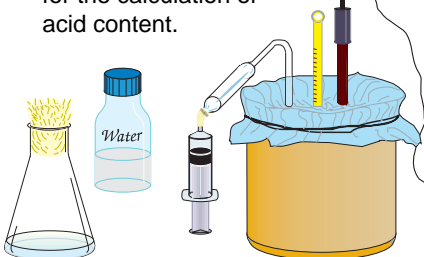
The production of sauerkraut is a traditional fermentation in which the sugars in the cabbage are fermented to acids by the naturally occurring bacteria that are found on the leaves. The cabbage is shredded and salted and under anaerobic conditions the sugars are converted to acids, ethanol, mannitol, esters and carbon dioxide. *Lactobacillus plantarum* is one of the important bacteria involved in the conversion of sugars and mannitol to lactic acid. The removal of mannitol is especially important as it imparts a bitter flavour to the sauerkraut.

Equipment and materials


300 g finely shredded cabbage
 300 cm³ 3% w/v sodium chloride solution
 1 dm³ glass beaker
 pH electrode and meter
 Temperature electrode (optional)
 15 cm³ bent glass pipette with 3 cm rubber tubing
 Restriction clip (Hoffman clip)
 Large plastic bag (approx. 34 cm x 26 cm)
 Scissors
 Adhesive tape
 Elastic bands
 Small metal weights
 3 x 99 cm³ sterile water for each population count
 Rogosa agar and GYLA plates (3 of each per count)
 (GYLA = Glucose Yeast Lemco Agar)
 Sterile 1 cm³, 2 cm³, 5 cm³ and 10 cm³ syringes
 Sterile spreader, and a capped beaker of IMS for flaming spreader 
 Burette containing 0.1 M sodium hydroxide solution
 Flasks containing 10 cm³ deionised water
 Phenolphthalein indicator solution and dropping pipette 

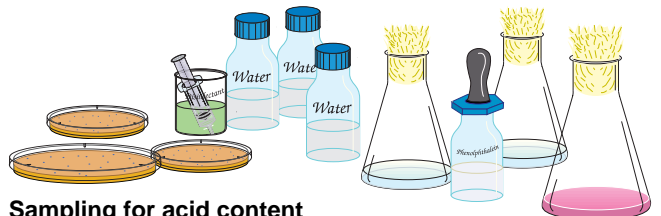
Procedure

- Place 300 g of finely shredded cabbage in the 1 dm³ beaker. Add sufficient sodium chloride solution to just cover the cabbage.
- Cut three sides of the plastic bag to give a single sheet of approximately 300 mm x 500 mm. Cut two small holes for the pH probe and modified pipette, approximately 150 mm in from each side on the central fold of the sheet. (A third hole will have to be cut if a temperature probe is used).
- Place plastic over the surface of the cabbage and insert probes and pipette through holes. Make as airtight a seal as possible around each probe with the adhesive tape. Secure the plastic around the beaker with two elastic bands. Press down with weights to exclude as much air as possible.
- Record initial pH (and temperature) and continue to record daily for two weeks.
- During this period, samples of the liquid should be taken for making bacterial population counts.
- Samples should also be taken for the calculation of acid content.




Sampling for population counts

- Prepare plates (Rogosa and Glucose Yeast Lemco Agar).
- The bent arm pipette provides safe and accurate sampling from the fermentation vessel.
- As aseptically as possible take 1 cm³ of liquid from the bottom of the sauerkraut container using a sterile 5 cm³ syringe attached to the bent arm pipette with the tubing.
- Add the sample to 99 cm³ of sterile water (10⁻²). Mix thoroughly and then aseptically remove 1 cm³ of the 10⁻² dilution and add to a second bottle of sterile water (10⁻⁴). Aseptically remove 1 cm³ of the second diluted solution and add to a third bottle of sterile water (10⁻⁶).
- Make lawns on both types of agar plates with 0.1 cm³ of each of the dilutions using three new sterile syringes. Flame the spreader with alcohol between each spreading. 
- After incubation of the plates for 24 - 48 hours (25°C) count the colonies and calculate the population of organisms present in the fermentation (number per cm³).



Sampling for acid content

- Aseptically remove 5 cm³ liquid from the fermentation and add to 10 cm³ deionised water. Titrate against 0.1M sodium hydroxide solution using a few drops of phenolphthalein solution as an indicator.  (*Good laboratory practice must be observed when using the indicator solution.*)
- Calculate the percentage of acid by applying the formula:

$$\% \text{ lactic acid} = \frac{\text{titre, cm}^3 \times \text{molarity of NaOH} \times \text{mol. mass of lactic acid}}{\text{cm}^3 \text{ sample} \times 10}$$

Assuming no acetic acid is present this value can be used as the amount of lactic acid produced by the fermentation. Care will need to be taken when determining the end point of each of the titrations. Consider how many replicates should be carried out to obtain a meaningful set of results.

Extension activities

- A student thinks that older cabbages contain more sugar and will therefore produce better sauerkraut more quickly. Investigate this idea by taking six old cabbages and six young cabbages and observing the time taken to obtain maximum acid production. Is there a statistical difference?
- Another student, Peter, suggests that the older the cabbages are the greater the number of bacteria they will have and the better the sauerkraut will be. Obtain population counts from at least six different samples of young and old cabbages to test this idea. Is there a significant statistical difference? Comment fully on Peter's suggestion.



Investigation Two

Two or three sugar substrate

Strains of the yeast *Saccharomyces cerevisiae* are used for the production of ales and the yeast *Saccharomyces carlsbergensis* is used for the production of lagers. An important difference between the two yeasts is that one can ferment raffinose completely but the other cannot. Traditionally, ales are produced from top fermenting yeasts with a fermentation period of three to five days at 15 - 20°C. Lagers on the other hand are produced from bottom fermenting yeasts, usually for seven to ten days at 6 - 8°C.

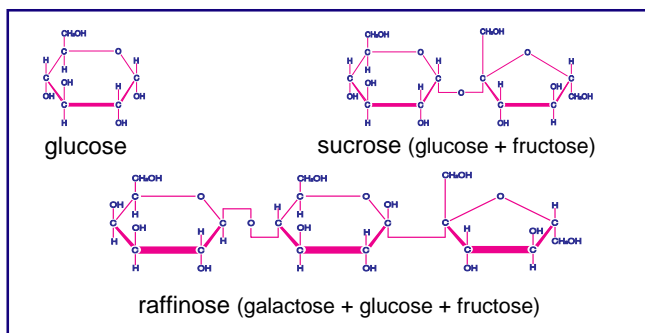
Equipment and materials

Culture of *S. cerevisiae* (e.g. Allinson's dried active baking yeast)
 Culture of *S. carlsbergensis*
 2 x malt agar plate
 40 cm³ GYEP broth
 (containing 2% glucose, 1% yeast extract, 1% peptone)
 400 cm³ RYEP broth
 (containing 5% raffinose, 1% yeast extract, 1% peptone)
 400 cm³ SYEP broth
 (containing 5% sucrose, 1% yeast extract, 1% peptone)
 4 x silicone rubber bung with a single hole
 4 x glass fermentation lock
 Non-absorbent cotton wool and greaseproof paper or aluminium foil
 Sterile water
 5 x Universal bottle
 4 x sterile Pasteur pipette
 Inoculating loop
 4 x wide-necked 250 cm³ flask
 Shaker (optional)
 4 x magnetic stirrer and follower (optional)
 Universal indicator solution (full range)
 4 x NCBE bubble logger
 4 x sterile 10 cm³ syringe

Procedure

Day 1

- 1 If yeast is a slope culture. Streak a loopful of each yeast culture from the stock culture bottles on to malt agar plates.
- 2 If yeast is a dried culture. Make a slurry of 1 g of yeast in 10 cm³ sterile water in a Universal bottle. Shake well to ensure an even slurry. Streak a loopful of the slurry on to a malt agar plate.
- 3 Incubate each plate at 25 - 30°C for 24 - 48 hours to check purity and to produce active cultures for the investigation.
- 4 Prepare 4 x 10 cm³ GYEP broth in Universal bottles. Autoclave for 15 minutes at 103 kPa (121°C).
- 5 Prepare 2 x 200 cm³ RYEP broth and 2 x 200 cm³ SYEP broth in four 250 cm³ wide necked flasks. (If magnetic stirrers are to be used then place a magnetic follower in each flask before sterilisation).



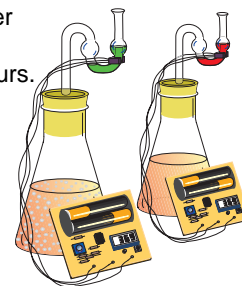
- 6 Fit each flask with a silicone rubber bung which has a non-absorbent cotton wool plug in the hole. Cover the bung with either greaseproof paper or aluminium foil. Autoclave for 15 minutes at 103 kPa (121°C).

Day 2 or 3

- 1 Aseptically inoculate two of the Universal bottles with a loopful of *S. carlsbergensis* and the other two Universal bottles with a loopful of *S. cerevisiae*.
- 2 Incubate at 25 - 30°C for 24 hours on a shaker or agitate frequently by swirling the bottles by hand for good aeration.

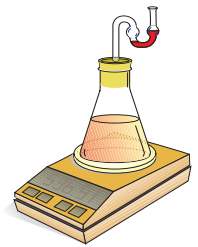
Day 3 or 4

- 1 Using aseptic technique remove the cover and cotton wool plugs from the bungs and carefully insert the glass fermentation locks. (See GLP safety information.)
- 2 Add 1 cm³ of universal indicator solution and 1 cm³ of water to each fermentation lock.
- 3 Label the flasks appropriately and select the best grown of each yeast culture. Then aseptically inoculate one flask of SYEP broth and one flask of RYEP broth with 5 cm³ of the swirled *S. carlsbergensis* culture using a sterile syringe. Repeat for the two remaining flasks using the culture of *S. cerevisiae*.
- 4 Attach a bubble logger to each fermentation lock (see bubble logger information) and place flasks on magnetic stirrers or mix contents by swirling frequently. Incubate at room temperature (15 - 20°C) and record the number of bubbles produced at suitable intervals over the next 48 - 72 hours. If a data logger or computer is to be used then the bubble logger should be connected to the logging device.
- 5 Compare the abilities of the two yeasts to ferment the two sugars.



Extension activities

- 1 A pair of students reasoned that the fermentation industry must be offered a variety of different sugars at different prices from the commodities markets. Stuart wanted to find out if his mother's baking yeast could ferment glucose better than raffinose. John wanted to investigate the idea that all yeasts would ferment monosaccharides better than trisaccharides. Consider how both of these ideas could be made into investigations and statistically valid data obtained.
- 2 Another group of students considered the temperatures at which ale and lager fermentations are carried out and came up with the following question. Does *S. carlsbergensis* ferment better than *S. cerevisiae* at 6 - 8°C? Consider the question and how this could form a statistically valid investigation.
- 3 Research the use of sugars and enzymes in the brewing industry.



Investigation Three

Balancing the loss of carbon dioxide

Yeasts ferment sugars anaerobically to produce alcohol and carbon dioxide. The mass of carbon dioxide lost can be measured by weighing the fermentation vessel during incubation to provide an indication of the rate of the fermentation. Brewing strains of the yeast *Saccharomyces cerevisiae* can ferment simple sugars but they cannot use polysaccharides such as starch. This is why grapes, containing natural sugars, are used directly for wine production but barley requires malting to break down the polysaccharides for beer production.

