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Preface

Hardly any other term in the field of biotechnology has been the subject of so much discussion among experts as white biotechnology at present. This term is an alias for "industrial biotechnology," an already established "heavyweight" that focuses on the production of the most diverse products (bulk and fine chemicals, enzymes, food and animal feed additives, pharmaceutically active substances and agrochemicals, auxiliary agents for process industries, etc.).

In some segments, white biotechnology has already captured leading market positions:

- In recent years the annual biotechnological production of amino acids exceeded one million tons.
- In vitamin production there have been several recent cases of a changeover from a chemical to a biotechnological synthesis process, a trend that is expected to increase.
- During the last 10 years the market volume for enzymes has increased by 50%.
- The successful launch of polylactide marked white biotechnology's breakthrough into the field of polymers and synthetics.

Today crude oil is the most important energy source and the most widely used chemical raw material. Both primary industry and polymer chemistry currently depend to a great extent on oil. However, it is only a matter of time before the world's oil reserves are depleted. Almost all studies presented to date agree that peak oil, i.e. the point in time when oil extraction reaches its highest level, will take place in the first half of the present century. The increasingly difficult development of new sources of oil have triggered initiatives worldwide to reduce national dependence on oil imports.

To summarize, there is no long-term alternative to developing a technology based firmly on renewable resources and industrial biotechnology may offer various solutions in this field. The tremendous pace of progress in the field of molecular biology has provided an unprecedented and promising launching pad for the development of further industrially relevant biocatalysts. Simultaneously, bioprocess engineering know-how is supporting efficient process development from titer plate format to shaker flasks to industrial scale. Thus, in principle, a basis exists for accelerating the development of new industrial bioprocesses in parallel with all disciplines concerned. In this book authors from different scientific and business areas of industrial biotechnology aim to give you an overview of the state of the art and ongoing developments.

Frankfurt and Kaiserslautern, October 2006

Dieter Sell
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Raw Materials

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Abstract Industrial fermentations need raw materials that fulfill the requirements of the organism (suitable carbon and nitrogen source, minerals and specific nutrients) and that are available in a high quantity and quality. This contribution gives a comprehensive overview, including the new trends and progress of recent years. The use of feedstock based on several raw materials such as sugar, starch, inulin and lignocellulose is discussed. Biomass-based raw materials are by far the most applied feedstocks for fermentation. However, there are also raw materials for fermentations derived from the petrochemical industry. These substrates are especially hydrocarbons, alcohols and carboxylic acids. Some applications are given in this chapter.

Keywords Raw materials · Carbohydrates · Lignocellulose · Oil & fat · Glycerol

1 Introduction

Most fermentation media consist generally of carbon sources, nitrogen sources, minerals and specific nutrients; in the case of aerobic microorganisms oxygen or an oxygen source is also a key nutrient. Media composition is mostly only chemically well defined in the laboratory whereas in industrial fermentations complex media are used.

Industrial fermentations use mainly chemoorganotrophic microorganisms that meet their energy and carbon demand by metabolising organic substrates. Furthermore, the carbon source is the main component by weight in fermentation media. Hence, this chapter will focus on carbon sources for chemoorganotrophs.

There is a broad variety of fermentation raw material and feedstock as carbon source available for industrial fermentations to bioproducts and biofuels. Carbohydrates derived from sugar or starch plants are the main fermentable carbon source in industrial fermentations. Moreover, fats and oils are applied as a single carbon source or in combination with carbohydrates. Furthermore, alcohols, hydrocarbons and other organic substrates are used. In recent years lignocellulosic raw materials have come into the focus of research.

Media for industrial fermentations have already been reviewed in several papers [1–5] that discussed raw materials and nutrients in detail. Thus in this chapter, a short comprehensive overview including new the trends and progress of recent years will be given.

2 Carbohydrate-Based Raw Materials

Carbohydrates [6, 7] are by far the most used raw material and include saccharose (also called sucrose) and molasses from sugar beet and sugarcane as well as hydrolysed starch products like glucose and dextrins. There is also fructose derived from inulin and other carbohydrate sources that are used as raw material. Carbohydrate-based sources for industrial fermentations were reviewed in detail for sugars-based raw materials [8] and starch-based raw materials [9] some years ago (see also [10, 11]). For ethanol fermentation several reviews discussing suitable raw materials have been published, for example [12–16]. Lignocellulosic materials containing cellulose and hemicelluloses became the focus of research, driven by efforts to produce cheaper bioethanol. There is currently an intensive ongoing research to use lignocellulosic raw materials as source for fermentable carbohydrates (for example [17–21]). Moreover, residue and waste biomass are being considered as fermentation feedstock [49]. Some are already used (whey, pulp waste liquor) while others (lignocellulosic residues) are mostly still under investigation.

2.1 Sugar-Based Feedstock

Sugar plants are plants containing the disaccharide saccharose (also called sucrose), which consists of glucose and fructose. The term sugar is used as a common synonym for the carbohydrate saccharose in agricultural science and botany. Sugar beet and sugarcane are the main source for saccharose and saccharose-rich molasses. Other alternative plant sources for saccharose are sweet sorghum, sugar maple and sugar palms. However, only sweet sorghum has some importance and is used as a sugar feedstock source for fermentations. The saccharose contents of the main sugar plants are given in Table 1.

The world production of beet and cane raw sugar amounted to about 155 million tons in 2004/2005 (23% from sugar beet and 77% from sugar cane) [22]. Main producers are Germany, France and USA for beet sugar as well as Brazil, Australia, India, Thailand, Mexico and China for cane sugar.

