Biofuels may be loosely defined as fuels derived from plant and animal sources. For many years their use was uneconomic owing to the easy availability of fossil fuels. Environmental concerns and increasing demand for oil have led to a need for alternative fuel sources. Among the biofuels in use or under consideration for future use are *ethanol, biodiesel, biogas, hydrogen, butanol* and *ethers*.

Ethanol

Ethanol, pure or blended with petrol, has been used as motor fuel since the 1970s oil crises. Its basic production method involves enzymatic *hydrolysis* of *starch* from plant sources by *amylases* – enzymes that break down starch. The product *sugars*, mainly glucose, are subsequently fermented by yeasts to yield ethanol that is then isolated and purified. Glucose and other sugars (including 5-carbon sugars such as xylose) can be fermented by other organisms to *butanol* – a superior fuel to ethanol.

Fig. 1 Maize stalks – a cellulose source

Fuel crops (e.g. sugar cane, cassava, corn and rapeseed) are now being widely grown. There are serious concerns that their wide cultivation, which is expected to increase, is already affecting food supply and the environment.

Much of a plant's glucose is tied up in *cellulose*. For more efficient fuel production this source must be unlocked. Using cellulose would also mean that many currently uncommercial plants (e.g.; switchgrass, bent, dog's mercury) and forestry plantations could theoretically be exploited. So too could compost and municipal waste (paper, clothing).

Yeasts do not have *cellulase* enzymes. Some bacteria and fungi do, as a walk in a forest will readily confirm. However, directly *incubating* cellulosic materials with these organisms is not an option – cellulose breakdown is a very slow process. *Starch* and cellulose are both *polymers* of glucose. Starch is an ideal energy storage substance because it is easily broken down into simple sugars. Cellulose is an ideal structural and material because it can withstand degradative processes. Scientists therefore are testing various cellulase *enzymes* in order to identify the most promising ones. The organisms that produce them can be isolated and grown in suitable media with a view to bulk manufacture of the enzymes. Preparations of cellulases, including combinations not found in nature, working *synergistically* may give viable rates of sugar release from cellulose.

Fig. 2 Wood chippings

Cellulose-metabolising organisms operate optimally in conditions of *temperature*, *pressure*, *pH* and *tonicity* that may be expensive to maintain. They may also utilise biochemical pathways that would generate contaminants or consume useful products. Inanimate enzymes provide purer products more quickly and their activity is more controllable.

One line of research involves the use of cellulases from *thermophilic* fungi. These enzymes can be added to a biomass preparation that is kept at a high temperature. Glucose is produced and simultaneously pasteurised, eliminating the risk of unwanted microbial action. Research is ongoing.

The breakdown of cellulose is greatly complicated by the structure of plant tissue and the linkage of cellulose to *hemicellulose* and *lignin* (phenolic acids in non-lignified tissues). These chemicals effectively sheathe the cellulose. A pre-treatment process involving high pressure and temperature and hydrolysis by dilute acid dissolves the lignin and breaks the hemicellulose down to component sugars (glucose, xylose, arabinose and others). Some of these sugars are fermentable to alcohol. The process is fairly effective although expensive. Some of the sugars are degraded, enzyme-inhibiting by-products may be formed and, in some cases, lignin is deposited, resulting in reduced access to the cellulose for enzymes. The current research objective is to develop a milder pre-treatment process, followed by the addition of enzymes, including *hemicellulases*. Preliminary tests have shown higher sugar yields.

As yet, *ligninase* enzyme action is not well understood but potentially useful candidates are being examined. Moreover, lignin is burned in some facilities as fuel, providing energy for the processes. Lignin – as wood – has been a biofuel for millennia.

Microscopic study of plant cells and tissues is ongoing. Computer programs are being developed with a view to understanding cell structure and developing strategies for enzyme combinations and use. Another line of research is the development of genetically modified plants – modified to allow easier breakdown of lignin-hemicellulose-cellulose complexes.

Biodiesel

Biodiesel is mainly produced by the base-catalysed reaction of plant oils with alcohol. An extra acid step is required if free fatty acids are used. (Animal lipids may also be used, e.g. tallow). Clearly, a successful cellulose-to-ethanol technology would help biodiesel production. There may in the future also be a role for lipase enzymes rather than acid/base catalysis. Lipases promote the *transesterification* of *triglycerides* and the esterification of fatty acids, and offer the prospect of cleaner products. Problems with enzyme stability, reaction rate and extent have not as yet been overcome. On another level, enzymes have been developed that are capable of degumming oil or removing unwanted *phospholipids*; this would be helpful in biodiesel plants in which refined oil was unavailable.