Equipment and materials

2 g dried baker's or brewer's yeast
 920 cm³ GYEP broth
 (containing 2% glucose, 1% yeast extract, 1% peptone)
 2 x Universal bottle
 2 x sterile Universal bottle
 2 x 500 cm³ wide necked flask
 2 x silicone rubber bung with a single hole
 Non-absorbent cotton wool
 Greaseproof paper
 Elastic bands
 2 cm³ silicone antifoam and 1 cm³ syringe
 2 x glass or plastic fermentation lock with lid or cotton wool plug in the exit vent
 Universal indicator solution (full range) and 1 cm³ syringe
 Balance suitable for weighing flasks up to 1000 g, sensitive to 0.1g
 Boiling water bath

Procedure

- 1 Prepare 920 cm³ of GYEP broth.
- 2 Transfer 450 cm³ of GYEP broth to each of two flasks and add 1 cm³ of antifoam to each with a syringe. Place a silicone bung containing a cotton wool plug into the neck of each flask.
- 3 Cover the bung with a double square of grease-proof paper and secure with an elastic band. Autoclave both flasks for 20 minutes at 103 kPa (121°C). At the same time autoclave two Universal bottles containing 10 cm³ of GYEP broth.
- 4 Weigh 1 g of yeast into each sterile Universal bottle.
- 5 When cool, aseptically add the yeast to each Universal bottle of broth.
- 6 Shake well to produce a yeast slurry.
- 7 Denature the yeast in one bottle by placing it in a boiling water bath for one hour.
- 8 After autoclaving the flasks remove the greaseproof paper covers and aseptically add the contents of one Universal bottle to flask A and the contents of the other to flask B.
- 9 Remove the cotton wool plugs and carefully insert a fermentation lock into each bung. (See GLP safety information.)
- 10 Add approximately 1 cm³ of Universal indicator solution and 1 cm³ of water to each fermentation lock with a syringe.
- 11 Record the mass of flasks A and B immediately and at suitable intervals during the next few days. Incubate at room temperature.
- 12 When no further loss in mass is recorded add a measured amount of glucose to the flasks and record any further loss in mass over the next few days.

Plot a graph of mass against time

Points for consideration

Glucose → ethyl alcohol + carbon dioxide.
 Can the alcohol concentration be worked out from this equation?

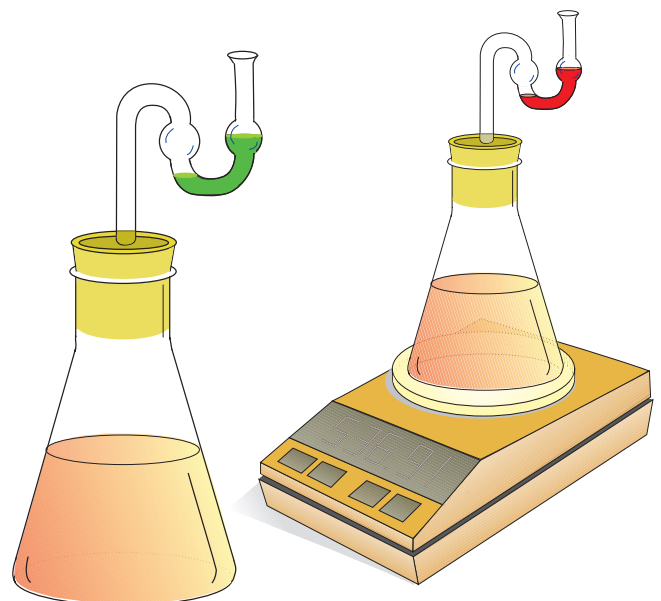
What factors have been ignored in the equation? What further information is needed to improve the quantitative nature of the investigation?

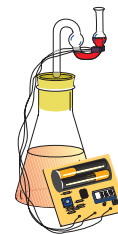
Work out the mole equivalents for the equation (and particularly for the carbon dioxide produced).

Would different sugars give the same mole equivalent of carbon dioxide?

Extension activities.

- 1 The sugar used in this investigation is glucose but what might happen if different sugars are used?
- 2 Compare the rate at which different strains of brewing and baking yeasts can utilise different sugars.
- 3 A group of students investigating the loss of carbon dioxide from sucrose and glucose argued that since glucose is a monosaccharide it would use the sugar more efficiently. They investigated the time taken for the rate of loss of carbon dioxide to become constant in six glucose and six sucrose containing flasks. They then applied a Mann-Whitney U test. Another group then worked out the slope of the lines using regression analysis and compared the gradients also using a Mann-Whitney U test. Finally a member of the group suggested that they could not use the gradient of the loss unless the points on the graph fall on an approximately straight line. Carry out the investigation and give your opinion.





Investigation Four

Yeast cells & enzyme - together they can do it

Although the brewing yeast *Saccharomyces cerevisiae* is able to ferment many simple sugars, such as the monosaccharide glucose and the disaccharide sucrose, to alcohol and carbon dioxide, it does not have an enzyme system to allow fermentation of the disaccharide lactose. However, by co-entrapping the yeast and the enzyme lactase (β -galactosidase), the yeast is able to ferment the sugars formed from the enzymic hydrolysis of lactose. In this investigation yeast cells and enzyme are immobilised together in a calcium alginate matrix.

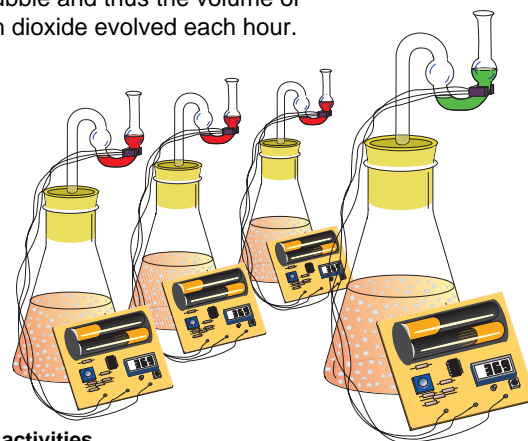
Equipment and materials

- 4 x 5 g baker's or brewer's yeast
- 4 x 100 cm³ beakers
- 4 x 50 cm³ water (deionised or distilled)
- Glass rod
- 4 x 50 cm³ 4% sodium alginate solution
- 2 x 10 cm³ lactase enzyme
- 4 x 200 cm³ 2% calcium chloride solution
- 4 x 250 cm³ wide neck flask
- 4 x magnetic stirrers and follower (optional)
- 6 x 10 cm³ syringes
- Tea strainer
- 2 x 150 cm³ 8% glucose solution in 0.5% calcium chloride
- 2 x 150 cm³ 8% lactose in phosphate buffer (0.1 M pH 7.0)
- 4 x wide necked bung with glass fermentation lock
- Universal indicator solution (full range) and 1 cm³ syringe
- 4 x NCBE bubble logger

Procedure

- 1 Place 50 cm³ water into a small beaker and add 5 g dried yeast.
- 2 Carefully stir the yeast into the water with a glass rod to ensure a thorough mix. Try not to mix air into the slurry.
- 3 Pour 50 cm³ 4% sodium alginate solution into the yeast slurry.
- 4 Carefully stir the sodium alginate solution into the yeast slurry to ensure a thorough mix. Again try not to stir air into the mixture.
- 5 For investigations involving co-immobilisation of the enzyme lactase with the yeast cells add 10 cm³ of lactase to the yeast slurry and sodium alginate solution. For investigations that do not use the enzyme lactase add a further 10 cm³ of water to the slurry.
- 6 Place 200 cm³ 2% calcium chloride solution into one of the flasks that is to be used for the fermentation. Add a magnetic follower and place on a magnetic stirrer and start stirring gently or mix by gently swirling the flask by hand.

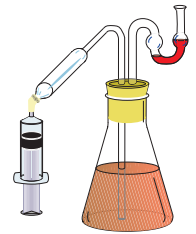
- 7 Draw the yeast-alginate mix up into a 10 cm³ syringe. Add the mixture drop by drop into the calcium chloride solution so that it forms small regular beads. To ensure the beads set fully, leave them in the calcium chloride solution for about ten minutes.
- 8 Separate the beads from the calcium chloride solution by using a tea strainer to hold them back in the flask.
- 9 Repeat the process three more times using the other flasks. The flasks containing immobilised yeast should be labelled 1 and 2. The flasks containing the co-immobilised yeast and enzyme should be labelled 3 and 4.
- 10 Add 150 cm³ 8% glucose solution to flasks 1 and 3. Add 150 cm³ 8% lactose solution to flasks 2 and 4.
- 11 Firmly place a bung with a fermentation lock in it, into each flask. Δ (See GLP safety information.) Add 1 cm³ of Universal indicator solution and 1 cm³ of water to each fermentation lock.
- 12 Attach a bubble logger to each fermentation lock. (See bubble logger information.)
- 13 Leave at room temperature (15 - 20°C) for up to 24 hours.
- 14 At the end of the investigation work out the volume of one bubble and thus the volume of carbon dioxide evolved each hour.



Flasks	Time in minutes					
	60	120	180	240	300	360
1 Yeast in glucose solution						
2 Yeast in lactose solution						
3 Yeast & lactase in glucose solution						
4 Yeast & lactase in lactose solution						

Extension activities

- 1 After a lesson on microbial growth and food hygiene a student, Kate, finding some mouldy food in the fridge at home, postulated that this was because fungi tend to be more tolerant of acid conditions than bacteria. Kate then started to consider whether the activity of enzymes from different microbes was influenced by different conditions. She came up with a hypothesis that fungal lactase would work better than bacterial lactase at a lower pH. Investigate this hypothesis and apply a statistical test to validate your hypothesis. Bear in mind that calcium chloride in the sugar solution helps to stabilise the beads during the fermentation and the buffer helps to control the pH of the lactose solution. Consider possible effects on any statistical investigations you may perform.
- 2 Consider the advantages and disadvantages of enzyme immobilisation and cell entrapment to the food industry.



Investigation Five

A sugary choice

In the absence of oxygen yeast cells obtain their energy from anaerobic fermentation, a process in which sugars are converted to alcohol and carbon dioxide. During fermentation the yeast *Saccharomyces cerevisiae* ferments different sugars at different rates. As the fermentation progresses it produces a change in the acidity of the medium. Thus there is a relationship between the acidity of the medium and the amount of fermentation. In this investigation the rate of fermentation is measured by the increase in acidity.

