PUPIL
RESEARCHER
INITIATIVE

Clear Solutions

Setting the Scene

You work for a company which is developing a new process to remove excess dyes from wastewater from factories which produce dyed fabrics. You have been asked to investigate the method to remove dyestuffs before your company puts more time and money into the process.

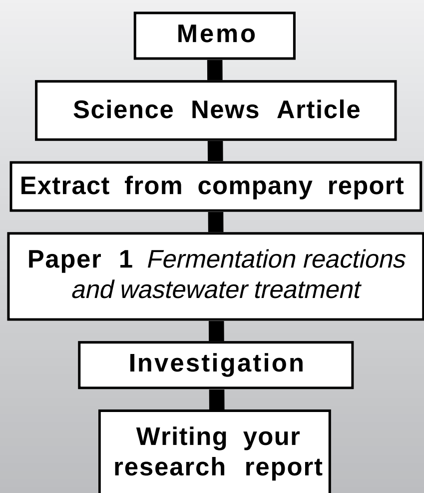
Pupil Research Brief

Study Guide

Syllabus Targets *Science you will learn about in this Brief*

- ❑ yeast cells convert sugar into carbon dioxide and alcohol by a process called fermentation
- ❑ fermentation is used to produce alcohol in beer and wine
- ❑ materials decay because they are broken down (digested) by microorganisms
- ❑ microorganisms digest materials faster in warm, moist conditions
- ❑ many microorganisms are more active when there is plenty of oxygen
- ❑ living things remove materials from the environment for growth and other purposes

Route through the Brief



Outcome Checklist

You will produce a research report for the company's Strategy Committee. A memo and an extract from a company internal report guide you through the Brief. You should make sure you produce the following items as you work through the Brief.

Science News article

- ❑ answers to questions and points raised by the notes written on the papers
- ❑ notes on biodegradable and non-biodegradable materials

Report

- ❑ ideas and notes for investigations

Paper

- ❑ report on investigation(s) into whether yeast columns might be a solution to the problem of removing dyes from wastewater

Colour Chemistry plc

Memorandum

From: Coordinator, Strategy Committee

To: Harjit Singh, R & D Controller

Subject: New project: biological treatment of wastewater

Date:

As part of the company strategy to do more work in the area of biotechnology a new project, described on the accompanying report extract, has been approved in outline by the Strategy Committee. The project looks at ways we can remove dyestuffs in the wastewater from factories which produce dyed fabrics. Your research group will have to carry out a feasibility study to see if it is worth pursuing further. The Strategy Committee needs a report as soon as possible.

The attached extract is taken from a report written by the research coordinator, outlining a number of possible projects we might wish to carry out. Only the 'dyes' project is described in the extract. There are some useful suggestions in the extract for investigations.

I have also sent you an article from the *The Researcher* and a paper by Kieran Evans from the *Journal of Clean Technology* which describe some of the background science. You will find these useful in planning your research.

The extract suggests a reference to a paper on immobiliser design *(Immobilising enzymes* by Ann Baker taken from the *Journal of Biotechnology*, December 1992, p67) which you will also find useful. This is available in the Pupil Research Brief *Catalytic Traps*. There is also an interesting short article on page 22 of *PRISM*, Issue 1, September 1996, called *Brewing Beer*. This describes how immobilised yeast can be used in the brewing industry.

After you have submitted your report, the Strategy Committee will decide whether we take the project any further.

Extract from report on future research projects

Project 3A

This project involves developing a microbial filter to treat the waste outflows from dyeworks. The outflows from these works contain significant amounts of reactive dyes which do not break down in the environment. Published work from other sources suggests the micro-organism, brewers yeast, (*Saccharomyces cerevisiae*) might be able to break down the dyes if the yeast growing conditions are carefully controlled. The costs of the system must be kept very low as the volume of liquid to be treated is very large. Immobilised yeast columns using alginate beads (Baker, 1992) might be a solution to the cost problem and initial results look promising.

The following are suggested for further investigation:

1 does the yeast break down the dye or just absorb it?

2 does the alginate absorb the dye?