Biogas

Methane, which is used for heat or as a vehicle fuel, is produced by anaerobic microbial decomposition of plant material, slurry, sewage and feed waste. If it is to become sustainable, fuel crops will have to be used. It has been shown that biogas production is lower in cellulose-rich plants. Enzymatic treatment to degrade cellulose and hemicellulose may improve yields.

Hydrogen

Hydrogen is potentially an excellent fuel but there are problems with transport and storage. At the moment, it is produced from fossil fuels or by electrolysis of water (which largely depends on fossil fuel for electricity production).

A method has been developed of converting starch to carbon dioxide and hydrogen by using enzyme combinations that do not occur in nature. These enzymes include *hydrogenases* and *dehydrogenases* and have been derived from plants, animals, bacteria and fungi. The process can occur at about 30 °C but researchers hope to develop the process to work at higher temperatures (as in a car)

In another development, microbial systems have been developed that digest cellulose to CO_2 and H_2. Because of the necessity to maintain optimal conditions for the bacteria, this method would be best suited to large scale production facilities.

One objective is production of H_2 for direct use in *fuel cells* that would generate electricity or drive electric vehicles (thereby eliminating the main problems associated with hydrogen use). An operator would fill a tank with starch solution, plant-derived cellulose suspension or waste as required. An all-enzyme process would be preferred because it would operate in relatively mild conditions, without the problems associated with maintaining living organisms.

Much recent work has focused on fuel cells. The *catalyst*, platinum, is expensive and can be easily poisoned by carnon monoxide (CO). Enzyme alternatives have been identified but there are problems with their stability, lifetime and efficiency. Immobilising enzymes on *nano-structured* materials may help address the issues.

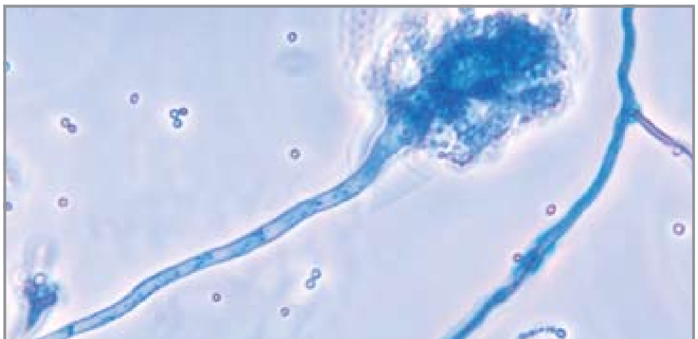


Fig. 3 Conidiophore of *Aspergillus fumigatus*

Enterprise Ireland (EI) is the government agency responsible for the development and promotion of the indigenous business sector. One of the key ways we do this is by working with universities, institutes of technology and other research institutions to translate publicly funded research into useable products, processes and services that are adopted by existing companies or are brought to market by new start-up companies. This process, called commercialisation, is an important aspect of the Irish research and innovation system.

Enterprise Ireland has established expert commercialisation teams working across the academic-industry interface in three key technology areas lifesciences & food, informatics and industrial technologies. Enterprise Ireland's Lifescience & Food Commercialisation Group (EI Bio) provides expertise, funding and support to industry and to third-level educational institutions in Ireland.

To find out more information on Enterprise Ireland and biotechnology visit: **www.sta.ie ,** **www.enterprise-ireland.com/commercialisation** or **www.biotechnologyireland.com**.

Some cellulase-producing microrganisms

Bacteria

Clostridium thermocellum
Ruminococcus albus
Streptomyces sp.

Actinomycetes

Streptomyces sp.
Thermoactinomyces sp.
Thermomonospora curvata

Fungi

Aspergillus fumigatus
Aspergillus niger
Chrysosporium
Fusarium solani
Phanerochaete
Schizophyllum commune
Sclerotium rolfsii
Sporotrichum cellulophilum
Talaromyces emersonii
Thielavia terrestris
Trichoderma reesei

Syllabus Reference

Leaving Certificate Biology

1.3.7 Metabolic Role of Biomolecules

- **Carbohydrates and lipids as primary sources of energy** for metabolic activity.
- **Proteins as enzymes.**

2.2.3 Enzymes

- **Definition of "enzymes"** – reference to their **protein nature, folded shape, and roles** in plants and animals. Special reference to their role in metabolism.
- Effect of **pH and temperature** on enzyme activity

H.2.2.7 Enzymes (Extended Study)

- The Active Site Theory to explain enzyme function and "specificity". Explanation of the term "optimum activity" under specific conditions as applied to pH range.
- **Heat denaturation** of protein.