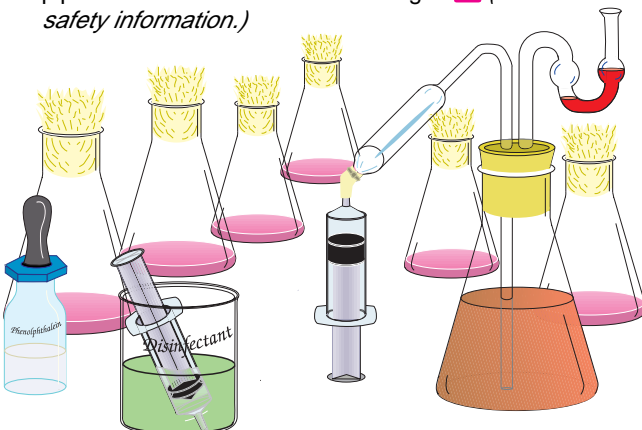
Equipment and materials

- 8 x 2 g dried baker's or brewer's yeast
- 200 cm³ 0.2 M fructose solution
- 200 cm³ 0.2 M galactose solution
- 200 cm³ 0.2 M glucose solution
- 200 cm³ 0.2 M lactose solution
- 200 cm³ 0.2 M maltose solution
- 200 cm³ 0.2 M raffinose solution
- 200 cm³ 0.2 M sucrose solution
- 8 x 0.5 g ammonium phosphate, NH₄ H₂PO₄ (or "yeast nutrient" from home brew shops)
- 8 x 0.5 g ammonium sulphate, (NH₄)₂ SO₄ (or "yeast nutrient" from home brew shops)
- 8 x 250 cm³ wide necked conical flask
- 8 x silicone rubber bung with two holes
- 8 x glass fermentation lock
- Universal indicator solution (full range) and 1 cm³ syringe
- 8 x 15 cm³ bent glass pipette with 3 cm rubber tubing
- 8 x restriction clip (Hoffman clip)
- 8 x glass rod
- 50 cm³ burette
- 8 x 20 cm³ syringe (or equivalent) for sampling
- 8 x 100 cm³ flask for titration
- 0.1 M sodium hydroxide solution (about 400 cm³)
- Phenolphthalein indicator solution and dropping pipette ⚠

Procedure

Day 1

- 1 Label eight 250 cm³ flasks: glucose, fructose, lactose, sucrose, galactose, maltose, raffinose and control (water). Add 200 cm³ of 0.2 M sugar solution to the named flasks and 200 cm³ of water to the control flask.
- 2 Add 2 g of dried yeast and then 1 g of ammonium salts to each flask (0.5 g each of ammonium phosphate and ammonium sulphate).
- 3 Ensure that the yeast is resuspended and the salts are dissolved in the sugar solution by carefully stirring each solution with a different glass rod.
- 4 Carefully and firmly insert the fermentation lock and bent pipette into the silicone rubber bungs. ⚠ (See GLP safety information.)



- 5 Place the bungs firmly into the neck of the flasks and add 1 cm³ of Universal indicator solution and 1 cm³ of water into the fermentation lock.
- 6 To assist the fermentation the flasks should be placed in an incubator at (20 - 25°C) or kept at room temperature (15 - 20°C).

Day 2

- 1 Set up a burette containing 0.1 M sodium hydroxide solution.
- 2 Swirl the flask to ensure a homogenous mix of culture and remove a total of 25 cm³ of sample (10 cm³ + 15 cm³) with a 20 cm³ sampling syringe.
- 3 Place the removed sample into a small flask and add two or three drops of phenolphthalein solution. ⚠ (Good laboratory practice must be observed when using the indicator). Titrate the sample against the alkali solution in the burette. Repeat the process for each sugar solution and the control.
- 4 Plot a histogram of the volume of the alkali used to neutralise each sugar solution. The histogram can be used to indicate the extent of fermentation.

Sugar	Volume of alkali used (cm ³) (over night cultures)
Glucose
Lactose
Galactose
Sucrose
Maltose
Fructose
Raffinose
Control (water)

Extension activities

- 1 Compare your data with the results of other groups which have duplicated the investigation. Are there enough replicates to be able to apply meaningful statistical analysis? If not, consider how another investigation could be designed for statistical tests to be applied.
- 2 Suzie was fascinated by all the different types of brewing yeasts she found in her local home brew shop. Some were for ales, some for lagers and some for wines. On her way home she wondered if all yeasts ferment the same sugar equally well. Design a project to explore this idea.

Investigation Six


How do they like it? - alcohol levels and pH




Pasteur's work in the late nineteenth century was important in showing that yeasts were responsible for the fermentation process. In 1875 Emil Hansen joined the new scientific laboratory at the Carlsberg brewery in Copenhagen where in 1883 he isolated the first pure culture of yeast. Many of today's alcoholic beverages use yeast strains that have been carefully selected and maintained over the last hundred years. These strains confer on the fermentation process specific features that produce unique products (e.g. aromas & flavours).

A. The effect of alcohol concentration on fermentation

Equipment and materials

Yeasts (ale, wine and champagne)
 60 cm³ 4% sucrose solution
 60 cm³ water
 12 cm³ ethanol 
 Non-absorbent cotton wool
 6 x 25 cm³ tube or 50 cm³ measuring cylinder
 Glass stirring rod and syringes (1 cm³, 5 cm³ and 10 cm³)
 6 x malt agar plate and inoculating loop

Procedure

- 1 Make 6 different 20 cm³ concentrations of ethanol in sucrose solution by measuring the amounts shown below. 

Final ethanol conc.	0%	1%	5%	10%	15%	20%
4% sucrose (cm ³)	10	10	10	10	10	10
water (cm ³)	10	9.8	9.0	8.0	7.0	6.0
ethanol (cm ³)	0.0	0.2	1.0	2.0	3.0	4.0

- 2 Transfer each solution to a tube or a measuring cylinder.
- 3 Select one type of yeast and add 0.1 g to each tube and stir carefully to resuspend.
- 3 Make tight plugs of non-absorbent cotton wool to fit the tubes or cylinders.
- 4 Leave the tubes at room temperature (about 15 - 20°C).
- 5 Record observations at regular intervals over 24 - 48 hours. Compare the effervescence and turbidity of each sample. How has the ethanol concentration affected cell activity and growth?

Other ethanol concentrations can be made to determine a more accurate threshold of tolerance. Compare different strains of yeasts, such as ale, wine and champagne.

If time allows population counts can be performed before and after incubation to determine any increase in biomass.

To test the yeast's viability, aseptically remove a sample of the yeast from each container and streak on to a malt agar plate.



Extension activities


- 1 Design an investigation to see if there is a correlation between cell number (population) and alcohol concentration. In the design of the investigation consider the number of replicates needed to ensure that a statistically valid test can be applied.
- 2 After fermentation the brewer must wait for the yeasts to sediment out before the brew can be bottled or barrelled. Brewers often prefer high-flocculating yeasts, which after fermentation fall quickly to the bottom of the vat. The concentration of the sugar maltose in the wort affects the rate of flocculation. Design a quantitative investigation to examine the effect of maltose on yeast flocculation and sedimentation.
- 3 An increase in the temperature of a fermentation normally causes an increase in the rate of reaction. Investigate the effect of temperature on the fermentation process using different yeast strains. Consider the implications for the brewing industry.

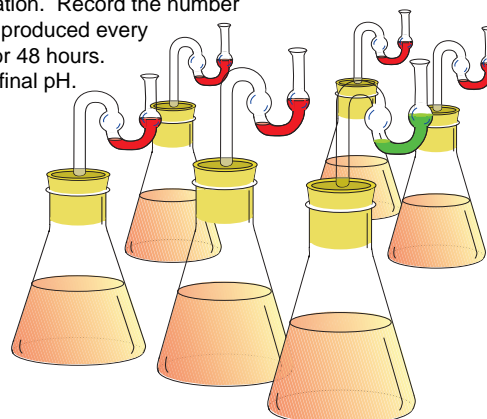
B. The effect of pH on fermentation

Equipment and materials

Yeasts (ale, wine and champagne)
 0.5 M phosphate buffer solutions (pH 4, 6, 7, 8 & 9)
 Culture ingredients: sucrose, yeast extract, peptone
 Non-absorbent cotton wool
 6 x 150 cm³ flask
 6 x Universal bottle containing 5 cm³ sterile water
 6 x NCBE bubble logger
 6 x glass fermentation lock
 6 x silicone rubber bung with single hole to fit flask
 Universal indicator solution and 1 cm³ syringe

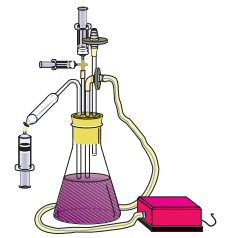
Procedure

- 1 Prepare 100 cm³ of six 0.5 M buffer solutions in the flasks (pH 4, 6, 7, 8, 9 and a second pH 7 as a control).
- 2 Add 2 g of sucrose, 1 g of yeast extract and 1 g of peptone to each of the six buffer solutions.
- 3 Carefully place a glass fermentation lock into each bung and place in the neck of the flasks.  (See GLP safety information.)
- 4 Add 1 g of appropriate yeast to each Universal bottle of sterile water and resuspend. Autoclave one of the samples to kill the yeast, for the control flask.
- 5 Add one bottle of swirled yeast slurry to each flask. Fit the bung containing the fermentation lock to the flask. Swirl carefully to mix the yeast slurry into the buffered medium.
- 6 Add 1 cm³ of Universal indicator solution and 1 cm³ of water to a fermentation lock. Repeat for all the other fermentation locks.
- 7 Attach a bubble logger to each fermentation lock. (See bubble logger information.) Use the bubble loggers to monitor the rate of fermentation. Record the number of bubbles produced every half hour for 48 hours.
- 8 Check the final pH.



Extension activities

- 1 As yeasts ferment sugar they also produce acids that change the pH of the medium. A student theorised that yeasts grow better in acid environments and thus there would be an increase in fermentation activity and an increase in bubble production in more acidic media. Plot a graph of experimental data and calculate whether there is a positive or negative correlation for bubbles produced against pH of the medium.
- 2 What happens to any correlation and hypothesis if alkaline conditions are considered?



Investigation Seven

Deep Purple! - a dark secret

The Gram-negative bacterium *Janthinobacterium lividum* (formerly known as *Chromobacterium lividum*) produces a deep purple pigment called violacein. The pigment is insoluble in water but soluble in alcohol and has antibiotic properties. A small signalling molecule (*N*-acyl homoserine lactone) found in some Gram-negative bacteria has created considerable interest among many researchers. These molecules, bacterial pheromones, act as regulatory systems to control physiological processes associated with population growth and pigment production.

Equipment and materials

Culture of *Janthinobacterium lividum*
 2 x glucose nutrient agar plate (GNA)
 500 cm³ glucose nutrient broth (GNB)
 (6.5 g Oxoid dehydrated nutrient broth in 500 cm³ deionised or distilled water, 5 g glucose, pH 7.0)
 Bioreactor
 2 x Universal bottle
 Sterile silicone antifoam
 Inoculating loop
 Sterile 1 cm³ syringe
 2 x sterile 10 cm³ syringe
 Sterile 3-way tap
 Aquarium pump and tubing
 Magnetic stirrer and follower (optional)

Procedure

Day 1.

- 1 Prepare two streak plates of *Janthinobacterium lividum* on glucose nutrient agar. Incubate for 24 - 48 hours at 25°C.
- 2 Prepare glucose nutrient broth and pour 450 cm³ into the bioreactor. Autoclave for 20 minutes at 103 kPa (121°C), allow to cool and store at 4°C until required.
- 3 Add 10 cm³ of glucose nutrient broth to each of two Universal bottles and autoclave for 15 minutes at 103 kPa.

Day 2 or 3.

- 1 Select the plate with best growth of *Janthinobacterium lividum*. Inoculate both broths in the Universal bottles with *Janthinobacterium lividum*. Incubate at 25°C for 24 hours. Incubate in a shaker if possible; if not, careful swirling of the bottles by hand every few hours assists growth of the bacterium.

Day 3 or 4.