Sugar beet (*Beta vulgaris*) is a temperate climate biennial root crop. It produces sugar during the first year of growth. It is sown in spring and harvested between autumn and early winter. Sugar beet is processed by extracting sliced beet cossettes with hot water (70 °C) to produce the raw sugar juice. The raw juice is purified to get the thin juice with an average sugar content of 16%. The thin juice is then concentrated in multiple steps, resulting in a thick juice with an average sugar content of 67%. The remaining wet residue of pressed, exhausted cossettes called pulp is dried, often pelleted and molassed for use as feed.

Sugarcane (*Saccharum officinarum*) is a perennial subtropical and tropical climate grass that is planted practically throughout the year in the equatorial belt; mostly in spring or late spring, or sometimes in autumn. The stalks are harvested after a minimum of 10 months or a maximum of 24 months after planting. The harvesting time depends on the sugar content and the stage of maturity. Sugar cane is harvested by hand or mechanically. The stalks are cut at the base and the leaves are removed. Afterwards, the plants develop new stalks, which allows up to eight seasons before new planting is necessary. Usually the harvesting and planting operations overlap in order to avoid storing the planting material. Sugarcane is processed by washing, chopping,

Table 1 Saccharose content of plants

Plant	Average % saccharose content
Sugar beet	16–24
Sugarcane	7–20
Sweet sorghum	7–15

and shredding the stalks. The shredded stalks are then extracted with water to produce the raw sugar juice with a sugar content of 10–15%. After clarification, the juice is concentrated by evaporation to get a thick syrup with an average sugar content of 60%. The remaining fibrous residue, called bagasse, is mostly burned for energy production.

The saccharose juice either from sugar beet or sugarcane is purified (refined) by crystallisation and centrifugation yielding 93–96% pure raw sugar syrup. Additional washing, centrifugation, and crystallisation operations yield > 99% pure white sugar. The remaining green run-off from the last crystallisations is called molasses, having still a carbohydrate content of 40–60%. The final molasses contain saccharose as well glucose and fructose.

Sweet sorghum (*Sorghum saccharatum*) is a warm climate cereal grass that is widely grown in Africa, Asia, and the Americans. After planting in spring, harvesting is similar to that of sugarcane. The sweet stems contain the saccharose. Due to an additional glucose content of 1%, crystallisation to produce white sugar is difficult. Hence, only sugar syrup is produced and used in the food industry and as feedstock for ethanol fermentation.

The different saccharose-containing fractions (raw juice, thick juice, saccharose, molasses) of the process may all serve as fermentation feedstock. Thus, saccharose of different purity is available as syrup or powder for fermentation. On the one hand, the pure character of saccharose, especially of crystallised white sugar, compared to molasses results in less interference in the fermentation process. On the other hand, saccharose is more expensive than molasses and does not contain additional nutrients such as nitrogen-sources, minerals or vitamins. Various more-or-less raw sugars of minor purity such as juices, syrups and green run-offs are available (Table 2). However some of them are only available during the harvest campaign. Thus, only crystallised sugar, refinery sugar liquids and molasses are available year around.

Beet and cane molasses are the cheapest and mostly used carbohydrate fermentation media of the sugar industry. Cane molasses accounted for approximately 80% of the world molasses production of about 48 million tons in 2004/2005 [23]. There are basically three types of molasses:

Table 2 Sugar content and purity of different feedstock from sugar beet processing [8]

Source	% Sugar	% Purity
Raw juice	13–17	89–91
Thin juice	13–17	91–93
Thick juice	61–70	> 91
Green run-off	63–70	77–90
Molasses	50–56	58–63

1. Beet or cane blackstrap molasses
2. Cane refinery blackstrap molasses
3. Cane high-test or inverted molasses

Blackstrap molasses are the final effluent during cane processing, where the mother liquor from the subsequent crystallisation steps still contains approximately 52 percent of total sugars. Beet molasses are produced by processes that are similar to those of sugarcane. Blackstrap molasses contains small amounts of complex polysaccharides and invert sugars as well as also various non-carbohydrate materials, including nitrogen-containing substances. The dark colour results from "browning reactions" (due to the Maillard reaction of the sugars with amino acids because of the heat and alkali used in processing). Blackstrap molasses is normally the cheapest sugar-based feedstock. Hence, it is most used for industrial fermentations.

Refinery blackstrap molasses is a product that differs from blackstrap molasses only in that it is the residual mother liquor that has accumulated in the recrystallisation refining of the crude cane-derived saccharose.

High-test or invert molasses contains approximately 70–75% sugar, and is produced in a manner different from that previously described as crystalline saccharose is not the desired final product. The sugarcane juice is partially inverted to prevent sugar crystallisation; that is, the sugar is partially hydrolysed to monosaccharides (either with heat and acid or enzymatically), purified, and finally concentrated to a syrup. Thus, high-test or invert molasses is strictly speaking an only moderately purified, inverted syrup that consists of glucose and fructose. It is preferred to blackstrap molasses because of the lower shipping charges on a sugar concentration basis and because of its lower levels of non-fermentable components. High-test molasses is produced only during years of sugar cane overproduction.

2.2 Starch-Based Feedstock

Starch plants contain the polysaccharide starch, which is the most abundant storage carbohydrate in the plant kingdom [24]. Starch is a mixture of the polysaccharides amylose (10–30%) and amylopectin (70–90%), both consisting of glucose units that are α-1,4-glucosidic linked together. However, amylose consists only of linear, unbranched chains of glucose, whereas amylopectin contains additionally α-1,6-linked side chains.

The most important starch sources are cereals (corn, wheat, rice), manioc, sweet potatoes and potatoes. The starch content depends on the plant species (Table 3). The world production of starch amounted to about 58 million tons in 2002 (roughly 69% from corn, 10% from manioc, 9% from sweet potatoes, 6% from wheat, 6% from potatoes, and less than 1% from other sources) [25]. Main producers are the USA, the European Union, China and Japan.