Leaving Certificate Chemistry

5.1 Sources of Hydrocarbons. Decomposition of animal waste and vegetation as methane sources. … Exothermic and Endothermic of an exothermic reactions

5.6 Other Chemical Fuels. Hydrogen's potential as a fuel.

Learning Outcomes

On completion of this lesson, students should be able to:

- Understand the desirability of converting cellulose to sugars for the production of biofuels
- Appreciate the difficulties involved in developing processes that are commercially viable
- Understand why the use of enzymes is desirable
- Recognise that enzymes may have other potential roles in the production of biofuels.

General Learning Points

- Biofuels are fuels derived from organic sources..
- Fuel crops producing starch and oils are a major source of biofuels
- The full fuel potential of plants is not being realised while the sugar present in cellulose is not being utilised.
- The breakdown of cellulose to sugars would have positive implications for the production of ethanol, hydrogen, biodiesel and biogas.
- Enzymatic conversion of cellulose to sugar is desirable. Conversion may be achievable under mild conditions, with high yields and rates of reaction.
- Modification of enzymes and the use of enzyme combinations not found in nature may achieve acceptable rates of conversion of cellulose to sugars.
- If cellulose can be utilised, non-food plants can become as a fuel source. Forestry products and cellulosic waste can also be used.
- Successful cellulose-to-ethanol technology could mean less land requirement for fuel crops.

Student Activity

1) Find out about cellulose, hemicellulose, lignin and starch. What are their functions in nature and what other uses have they?
2) Hydrogenases are ancient enzymes, dating from a time when there was no oxygen in the air. Find out about their roles in nature.
3) What agricultural crops and short-cycle trees might be planted in Ireland to meet our energy needs?
4) The use of biofuels is one approach to solving our energy problems. What other approaches are there? Discuss the relative merits and demerits of reliance on biofuels.
5) Add a piece of liver to hydrogen peroxide solution in a test tube. Note the evolution of oxygen gas because of the action of the catalase enzyme. Now obtain some liver and crush it in water to obtain an extract. Filter to remove liver fragments. Add this extract to hydrogen peroxide solution and see if you have succeeded in producing an active enzyme extract.
6) Make up a starch solution. Test some of it for starch and sugars. Add some amylase to the original solution. After some time, confirm that it has been enzymatically converted to sugar. Now add yeast to this solution and leave in a warm place. Test for the evolution of carbon dioxide and, if you have time, try distillation to obtain ethanol. You have now simulated the production of alcohol in a biofuel plant.
7) Examine a block of wood or some wild grass. Simply think of the difficulty of refining them to sugars. That is the challenge of cellulose-to-fuel technology.

True/False Questions

(a) Ethanol is the only alcohol that can be used as a biofuel. **T F**

(b) Lignin has long been used as a fuel. **T F**

(c) Non-food plants are potential sources of fuel. **T F**

(d) Compared with other processes, enzymes can work under milder conditions, are more specific and give good yields. **T F**

(e) It can be difficult to maintain optimal conditions for cellulase-producing organisms. **T F**

(f) Starch and cellulose are built of glucose units. **T F**

(g) All sugars are easily metabolised to alcohol. **T F**

(h) GM technology may be a useful complement to enzymes in biofuel production. **T F**

(i) The costs and availability of labour, fuel, fertilisers, pest control are factors involved in growing fuel crops. **T F**

(j) Cellulose-to-ethanol conversion is now a proven commercial technology. **T F**

Check your answers to these questions on www.sta.ie

Examination Questions

Leaving Cert Biology, 2007 (H)Higher Level

7.(a) (i) What is meant by an enzyme?

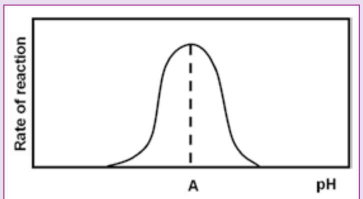
(ii) Give an example of a protein that has a structural role.