3 is there any link between the amount of yeast in the alginate beads and dye removal?

4 what conditions produce the fastest growth of yeast?

5 what is the best feedstock for the yeast cells?

References

Baker, A. (1992), *Immobilising enzymes*, Journal of Biotechnology, December, p67

Science News

This paper describes work done by a university colour chemistry research group. Make sure you are familiar with the terms biodegradable and non-biodegradable.

Bugs that degrade the undegradable

Any secondary school student will be able to tell you that materials can be either biodegradable or non-biodegradable. Micro-organisms in the environment break down biodegradable materials like paper and kitchen wastes into harmless compounds. Non-biodegradable materials like certain plastics and glass cannot be broken down by microorganisms and so could last forever.

These everlasting materials can be quite a problem and empty plastic washing-up bottles turn up on beaches across the world. Nowhere is safe from our waste any more. A number of manufacturers have been slow to address important environmental issues, and use fully biodegradable packaging, even though it is sometimes claimed that some types of plastics are biodegradable nowadays.

But the distinction between biodegradable and non-biodegradable might change thanks to the work of research chemists at University of Leeds. The Leeds group have been looking at dyes which were thought to be non-biodegradable, or difficult to degrade using current techniques. These dyes belong to a group of chemicals called reactive azo-dyes. These have a strong nitrogen-nitrogen double bond in their molecule. They combine chemically with fabrics to give a very high quality colour that does not fade or wash out. Unfortunately the dyes do not break down in the environment. People have begun to complain about the coloured effluent in waterways flowing past dyeworks that use these reactive dyes.

One way to clean the water is to filter it with charcoal. This works quite well but leaves the dyeworks with a mass of dyed charcoal, with every new batch of wastewater needing more charcoal for treatment. The Leeds group found a bacterium which was able to break down the dye.

It began to look as if the non-biodegradable dye was actually biodegradable after all. Work is now proceeding to develop a treatment system that uses these bacteria to break down the dye before the wastewater is released. It is hoped that the new biological waste treatment scheme will be cheaper, more effective and, of course, more environmentally friendly than the old system.

When you have read this article go on to the paper on fermentation reactions and wastewater treatment - this talks about the possibility of using yeast to help break down waste products.

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Fermentation reactions and wastewater treatment

Kieran Evans

Abstract

This paper looks at recent research into treatment of wastewater using the yeast *Schizosaccharomyces cerevisiae* and outlines some of the problems likely to be encountered before fully biological systems can be developed to replace current filtration plants.

Introduction

Yeasts are a group of single-celled fungi that can grow in a wide variety of different habitats. They cannot carry out photosynthesis and so need to obtain food and energy from the environment. This food can come from a wide range of organic chemicals including sugars and starches. The yeast most familiar to human beings is probably *Schizosaccharomyces cerevisiae* which grows on the surface of many fruits. *S. cerevisiae* and its close relatives ferment the sugars of the fruits to alcohol and carbon dioxide and have been used for thousands of years for brewing and wine-making. The same yeast is also familiar as the yeast used to make bread.

All yeasts are able to respire aerobically and anaerobically. In oxygen rich (aerobic) conditions they obtain energy from the same basic respiratory reaction as human beings. Glucose (a sugar) is converted by yeast to carbon dioxide and water. When no oxygen is present yeasts switch to anaerobic respiration to produce alcohol and carbon dioxide. This anaerobic respiration is often called fermentation and shows how important yeast is in the brewing industry.

Maybe yeast can get energy from breaking down dyes?

Fermentation reactions

The main fermentation reaction is the breakdown of glucose to alcohol and carbon dioxide. The brewing industry has studied this over many years in great detail and we have a good understanding of the conditions that affect the rate of production of alcoholic drinks from sugary or starchy feedstocks. Other papers deal with this more fully and we will not repeat those details here.

Our interest is in the range of other reactions that yeast can use to produce energy to survive and grow. Many of these reactions are now also called fermentations. Table 1 summarises some of the available data on sugar fermentation by yeast.