- 1 Allow the bioreactor to come to room temperature.
- 2 Aseptically add the sterile 3-way tap to the bioreactor.
- 3 Use a sterile 1 cm³ syringe to add 1 cm³ of sterile antifoam to the bioreactor via the 3-way tap.
- 4 Add 10 cm³ of *Janthinobacterium lividum* culture (select the culture with best growth) using a sterile 10 cm³ syringe via the 3-way tap.
- 5 Replace the used 10 cm³ syringe with a new sterile syringe. The used syringe should be discarded to disinfectant solution.
- 6 Connect the air supply to the bioreactor and adjust the air flow so that the medium is aerated and continue aeration for 24 - 48 hours. Incubate at 25°C.

Day 4 or 5.

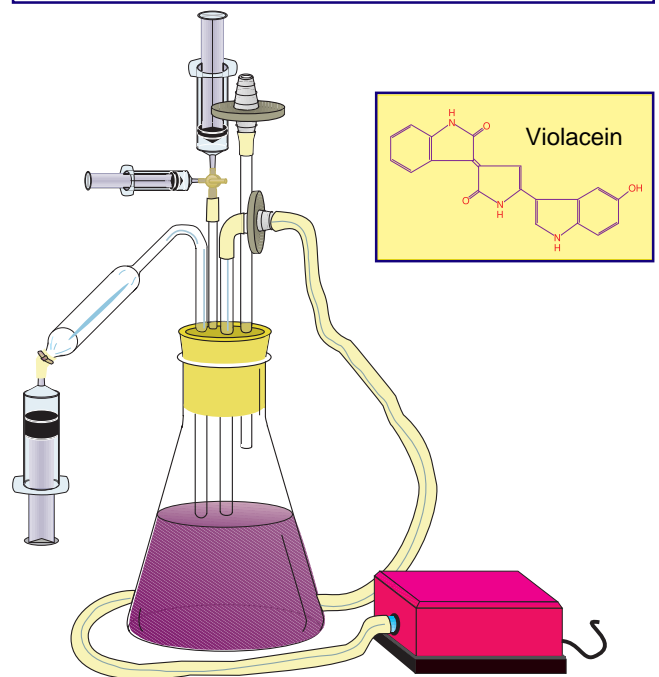
- 1 Record the colour and how well the culture has grown in the bioreactor. The intensity of the purple colour depends on the environmental conditions.

Points for consideration

Would the addition of an inducer such as *N*-acyl homoserine lactone to the broth influence the production of violacein? How can any changes be quantified?

Are signalling molecules that influence production of the purple pigment (violacein) in *Janthinobacterium* produced by any of the following organisms: *Micrococcus luteus*, *Erwinia carotovora*, *Escherichia coli*, *Rhizobium leguminosarum*?

How can any synergistic relationships be quantified?

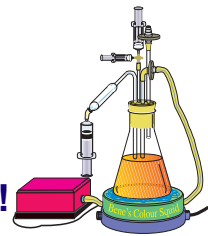


Extension activities

- 1 What is the correlation between bacterial cell count and pigment production? Plot a graph of pigment production against number of bacterial cells. Compare the correlation between pigment production and cell count in this activity with another coloured bacterium like *Micrococcus roseus*.
- 2 Is there a correlation between pigment production and the presence of Gram-negative bacteria? Is this the same for Gram-positive bacteria? Plot graphs and apply statistical tests to confirm any correlation.
- 3 Investigate the factors that influence cell growth and pigment production e.g. incubation time, degree of aeration and light. Extract the pigment from the cells by using a suitable technique and if possible purify the pigment by using a mini-purification column.
- 4 The literature suggests that the pigment has antibiotic properties. Design an investigation to examine the claim and evaluate its potency.

Investigation Eight

Nothing's for free - you gain some, you lose some!



During cellular respiration complex organic substances are broken down to simpler compounds releasing chemical energy that is essential for cell growth and other activities. Since all living cells need energy this is a universal process. In investigations that evaluate microbial growth it is essential to link biomass formation or product production with substrate use. If the loss of a sugar substrate from a fermentation is measured and the increase in the biomass is recorded then the yield coefficient for the fermentation process can be calculated.

Equipment and materials

Fresh dried bakers' yeast, *Saccharomyces cerevisiae*
500 cm³ of GYEP broth
(10% glucose, 1% yeast extract, 1% peptone)

Bioreactor

3 x Universal bottle

10 cm³ sterile water in a Universal bottle

2 x malt agar or glucose nutrient agar plate

Sterile silicone antifoam

Inoculating loop

3 x sterile 1 cm³ syringe

Sterile 3-way tap

Aquarium pump and tubing

Magnetic stirrer and follower

Containers and sterile 10 cm³ syringes for sampling

Procedure

Day 1.

- 1 Prepare and autoclave a bioreactor with 450 cm³ of GYEP broth and two Universal bottles with 10 cm³ of broth. N.B. Long exposure to high temperature can caramelize sugar-rich media; therefore care must be taken when autoclaving i.e. use 15 minutes at 103 kPa (121°C). After autoclaving the bioreactor should be stored at 4°C until needed. (If the bioreactor is to be stirred by a magnetic stirrer then add a magnetic follower before autoclaving).
- 2 Aseptically weigh out 1 g of dried yeast from a fresh pot or sachet into a sterile Universal bottle. Aseptically add 10 cm³ of sterile water to the yeast. Shake thoroughly to resuspend the yeast.
- 3 Aseptically streak a loopful of yeast culture onto two malt agar plates using an inoculating loop. Leave to grow overnight at 25°C.

Day 2 or 3.

- 1 Select the plate with best growth of yeast. Using a wire loop inoculate both broths in the Universal bottles with one or two yeast colonies from the agar plate. Incubate at 25°C for 24 hours. Incubate in a shaker if possible; if not, careful swirling of the bottles by hand every few hours assists growth of the yeast.

Day 3 or 4.

- 1 Allow the bioreactor to come to room temperature and aseptically add 1 cm³ of sterile antifoam.
- 2 Select the *Saccharomyces cerevisiae* culture with best growth. Inoculate the bioreactor with 1 cm³ of the broth culture and turn on the stirrer and aerator to mix the yeast inoculum into the broth. Turn off the stirrer and aerator after ten minutes. Using the sampling unit and a sterile syringe remove 1.5 cm³ of broth so that the initial yeast population and glucose content can be estimated.

It is important that measurements are made without delay to give reliable initial values. If this is not possible then place the sample in a fridge and test later.

- 3 The bioreactor should be incubated at 25°C. Initially samples should be taken every 6 hours if possible but at least every 12 hours. More frequent samples should be taken once a change has been noted, e.g. hourly. The bioreactor should be monitored for about 24 - 48 hours. The population of yeast cells and glucose levels should be measured for each sample.

An easy way of measuring the glucose content of the broth is to use semi-quantitative diabetic glucose test strips e.g. Roche *Diabur-Test® 5000*. If the solution is too concentrated, or more accurate results are needed, then dilutions can be made and percentages calculated.

A yield coefficient can be calculated from the glucose loss from the broth and the biomass increase of the yeast. The loss of glucose can be measured easily by the use of glucose test strips. There are various methods for measuring biomass. Consider them all and then use the most appropriate one to measure the increase. Mathematically the yield coefficient for biomass production can be expressed as:

$$\text{Yield coefficient } Y_{x/s} = - \frac{dx}{ds}$$

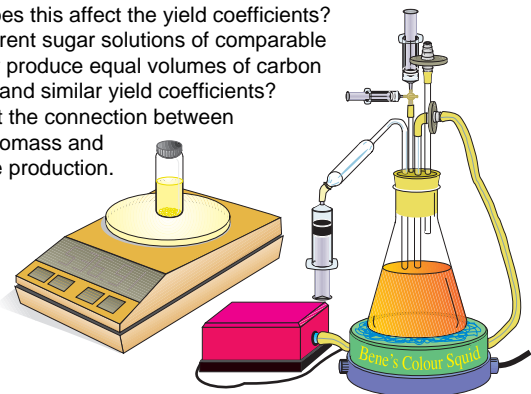
Where ds is the decrease in substrate concentration corresponding to a small increase in microbial biomass, dx. The negative sign indicates that x and s vary in opposite senses. Providing the growth conditions remain constant the yield coefficient remains constant. In the following expression x₀ and s₀ represent the initial biomass and substrate concentration respectively and x and s the values at time t during microbial growth.

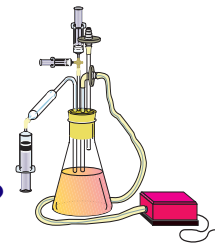
$$(x - x_0) = Y_{x/s} (s_0 - s)$$

N.B. The yield coefficient varies with the growth conditions.

Extension activities

- 1 Do different microbes produce different yield coefficients?
- 2 Do different sugar substrates, in anaerobic fermentations, produce different volumes of carbon dioxide? If so, does this affect the yield coefficients? Do different sugar solutions of comparable molarity produce equal volumes of carbon dioxide and similar yield coefficients?
- 3 Find out the connection between yeast biomass and Marmite production.





Investigation Nine

Ester production - a fragrant or smelly fermentation?

Esters are responsible for fruity flavoured aromas and are formed by the condensation of an alcohol with an acid. Bacteria such as *Lactobacillus*, *Lactococcus* and *Pseudomonas* produce esters such as ethylacetate, ethylbutyrate, ethylisovalerate and ethylhexenoate. The yeasts such as *Hansenula anomala*, *Candida utilis* and *Pichia anomala* also produce esters during fermentation. Since these esters are very volatile their aroma can often pervade a whole laboratory. Many of these are of commercial importance.

Equipment and materials

Culture of *Pichia anomala*
 2 x malt agar plate
 450 cm³ GYEP broth
 (10% glucose, 1% yeast extract, 1% peptone)
 20 cm³ GYEP broth
 (2% glucose, 1% yeast extract, 1% peptone)
 Bioreactor
 2 x Universal bottle
 Sterile silicone antifoam
 Inoculating loop
 Sterile 1 cm³ syringe
 2 x sterile 10 cm³ syringe
 Sterile 3-way tap
 Aquarium pump and tubing

Procedure

Day 1.

- 1 Prepare two streak plates of *Pichia anomala* on malt agar medium. Incubate for 24 - 48 hours at 30°C.
- 2 Prepare GYEP broth (10% glucose) and pour 450 cm³ into a bioreactor. Autoclave for 15 minutes at 103 kPa (121°C), allow to cool and store at 4°C until required.
- 3 Add 10 cm³ of GYEP broth (2% glucose) to each of two Universal bottles and autoclave for 15 minutes at 121°C.

Day 2 or 3.

- 1 Select the plate with best growth of *Pichia anomala*. Inoculate both broths in the Universal bottles with *Pichia anomala*. Incubate at 25°C for 24 hours. Incubate in a shaker if possible; if not, careful swirling of the bottles by hand every few hours assists growth of the culture.

Day 3 or 4.

- 1 Allow the bioreactor to come to room temperature.
- 2 Aseptically add the sterile 3-way tap to the bioreactor.
- 3 Use a sterile 1 cm³ to add 1 cm³ of sterile antifoam to the bioreactor via the 3-way tap.
- 4 Select the culture with best growth. Using aseptic technique and a sterile 10 cm³ syringe add 10 cm³ of *Pichia anomala* culture via the 3-way tap.
- 5 Replace the used 10 cm³ syringe with a new sterile syringe. The used syringe should be discarded into disinfectant solution.
- 6 Connect the air supply to the bioreactor and adjust the air flow so that the medium is gently aerated. The air flow should be sufficient to mix the yeast cells and medium to ensure an aerobic fermentation but not so strong that the volatile compounds are all driven off. Incubate for 24 hours at 25°C.

Day 4 or 5.