7.(b) Answer the following questions in relation to an investigation that you carried out to determine the effect of temperature on enzyme action.

(i) Name the enzyme that you used.
(ii) Name the substrate of the enzyme.
(iii) State one factor that you kept constant during the investigation
(iv) How did you keep this factor constant?
(v) How did you vary the temperature?
(vi) How did you measure the rate of activity of the enzyme?
(vii) What was the result of your investigation?

Leaving Cert Biology, 2006 (H)Higher Level

3. The graph shows how the rate of reaction of a carbohydrate-digesting enzyme in the human alimentary canal varies with pH.



(a) Name a carbohydrate-digesting enzyme in the human alimentary canal
(b) Where in the alimentary canal does this enzyme act?
(c) State the enzyme's product (s)
(d) What is the pH at A?
(e) A is said to be the enzyme's .... pH
(f) Suggest a temperature at which human enzymes work best
(g) What term best describes the shape of an enzyme?

Leaving Cert Biology, 2006 (H) Higher Level

7.(a) Immobilised enzymes are sometimes used in bioreactors.
(i) What is a bioreactor?
(ii) State one advantage of using an immobilised enzyme in a bioreactor.

7.(b) Answer the following questions in relation to an experiment that you carried out to immobilise an enzyme and use that immobilised enzyme.
(i) Name the enzyme that you used
(ii) Draw a labelled diagram of the apparatus that you used to immobilise the enzyme.
(iii) Describe how you used this apparatus to immobilise the enzyme. In your answer name the solutions that you used and explain their purpose.
(iv) Describe briefly how you used the immobilised enzyme.

Other Questions

Leaving Cert. Ag. Science (H): 2007, Qs.3 (a), 5; 2005, Qs. 3, 5

Leaving Cert. Ag. Science (O): 2007, Q. 10(b)

For further examples of past questions check www.sta.ie

Did You Know?

In the event of success in developing a commercial cellulose-to-ethanol technology, some scientists envisage using GM technology to develop plants that would produce more cellulose and would grow in dry, hot, saline conditions. They would be further modified for pest resistance. The aim would be to utilise marginal land and grow fuel crops requiring less maintenance.

The European sugar beet industry has declined enormously in recent times. However, it produces actual sugar and, if its cellulose can be exploited, it may become a viable crop again.

In experiments, enzymes have been added to animal feeds to degrade the cellulose and improve their digestibility. Results are encouraging.

Yeast may be the organism of choice for fermentation but conditions in a cellulose reactor can be severe. By-products and rising ethanol concentrations often kill the yeast. Through genetic engineering, scientists have developed a strain of *Zymomonas bacteria*, tolerant to severe conditions, which can ferment not only glucose but even sugars that yeast cannot metabolise.

American scientists are working on corn engineered to produce cellulases and sequester them in seed cell vacuoles. These cellulases, released in the grinding process, will hopefully cause immediate sugar production.

Biographical Notes

Ray Wu (Aug. 14, 1928–Feb. 10, 2008)

Ray Wu was born in Beijing, China in 1928. In 1948 the he moved to the US where his father, Hsien Wu, had earlier trained as a biochemist and later as professor of biochemistry in the University of Alabama. Ray was the first person to explain the denaturation of protein as a change in its folding structure and not a chemical change in the protein chain.

Ray Wu is considered to be one of the fathers of genetic engineering. He was the first person to determine the order of the nucleotide bases, adenine, thymine, guanine and cytosine in a large segment of DNA, and the techniques he developed underlie today's techniques.

He developed a transgenic variety of rice (i.e. a variety that incorporated genetic material from another species) that could be grown in hostile environments. This alone boosted food production in many places. He established a system to facilitate the education of Chinese students in the US.

He published more than three hundred articles on science; yet he remained a humble and generous person with a warm smile.

Revise The Terms

Can you recall the meaning of the following terms?

amylase, bacteria, biodiesel, biofuel, biogas, butanol, catalyst, cellulase, cellulose, dehydrogenase, enzyme, ethanol, ether, fuel cell, GM, GMO, hemicellulase, hemicellulose, hydrogen, hydrogenase, hydrolysis, immobilised enzymes, incubating, lignin, ligninase, lipase, nano-structured, pH and tonicity, phospholipid, polymer, pressure, starch, sugars, synergistic, temperature, thermophilic, transesterification, triglyceride.

Check the Glossary of Terms for this lesson at www.sta.ie