Sugar	Fermented by yeast
glucose	yes
fructose	yes
sucrose	yes
lactose	no

Table 1. Sugars fermented by yeast

Other researchers have studied a range of other reactions to try to work out exactly the range of substances yeast is able to breakdown. At the moment we know that the list is long and gets longer with each passing year. It is also true that yeasts may take part in cooperative fermentations with other microorganisms. Brewers are already aware of this as a problem. Yeast breaks down glucose to alcohol and carbon dioxide but any *Acetobacter* bacteria in the fermenting mixture will convert this alcohol to ethanoic acid and water. Vinegar is a solution of ethanoic acid and no brewer wants to sell beer with vinegar flavour! In the environment, all microorganisms exist in mixed cultures and so yeast might fail to ferment something in the clean laboratory reactor but manage it quite well as part of the mixed culture in a river.

Fermenter technology

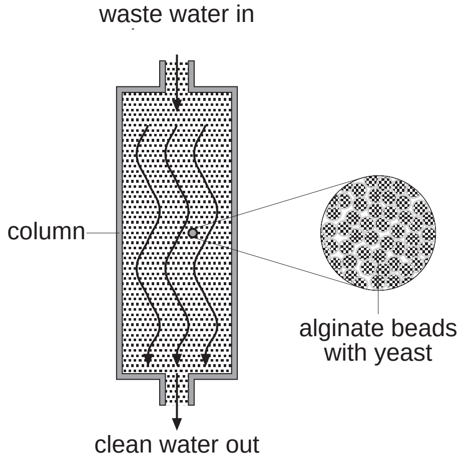
Fermenters are simply containers that allow scientists to observe and control a fermentation reaction. Again, much of our understanding of fermenter design and operation comes from the brewing and dairy industries.

The vats producing beers or wines and yogurts or cheese are some of the earliest fermenters. Modern laboratory fermenters are usually made of glass or stainless steel and are easy to sterilise so that the reactions of a single, pure culture can be investigated. Factors studied will include:

- temperature
- food supply
- oxygen levels
- pH
- a range of other specialist additives

See the Report Extract from the Strategy Committee - points 4 and 5 could be answered by following up some of the suggestions in this list

The move from a laboratory procedure to an industrial one always involves a change of scale and may need a complete redesign of the fermenter. A modern development uses immobilised microorganisms (see Figure 1).



See 'Immobilising enzymes' Baker, A. (1992) Journal of Biotechnology, December, p67 - this outlines a method for producing alginate bead columns to immobilise the yeast - a copy is in the Pupil Research Brief 'Catalytic Traps'.

Figure 1. Yeast immobilisation column

The microbe is packaged in spheres of alginate which allows chemicals to pass easily from the liquid passing over the alginate beads (bathing solution) to the yeast cells and out of the cells into the bathing solution. The alginate spheres can be packed into a column and the liquid feedstock added at the top. The liquid percolates between the spheres and the microbe carries out the fermentation as normal. The product is easily collected at the base of the column relatively free of contamination by microbes. More complex, longer fermentation reactions need taller columns to give the liquid more time in contact with the microbes. This system also means a traditional batch process can be converted to a continuous feed system with an increase in efficiency.

Fermentation and water purification

The section below makes some useful suggestions which will help you plan the investigations.

New fermenters using immobilised microbes could be very useful in water purification plants. A suitable micro-organism could be cultured by traditional methods and then immobilised in columns. The wastewater could be fed in and the output collected at the base and passed to the outside world or on to another column for further processing. Compounds that are difficult to break down may need to pass through the system a number of times or even have to pass through mixtures of immobilised microorganisms before breakdown is complete. Recent work by genetic engineers suggests it may be possible to design microbes with the perfect combination of fermentation pathways to deal with any waste product. A microbe that could digest plastic waste would be a great asset to a waste disposal company - but imagine what havoc it might cause if it escaped into our plastic-rich environment.

Work with yeasts and dyeworks' wastes is being done in a range of institutions. Waste outflow from the dye vats always contains unused dyes. These can change the colour of natural waterways and many water companies are now insisting dyeworks should clean their wastewater before it is released.