- 1 Using good laboratory practice - smell the result! ⚠

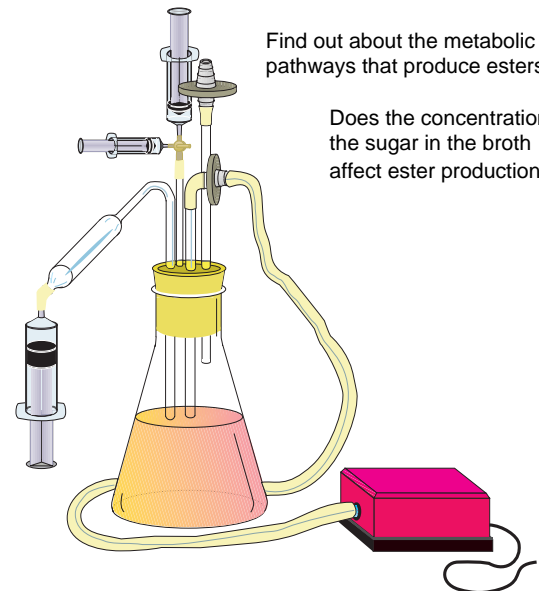
The air flow can now be increased to help drive off the volatile compounds, this should intensify the aroma.

Points for consideration

Consider different methods that might be used to extract and concentrate the esters formed in the fermentation.

Find out about the metabolic pathways that produce esters.

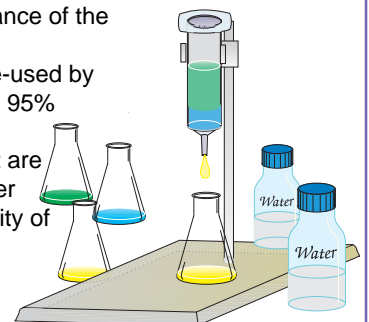
Does the concentration of the sugar in the broth affect ester production?



Demonstration of downstream processing

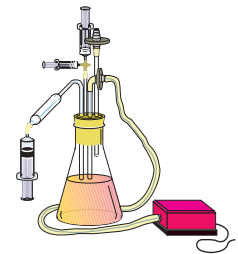
The separation of food pigments by an Isolute column

- 1 Charge the Isolute column by allowing 2 cm³ of a 95% solution of ethanol to drain through the column by gravity. ⚠
 - 2 Add 0.5 cm³ of green food dye and collect the pigment that comes through in a small flask.
 - 3 Wash the column with 1 cm³ of water to remove the last traces of the first dye. Collect the sample in a second flask.
 - 4 Change the charge in the column by passing 2 cm³ of 20% ethanol solution through and collect the dilute ethanol solution in a third flask.
 - 5 Compare the appearance of the different solutions.
 - 6 The column can be re-used by washing with 2 cm³ of 95% ethanol solution.
- If time and equipment are available then consider ways in which the purity of the coloured products could be evaluated and measured.



Investigation Ten

Dextran production - a sticky fermentation



The bacterium *Leuconostoc mesenteroides* converts sucrose to dextran, a glucose polysaccharide which is used commercially, e.g. in hospitals as a plasma substitute for treating haemorrhages and burns. Whereas it is usual for polysaccharides to be produced intracellularly, *Leuconostoc mesenteroides* synthesises dextran extracellularly. This is achieved by the enzyme dextran sucrose which acts outside the cell by splitting sucrose into fructose and glucose and assembling the glucose molecules to form dextran. The mass of the dextran produced varies with sucrose concentration and temperature of incubation.

A. Dextran production

Equipment and materials

Culture of *Leuconostoc mesenteroides*
 2 x GNA (glucose nutrient agar) plate
 20 cm³ starter broth (glucose nutrient broth + 4% sucrose)
 400 cm³ fermentation broth
 (17% sucrose, 0.14% yeast extract, 4 x pH 7.0 tablets)
 Bioreactor
 200 cm³ medical flat or similar bottle
 2 x Universal bottle
 Sterile silicone antifoam
 Inoculating loop
 1 cm³ sterile syringe
 Sterile 3-way tap
 2 x 10 cm³ sterile syringe
 Aquarium pump and tubing
 2 dm³ plastic beaker or deep sided tray
 250 cm³ flask, cotton wool, gauze, elastic band, greaseproof paper

Procedure

Day 1.

- 1 Prepare two streak plates of *L. mesenteroides* on glucose nutrient agar. Incubate at 30°C for 2 - 3 days.
- 2 Add 10 cm³ of starter broth to each of two Universal bottles and autoclave for 15 minutes at 103 kPa (121°C).
- 3 Prepare 400 cm³ fermentation broth. Pour 300 cm³ into a bioreactor and the remainder into a 200 cm³ bottle. Autoclave for 20 minutes at 103 kPa (121°C).

Day 3 or 4.

- 1 Select the plate with best growth of *L. mesenteroides*. Inoculate both broths in the Universal bottles with *L. mesenteroides*. Incubate at 25°C for 24 hours. Incubate in a shaker if possible; if not, careful swirling of the bottles by hand every few hours assists growth of the bacterium.

Day 4 or 5.

- 1 Place the bioreactor in the 2 litre plastic beaker or tray.
- 2 Aseptically add 2 cm³ sterile antifoam to the bioreactor via the 3-way tap. Select the starter culture with the best growth. Inoculate the bioreactor with 5 cm³ of culture and gently aerate to mix.

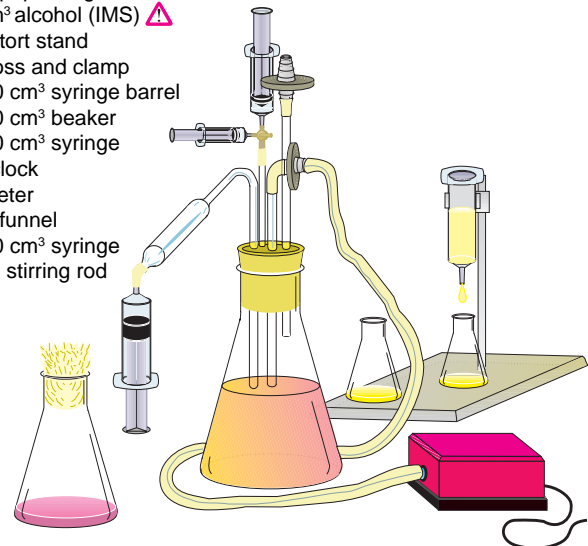
Day 5 or 6.

- 1 Observe the growth of *L. mesenteroides*. Before any further investigations are carried out the sample must be autoclaved. Aseptically transfer a sample from the bioreactor into a 250 cm³ flask, plug with cotton wool wrapped in gauze, cover with greaseproof paper held in place with an elastic band and autoclave for 20 minutes at 103 kPa (121°C). This will kill the bacteria so that the physical properties of the culture can be investigated safely.

B. Dextran investigations

Equipment and materials

Autoclaved sample of uninoculated fermentation broth
 Autoclaved sample of fermentation broth
 Filter paper, e.g. Whatman No.1, 11 cm diameter
 10 cm³ alcohol (IMS) ⚠
 2 x retort stand
 2 x boss and clamp
 2 x 20 cm³ syringe barrel
 2 x 50 cm³ beaker
 2 x 20 cm³ syringe
 Stopclock
 pH meter
 Filter funnel
 2 x 10 cm³ syringe
 Glass stirring rod



- 1 Compare the pH of the broth culture with that of the uninoculated broth medium.
- 2 Compare the viscosity of the broth culture with that of the uninoculated broth medium. Using the retort stands, bosses and clamps, set up two 20 cm³ syringe barrels over two 50 cm³ beakers. Use a syringe to add 20 cm³ of the autoclaved broth culture into one syringe barrel and 20 cm³ of the uninoculated broth into the other. Record the time taken for the samples to pass through the syringe barrels.
- 3 Determine the mass of dextran produced in the broth. Weigh the folded dry filter paper. Add 10 cm³ alcohol to 10 cm³ autoclaved broth culture and stir well. The dextran will precipitate out. Filter through the folded filter paper and allow to dry. The mass of dextran obtained can then be calculated. Determine the total produced by the fermentation.

Extension activity

A class of students used the viscosity of the broth as an indication of the amount of dextran produced in the fermentation. They carried out a range of investigations that involved varying the temperature of the fermentation, the sucrose concentration and the sugar used as the substrate. Make predictions as to the outcome of the different investigations. Any investigations that are carried out should have statistical tests applied to them.

Investigation Eleven

Some sticky investigations - by gum!



Alginate from seaweed has long been used as a thickener in cooking. Gums produced by microorganisms have a wide range of applications in the food industry. The gums can be used on their own or mixed with gums from plants to form synergistic gels. The interaction of these polysaccharides is important in the food processing industry for determination of characteristics such as flavour release and food texture. In 1967 xanthan was the first microbial polysaccharide to be produced commercially and was then approved for food use in 1969 to help improve 'mouthfeel' in processed foods.

A. Alginate beads

Equipment and materials

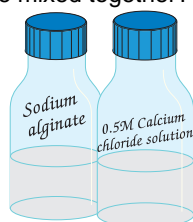
- 10 cm³ 1% sodium alginate soln. (from marine algae or bacteria)
- 100 cm³ 0.5 M sodium chloride solution
- 150 cm³ 0.5 M calcium chloride solution
- 100 cm³ 0.5 M strontium chloride solution
- 4 x Pasteur pipette
- 1 cm³ syringe with short rubber tube
- 4 x beaker 250 cm³
- 1 cm³ 0.1 M EDTA solution (sodium salt) ⚠
- Waterbath and 100 cm³ flask

Procedure

- 1 Place 100 cm³ of the salt solutions (sodium chloride, calcium chloride and strontium chloride) into three different labelled beakers .
- 2 Attach the wide end of a Pasteur pipette to the short piece of rubber tubing on the 1 cm³ syringe. Draw up 1 cm³ of sodium alginate solution into the Pasteur pipette by slowly withdrawing the syringe plunger to the 1 cm³ mark.
- 3 Add the alginate solution dropwise into one of the salt solutions by very gently returning the syringe plunger and observe the effect.
- 4 Repeat the process for the other two salt solutions.
- 5 Compare any alginate beads formed in the different solutions.
- 6 Prepare more beads by dropping 1 cm³ of alginate solution into 50 cm³ of calcium chloride solution in a flask and place in a boiling water bath for 5 minutes. ⚠ (*Good Laboratory Practice must be observed when boiling liquids*). What effect does this treatment have on the beads?
- 7 Very carefully add three or four drops of EDTA solution, a chelating agent, to the beaker containing the beads. ⚠ (*Good laboratory practice must be observed when using the chelating agent*). What conclusion can you draw about the formation of beads and their maintenance? How might the results from this investigation be of relevance to the food industry?
- 8 If time allows, predict and test what might happen if other salt solutions e.g. cupric chloride, magnesium chloride and ferric chloride, are used. What might happen if beads from different salts are mixed together?

Extension activity

A student suggested that calcium chloride might be better than sodium chloride for bead formation. Devise an investigation to test this idea using the Mann-Whitney U test.



B. Xanthan gum

Equipment and materials

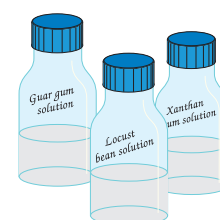
- 50 cm³ 0.25% xanthan gum solution
- 50 cm³ 0.25% locust bean gum solution
- 50 cm³ 0.25% guar gum solution
- 10 cm³ 2 M calcium chloride solution
- 4 x 5 cm³ syringe
- 5 x test tube with bung or plastic Universal bottle
- Waterbath

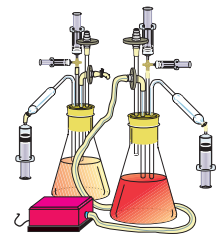
Procedure

- 1 Using a syringe add 5 cm³ of xanthan gum solution to a test tube or bottle. Add 5 cm³ of locust bean gum solution and mix thoroughly by shaking.
- 2 Repeat using xanthan gum and guar gum solutions.
- 3 Add 5 cm³ of xanthan gum solution and 5 cm³ of locust bean gum solution to 10 cm³ of water.
- 4 Repeat for xanthan gum and guar gum solutions and water.
- 5 Mix 10 cm³ of xanthan gum and 10 cm³ of locust bean gum solutions with 10 cm³ of 2 M calcium chloride solution.
- 6 Compare the five mixtures and comment on the quality of gelling. Devise a method of measuring the relative viscosities of the different gels formed.
- 7 Heat each mixture to 90°C in a water bath, then mix by shaking. Observe any changes, allow to cool and note any further changes. ⚠ (*Good Laboratory Practice must be observed when heating liquids*).
- 8 Use water baths at different temperatures e.g. 40°C, 50°C, 60°C to determine the melting point of the gels.
- 9 If time allows, prepare a number of mixtures of equal volumes (5 cm³) of 0.25% xanthan gum solution and 0.25% locust bean gum solution. Then add increasing volumes of water to each to determine the minimum gum concentration required for synergistic gel formation. Consider how work in this area of gel formation might be of relevance to the food industry.

Extension activity

A student suggested that there might be a positive correlation between calcium chloride concentration and viscosity of the gel. Design an investigation to test this idea using a suitable statistical test.





Investigation Twelve

Probably the best yeast in the world

The yeast *Saccharomyces cerevisiae* (K5-5A) used in this investigation is an isolate from the Carlsberg laboratory in Copenhagen. In 1875 a Danish brewer, Carl Jacobsen, built a scientific laboratory alongside his brewery. He appointed a specialist, Emil Christian Hansen who continued work started earlier by Louis Pasteur in France. Pasteur had shown the need for good hygiene to protect beers from infectious contamination and that yeasts were responsible for the fermentation. Hansen isolated the first pure strain of brewer's yeast- *Saccharomyces carlsbergensis*.

Production of yeast pigment

Equipment and materials

- Culture of *Saccharomyces cerevisiae* (K5-5A)
- 2 x malt agar plate
- 1 dm³ GYEP broth (2% glucose, 1% yeast extract, 1% peptone)
- 2 x bioreactor
- 3 x Universal bottle
- Sterile silicone antifoam
- Inoculating loop
- 2 x sterile 1 cm³ syringe
- 2 x sterile 3-way tap
- 8 x sterile 10 cm³ syringe
- Aquarium pump and tubing

Procedure

Day 1.

- 1 Prepare two streak plates of *Saccharomyces cerevisiae* (K5-5A: a Karl 1 mutant) on malt agar. Incubate at 25 - 30°C for 24 - 48 hours.
- 2 Prepare GYEP broth and pour 450 cm³ into each of the two bioreactors and 10 cm³ into three Universal bottles.
- 3 Autoclave both bioreactors and the Universal bottles for 20 minutes at 103 kPa (121°C).
- 4 Allow to cool and store at 4°C until needed.

Day 2 or 3.

- 1 Choose the streak plate with the darkest red colonies. Using aseptic technique transfer a colony to each of three sterile Universals of broth using a wire loop.
- 2 Incubate at 25 - 30°C for 24 hours. Incubate in a shaker if possible; if not, careful swirling of the bottles by hand every few hours assists growth of the yeast.

Day 3 or 4.

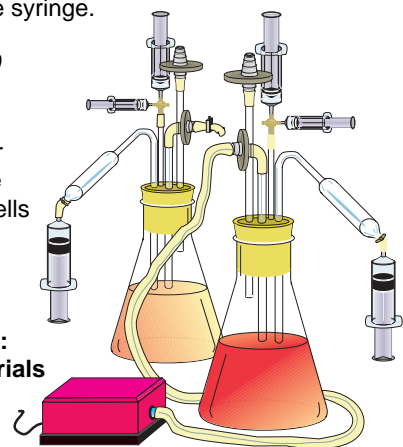
- 1 Allow the bioreactors to come to room temperature.
- 2 Aseptically add the sterile 3-way taps to the bioreactors.
- 3 Aseptically add 1 cm³ of sterile antifoam to each bioreactor using a sterile 1 cm³ syringe connected to the sterile 3-way tap.
- 4 Aseptically add 10 cm³ of K5-5A yeast inoculum from one of the Universal bottles using a sterile 10 cm³ syringe via the 3-way tap. Connect the air supply to the bioreactor and adjust the air flow of the aquarium pump so that the fermenter culture is well aerated.
- 5 Aseptically add 10 cm³ of K5-5A yeast inoculum from the second Universal bottle to the second flask. Do not connect an air flow to this fermenter.
- 6 Incubate the bioreactors for three to four days at 25°C.
- 7 Take samples every day for estimating yeast cell population. Plot cell population against incubation time and compare the results for the two conditions of aeration.

Estimation of total cell population

Aseptically remove from the bioreactor a culture sample (2 - 3 cm³) using the side arm sampling device and a sterile syringe.

(See *Preparing a bioreactor for use.*)

Either use a cell counting chamber or the Breed smear method to estimate the population of cells per cm³ of culture.



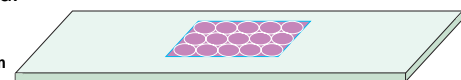
Breed Smear Method:

Equipment and materials

- Beaker of disinfectant
- Microscope
- 4 x microscope slide
- Waterproof marker pen
- 4 x graduated micropipette tip or Pasteur pipette & inoculating loop

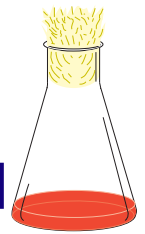
- 1 Using a waterproof marker pen accurately draw a 20 mm by 10 mm rectangle on a microscope slide.
- 2 Into the middle of the rectangle place either a 10µl volume of culture using a graduated micropipette tip or a single drop of known volume from a Pasteur pipette.
- 3 Very carefully spread the sample evenly over the whole of the rectangle using either the micropipette tip or a sterilised inoculating loop. Discard contaminated material and any excess culture to disinfectant.
- 4 Allow to dry and fix by very gently warming in a Bunsen burner flame. ⚠ Use a suitable simple staining procedure and view under a microscope. Determine the field of view, for that magnification, and the number in the 20 mm by 10 mm rectangle.
- 6 Since there is a defined volume spread over a known area and the number of fields of view that make up that area are known, the volume of a single field of view can be calculated.

Area = 10 mm x 20 mm
Volume = 10 µl
If there are 1,000 fields of view
Then 1 field of view = 0.01 µl



Cells in a single field of view can be counted. After counting a number of fields of view an estimate of the population can be made.

Consider the advantages and shortcomings of this method for estimating cell population. How could it be improved?



Investigation Thirteen

Probably the best pigment in the world

Some higher fungi produce very brightly coloured fruiting bodies from which pigments can be extracted. Different species are found in different habitats and many of the pigments obtained are very specific to a given region or country. These pigments have been used to dye wool for many centuries. Today there are still established cottage industries producing wool garments coloured by fungal pigments. However, yeasts, which are also fungi, have not been used traditionally as a source of pigment for dyeing.

A. Extraction of pigment from red yeast.

Equipment and materials

An actively growing culture of red yeast *Saccharomyces cerevisiae* (K5-5A) from Investigation Twelve
 Beaker of disinfectant
 2 x 50 cm³ sterile conical flask
 Cotton wool (non-absorbent)
 4 x sterile 10 cm³ graduated pipette, plugged with cotton wool
 2 x sterile centrifuge tube, plugged with cotton wool
 Centrifuge
 Sterile water, approx. 50 cm³
 Balance
 Pipette and capped tubes for collecting pigment

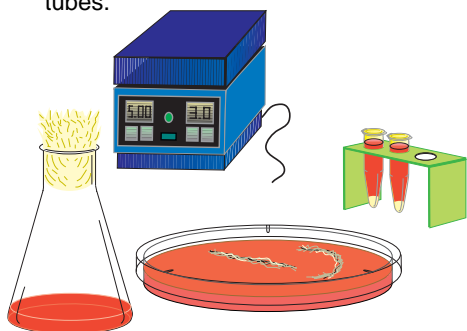
Procedure

Day 1.

- 1 Switch off the air pump from the actively growing culture and allow yeast cells to settle to the bottom of the bioreactor. Place in fridge overnight to assist the settlement.

Day 2.

- 1 Very carefully decant the majority of the supernatant into disinfectant solution. **⚠** (Good Laboratory Practice should be followed.)
- 2 Swirl the remainder of the broth in the bioreactor and transfer to a 50 cm³ flask. Plug the flask with cotton wool.
- 3 Pipette two 10 cm³ aliquots into each of two centrifuge tubes. Check tubes are balanced and spin at a minimum of 3,000 rpm (1,000 g) for 3 minutes.
- 4 Decant the supernatant into disinfectant.
- 5 Add 10 cm³ of sterile water to each tube. Mix well, check tubes are balanced and spin as before. Decant supernatant into disinfectant.
- 6 Add 10 cm³ of sterile water to each tube. Mix with the end of a pipette and transfer to a second 50 cm³ flask. Plug with cotton wool and incubate at 37°C for 48 hours to autolyse.
- 7 Pipette pigment into capped tubes e.g. microcentrifuge tubes.



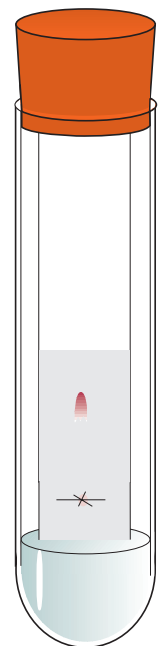
B. Chromatography of the pigment.

Equipment and materials

Decanted pigment from yeast culture (K5-5A)
 Whatman No.1 filter paper, 2 cm x 15 cm
 Boiling tube with bung
 Micropipette
 (made by drawing out the end of a Pasteur pipette in a Bunsen burner flame)
 Drawing pin
 20 cm³ solvent: **⚠**
 glacial ethanoic acid : conc. HCl : water; 30 : 3 : 10
⚠ (Good laboratory practice must be followed in the preparation and use of this solvent, including the use of a fume cupboard).

Procedure.

- 1 Draw a fine pencil line 2.5 cm from one end of the filter paper and mark a light cross on the centre of the line.
- 2 Using a micropipette, spot the pigment solution on to the cross on the filter paper. Repeat approximately 15 times, allowing the spot to dry between each application.
- 3 Fold over the other end of the filter paper and pin to the base of the bung.
- 4 **In the fume cupboard** pour 10 cm³ of solvent into the boiling tube and carefully place the bung with the filter paper into the tube. **⚠** (Check that the base of the filter paper is in the solvent but that the spot is not covered by the solvent).
- 5 Support the boiling tube vertically in a rack and leave to run until the solvent front is about 2 cm from the base of the bung. (About 1 hour).
- 6 Remove the filter paper from the boiling tube, marking the solvent front with a pencil line. Leave to dry in the fume cupboard.
- 7 Observe the chromatogram and make any relevant measurements.

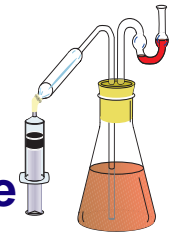


Extension activities

- 1 Compare the chromatography of flower and fruit pigments.
- 2 Find out about procedures for dyeing wool and then investigate the possibility of developing a protocol using the yeast pigment.
- 3 Alternative methods of pigment extraction involve the use of either sodium hydroxide and detergent solutions, or enzymes which lyse the cells rapidly. Investigate any advantages there may be in using these methods instead of autolysis.

Investigation Fourteen

Vibrio natriegens - for a speedy growth curve



Vibrio natriegens is a unicellular Gram-negative marine bacterium that inhabits estuarine muds. This organism is of value in population studies since it can grow quickly and has a short lag phase. Therefore under ideal conditions it can show a complete growth curve of lag phase, exponential growth and stationary phase in just a few hours. This is because its generation time, and thus a doubling of the cell population, is only a few minutes compared to an hour or more for many other microorganisms.

Equipment and materials

Culture of *Vibrio natriegens* on 2% saline nutrient agar (pH 7.5)

2 x 2% saline nutrient agar plate

(a further 48 plates may be needed, see below)

3 x Universal bottle

156 cm³ of 2% saline nutrient broth (pH 7.5)

250 cm³ wide-necked flask

Silicone rubber bung with two holes

Glass fermentation lock

Universal indicator solution (full range) and 1 cm³ syringe

15 cm³ bent glass pipette with 3 cm narrow rubber tubing

Restriction clip (Hoffman clip)

Non-absorbent cotton wool and aluminium foil

Inoculation loop

12 x 10 cm³ sterile syringe

Large beaker half filled with disinfectant solution for disposal ⚠

Water bath and thermometer

10 x sterile Universal bottle

Ordinary graph paper and 3-cycle semi-log paper

Determination of cell population:

Colorimeter, spectrophotometer or turbidity meter with cuvettes

or

30 x sterile plugged Pasteur pipette

1 cm³ syringe with 3 cm wide rubber tubing (to fit over syringe barrel and pipette)

80 x 9 cm³ of sterile saline (0.85%) in Universal bottle

Sterile spreader, and a capped beaker of IMS for flaming spreader

48 x 2% saline nutrient agar plate

Procedure

Day 1.

- 1 Prepare two streak plates of *Vibrio natriegens* on 2% saline nutrient agar. Incubate for 24 hours at 30°C.
- 2 Prepare 2% saline nutrient broth and pour 96 cm³ into the wide-necked flask and 20 cm³ into each of the three Universal bottles.
- 3 Carefully place the modified glass pipette and the glass fermentation lock into the bung and place in the neck of the flask. ⚠ (See GLP safety information.) Place a cotton wool plug into the neck of the fermentation lock and loosely cover with foil. Place the clip on the rubber tubing and close.
- 4 Autoclave flask and bottles for 20 minutes at 103 kPa (121°C), allow to cool and store at 4°C until required.

Day 2.

- 1 Select the plate with best growth of *Vibrio natriegens*. Using aseptic technique transfer a colony of bacteria into two sterile Universal bottles of saline broth using a wire loop.
- 2 Incubate at 25 - 30°C for 24 hours. Incubate in a shaker if possible; if not, careful swirling of the bottles by hand every few hours assists growth of the bacteria.

Day 3.

- 1 Allow the overnight cultures to cool to room temperature to reach lag-phase; this usually takes about an hour.
- 2 Set up the water bath at 30°C.
- 3 Select the culture with best growth. Add 6 cm³ of the culture

to 94 cm³ of sterile broth in the flask, swirl and immediately take a 2 - 3 cm³ sample and place in a sterile Universal bottle. Label and store at 4°C.

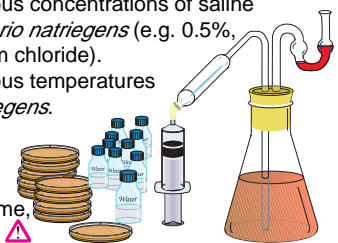
- 4 Incubate the flask in the water bath at 30°C for the next two to three hours and take samples every twenty minutes for growth measurements using aseptic technique.
- 5 If a spectrophotometer or turbidity meter is to be used then calibrate using a sample of clear broth and a sample of overnight culture to give the range. (A reading of between 0.02 to 0.05 units at 550nm is expected). Samples of culture should be removed aseptically and disposed of in disinfectant when finished with. ⚠
- 6 Record the results from the spectrophotometer and plot the values on ordinary graph paper and semi-log paper to show the growth curve and generation time.
- 7 If the spread plate method is to be used for determining cell numbers, serial dilutions should be prepared. Aseptically add 1.0 cm³ of the broth culture to 9.0 cm³ of saline to obtain the first dilution (10⁻¹). Take 1.0 cm³ of the diluted broth culture to 9.0 cm³ of saline to obtain the second dilution (10⁻²). A series of dilutions should be made in a similar way to give dilutions in the range of 10⁻⁶ to 10⁻⁸. Using a sterile spreader spread 0.1 cm³ of one dilution evenly over the surface of a saline agar plate Repeat for the more dilute culture to give a pair of plates (e.g. 10⁻⁶ and 10⁻⁷ or 10⁻⁷ and 10⁻⁸). Duplicate plates should be set up for each dilution to add to the accuracy of the investigation.
- 8 All sampling equipment should be discarded to disinfectant immediately after use. ⚠
- 9 Incubate the plates overnight at 30°C.
- 10 Samples and dilutions can be stored in a fridge if it is necessary to refer to or use them again. All cultures, samples and dilutions should be sterilised by autoclaving when finished with.

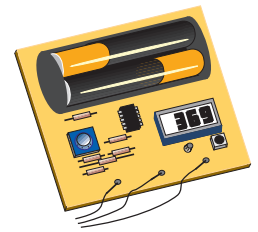
Day 4.

- 1 Examine all plates and select the most appropriate of each pair (30 - 300 colonies) and count the number of colonies on each. Calculate the number of bacterial cells per cm³ of each sample. Plot a growth curve of log number against time and calculate the mean generation time.

Extension activities

- 1 Investigate the effect of various concentrations of saline solution on the growth of *Vibrio natriegens* (e.g. 0.5%, 1.0%, 1.5% and 2.0% sodium chloride).
- 2 Investigate the effect of various temperatures on the growth of *Vibrio natriegens*.
- 3 Investigate the effects of antimicrobial agents on the growth of *Vibrio natriegens*, e.g. detergent (SDS), lysozyme, penicillin and chlorophenicol. ⚠





Information One

The bubble logger

The bubble logger can be used to measure the rate of fermentation by recording the number of bubbles produced with time. By working out the volume of a bubble and then the total number of bubbles produced in a given time, one can calculate the total volume of carbon dioxide produced to give an idea of the progress of the fermentation.

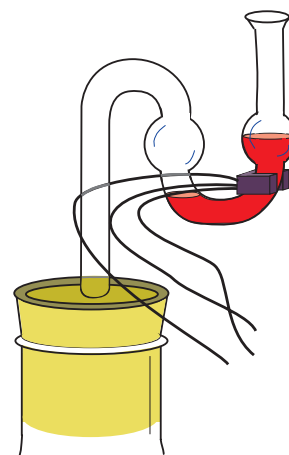
The bubble logger can be used on its own or connected to a datalogger or computer.

Inserting glass fermentation lock into silicone rubber bung

Great care must be taken when inserting the glass fermentation lock into a silicone rubber bung as it is not made of toughened laboratory glass. A glove or cloth should be used to protect the hand when fitting the lock into the bung. Use a little silicone grease with water, but if this is not available then use a little soap solution or washing up liquid as the lubricant. When gently pushed and twisted the end of the glass fermentation lock should fit easily into the bung to a depth of about one centimetre. No excess force should be used.

Placing the sensor on to the glass fermentation lock

The sensor unit, composed of the infrared emitter and the infrared receiver, should be placed over the ascending arm of the fermentation lock. It should be located about five millimetres below the exit expansion bulb. By careful manipulation it is possible to place one wire on one side of the arch of the fermentation lock while the other two pass the other side (see drawing). This helps to hold the sensor in place and makes it more difficult for it to fall off the fermentation lock.



Purpose of liquid in fermentation lock

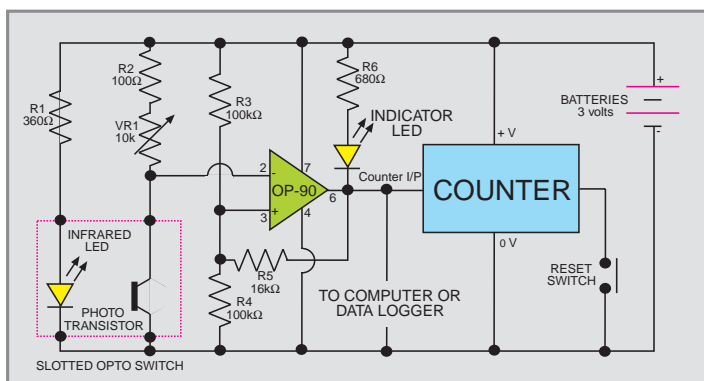
By using Universal indicator solution in the fermentation lock the acidity of the gas from the fermentation can be noted. If exactly 1 cm³ of Universal indicator solution and 1 cm³ of water are used each time then comparisons can be made between different fermentations. The total volume of gas generated can be worked out by calculating the volume of a single bubble and noting the total number of bubbles produced.

Adjusting the logger

The variable resistor (VR1) allows the sensor to be adjusted to suit the various light conditions that may be encountered. The device should never be placed in direct sunlight or where later in the day the sun's rays will fall directly on the sensor. The indicator LED will help to reassure the user that the device has been correctly adjusted. The LED should flash once each time a bubble passes, adding one to the value on the counter display. If it adds two for each bubble, further adjustment will be needed.

Zeroing the counter

A reset button has been included in the circuit so that the digital display on the counter can be zeroed. Remember that zeroing will lose any previously logged value.



Checking calibration of the logger

The logger can be checked by recording the circuit voltage that passes under three different conditions. The approximate logger voltage should be:

- off the fermentation lock, 1.7 volts
- on the fermentation lock filled with air, 1.5 volts
- on the fermentation lock filled with universal indicator solution, 1.3 volts.

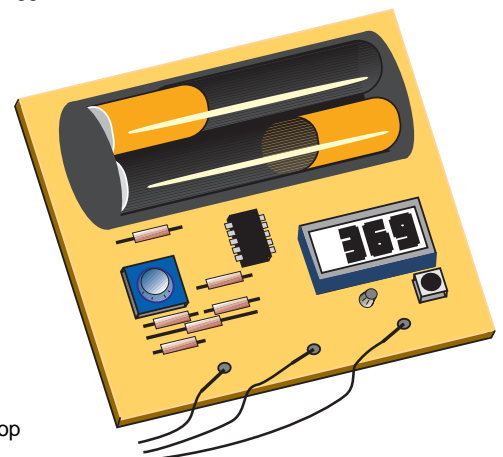
The variable resistor can be used to fine tune the device when it is in place on the fermentation lock to ensure it only registers once for each bubble.

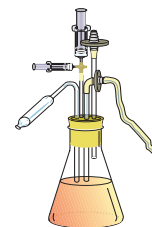
Logging to

The bubble logger records the number of bubbles that pass the sensor. If it is also connected to either a data logger or computer the number of bubbles produced can be recorded against time automatically.

Battery power

The bubble logger requires two A2 batteries which should allow the logger to run non-stop for at least a week. Long life alkaline batteries should extend this to about two weeks.





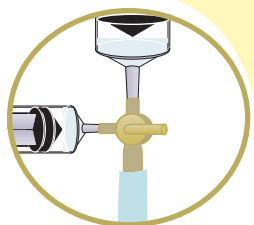
Information Two

Principles of a bioreactor

Before a bioreactor can be used for microbial growth investigations the vessel and its contents must be sterilised by autoclaving. Autoclaving involves using steam under pressure and ensures the complete destruction of microorganisms and their spores. The bioreactor must be correctly prepared to ensure successful sterilisation. The individual components of the bioreactor must be clean and then carefully assembled. Care should be taken to ensure the correct vents are fully open or closed for autoclaving. The assembled bioreactor should be filled with broth just before autoclaving. The autoclave time is worked out by choosing a temperature (e.g. 121°C) and calculating total sterilisation time. The total time consists of (a) heat penetration time, (b) holding time to kill all organisms and (c) safety margin (e.g. 5+10+5 = 20 mins). It is important to close the addition/inoculation port immediately after autoclaving so that the bioreactor remains sterile.

Syringes for additions and inoculation

Syringes allow for the accurate addition of the culture and any chemicals required for the investigation. The nozzle of the sterile syringe fits into a three-way tap. Aseptic technique must be observed when fitting and changing syringes. A fresh sterile syringe should be used for each new addition. Syringes are fitted after autoclaving and just before use. **The addition/inoculation port is open during autoclaving and closed immediately afterwards.**

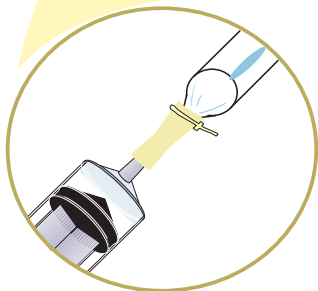


Three-way tap

The three-way tap allows for the addition of the inoculum, sterile silicone antifoam as well as any liquids such as acid or alkali. The tap allows the vessel to be isolated from the syringes. A small length of silicone tubing links the glass inlet tube to the plastic tap. The rubber tube should be disinfected with alcohol just before fitting the tap. The tap is supplied in a sterile wrapping and fitted aseptically. **The tap is fitted after autoclaving and just before use.**

Bung

Before any glass tubing is inserted into the silicone bung the glass should be smeared with a small amount of silicone grease. Both the glass and the bung are then moistened with water - the glass should slide into the bung easily. Silicone bungs are used because they can be autoclaved many times without deteriorating.



Syringe attachment

Very carefully open the sterile syringe packet at the plunger end, retaining the barrel in the packaging. Withdraw the plunger so that the rubber piston is in the middle position. The syringe is then removed from the packet and aseptically attached to the rubber tube which should be disinfected with alcohol just before fitting.

Sampling

Check that the syringe plunger is withdrawn to the middle position. The piston is slowly and very carefully withdrawn so that culture from the vessel is drawn up the tube and falls into the expanded region of the bent pipette. Then slowly and gently push back the piston so a little air bubbles through the broth retained in the expanded region. The piston is gently withdrawn again and the broth enters the syringe. Careful repetition of this process should ensure all the sample ends up in the syringe - none in the expanded region. The remaining broth in the sampling tube should be returned to the same level as the broth in the flask. With practice and patience exact volumes can be withdrawn.

Air exit filter

The exit filter ensures that air leaving the vessel does not contaminate the laboratory. For autoclaving the filter should be protected by placing a small plug of non-absorbent cotton wool into the exposed hole and then covering the whole filter with aluminium foil. The foil and cotton wool plug are removed just before use. **The vent is left open to allow expanded air to escape through the filter.**

Air entry filter

The air filter ensures that air being supplied to the vessel is sterile. The filter must be protected during autoclaving by placing non-absorbent cotton wool in the aperture and covering with aluminium foil. Remember, all glass to silicone tubing should have a cable tie to prevent the silicone tubing working free during autoclaving. **The tubing on the vessel side to the filter is clamped with a clip during autoclaving to prevent broth from expanding into the filter.**

Medium

Medium should be autoclaved within the vessel to minimise the risk of contamination. Ample space should be left at the top of the flask to prevent any froth formed from the medium entering the exit filter (e.g. 450 cm³ medium in 500 cm³ flask). Excessive frothing can be prevented by the addition of sterile silicone antifoam solution. This should be added aseptically via the addition port before the system is inoculated.

Disposal of broth and contaminated material

The bioreactor containing culture should be autoclaved after use. Any other contaminated equipment, samples taken or cultures associated with the bioreactor should be autoclaved or disinfected. Autoclaving is more reliable than disinfecting to ensure sterilisation and is to be preferred. **Sterilisation is absolute!** Plastic syringes and three-way taps can be disinfected with a suitable chemical disinfectant. This may also be satisfactory for some glassware (e.g. microscope slides) or very small samples of culture.

Background reading:

Books:

Microorganisms & Biotechnology
Peter Chenn
John Murray 1997 ISBN 0 7195 7509 5

Microbiology & Biotechnology
Alan Cadogan & John Hanks
Biology Advanced Studies
Nelson 1995 ISBN 0 17 448227 2

Microorganisms & Biotechnology
Jane Taylor
University of Bath Science 16-19
Nelson 1990 ISBN 0 333 48320 0

Microbes, Medicine & Biotechnology
Ken Mannion & Terry Hudson
Series Editor: Mike Coles
Collins Educational 1996 ISBN 0 00 322392 2

Microorganisms in Action
Investigations
Peter Freeland
Hodder & Stoughton 1991 ISBN 0 340 53922 4

Microorganisms, Biotechnology & Disease
Pauline Lowrie & Susan Wells
Cambridge University Press 1991 ISBN 0 521 38746 9

Microbiology & Biotechnology
Pauline Lowrie & Susan Wells
Cambridge University Press 1994 ISBN 0 521 42204 3

Maths for Advanced Biology
Alan Cadogan & Robin Sutton
Thomas Nelson and Sons Ltd. 1994 ISBN 0 17 448214 0

Microorganisms & Biotechnology
John Adds, Erica Larkcom & Ruth Miller
Nelson 1998 ISBN 0 17 448269 8

Science with Technology
Control in Action: Designing a Fermenter
Jim Sage & Robert Sharp
The Association for Science Education 1995 ISBN 0 86357 235 9

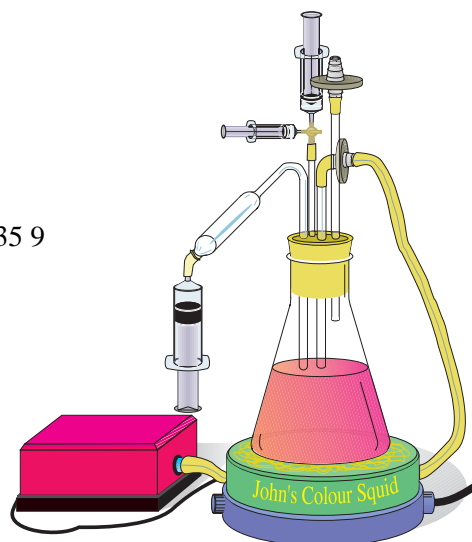
Software:

Bacterial Growth 3
A computer-based practical for microbiology students
Scotcal Software
76, Heol Gwenallt, Gorseinon, Swansea, Wales, SA4 4JN.
WWW: <http://www.demon.co.uk/scotcal/index.html>

Websites:

National Centre for Biotechnology Education
<http://www.reading.ac.uk/NCBE>

Society for General Microbiology
<http://www.socgenmicrobiol.org.uk>



Good Laboratory Practice - GLP for all!



Just like any other practical activity in a laboratory all these investigations require the user to adopt good laboratory practice. Given here are a few brief notes and hints to help those involved in the various activities to carry them out safely. Remember that before any practical activity is undertaken a risk assessment should be performed to ensure there is minimal hazard to all concerned. If there is any doubt about the assessment of the risk, reference must be made to safety texts or expert advice taken.

Safe microbiology

The practical activities selected in this package and the microorganisms suggested present minimum risk given good practice. It is therefore essential that good microbiology laboratory practice is observed at all times when working with any microbes.

There are five areas for consideration when embarking on practical microbiology investigations which make planning ahead essential.

- 1 Preparation and sterilisation of equipment and culture media.
- 2 Preparation of microbial cultures as stock culture for future investigations and inoculum for current investigation.
- 3 Inoculation of the medium with the prepared culture.
- 4 Incubation of cultures and sampling during growth.
- 5 Sterilisation and safe disposal of all cultures and decontamination of all contaminated equipment.

Good organisational skills and a disciplined approach ensure that every activity is performed both safely and successfully.

Protection

Food or drink should not be stored or consumed in a laboratory that is used for microbiology. One should not lick labels, apply cosmetics, chew gum, suck pens or pencils or smoke in the laboratory. Hands should be washed with disinfectant soap after handling microbial cultures and whenever leaving the laboratory. If hand contamination is suspected then the hands should be washed immediately with disinfectant soap. To ensure that any wounds, cuts or abrasions do not get infected or infection is passed on, protect them by the use of waterproof dressings or wear disposable surgical gloves.

General personal safety

Each individual embarking on these or any other microbial investigation is responsible for their own safety and also for the safety of others affected by their work (other students, technicians, teachers). The individual must include in the planning and performance of the investigation a risk

assessment to assess any hazard that the investigation may pose and ways of minimising it. Points to consider include safe storage and culturing of microorganisms, emergency procedures such as dealing with spillages and safe disposal of all contaminated material. No one should perform any microbiological procedures without receiving appropriate training from a competent person. To minimise the chance of contamination of the user, any other individual, the environment or the microbial culture good laboratory practice is required. GLP requires us to consider all cultures as potentially pathogenic.

Aseptic technique

Sterile equipment and media should be used to transfer and culture microorganisms. Aseptic technique should be observed whenever microorganisms are transferred from one container to another. Contaminated equipment should preferably be heat sterilised by either incineration or autoclaving. A suitable chemical disinfectant can be used but this may not ensure complete sterilisation.

Electrical safety

Many of the investigations use bioreactors that require aeration and this is usually supplied by the use of an aquarium air pump. Care should be taken to ensure that no liquid comes into contact with electrical mains power. The same care should apply if a magnetic stirrer is to be used to mix the growth medium in a bioreactor.

Glassware

Great care must be taken when assembling the glassware for the bioreactor. The insertion of the glass fermentation lock used in some of the investigations requires particular care as it is not laboratory grade glass. Hands should be protected during the insertion of the glass into the bung. Both the bung and the glass should be lubricated with water and either a small amount of silicone grease or washing up liquid. Very gentle twisting should be used to assist fitting but not too much so that it breaks! (See information 1. The bubble logger)



NATIONAL CENTRE FOR BIOTECHNOLOGY EDUCATION

School of Food Biosciences

The University of Reading

Whiteknights, Reading, RG6 6AP

Tel: 0118 9873743 Fax: 0118 9750140 <http://www.ncbe.reading.ac.uk>

Published by The Society for General Microbiology

© John Schollar and Benedikte Watmore ISBN 0 9536838 0 X

