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Preface

Industrial biotechnology is one of the most promising new approaches to pollution prevention, resource conservation, and cost reduction. It is often referred to as the third wave in biotechnology. If developed to its full potential, industrial biotechnology may have a larger impact on the world than health care and agricultural biotechnology. It offers businesses a way to reduce costs and create new markets while protecting the environment. Also, since many of its products do not require the lengthy review times that drug products must undergo, it's a quicker, easier pathway to the market. Today, new industrial processes can be taken from lab study to commercial application in two to five years, compared to up to a decade for drugs. The application of biotechnology to industrial processes is not only transforming how we manufacture products but is also providing us with new products that could not even be imagined a few years ago. Because industrial biotechnology is so new, its benefits are still not well known or understood by industry, policymakers, or consumers. From the beginning, industrial biotechnology has integrated product improvements with pollution prevention. Nothing illustrates this better than the way industrial biotechnology solved the phosphate water pollution problems in the 1970s caused by the use of phosphates in laundry detergent. Biotechnology companies developed enzymes that removed stains from clothing better than phosphates, thus enabling replacement of a polluting material with a non-polluting bio-based additive while improving the performance of the end product. This innovation dramatically reduced phosphate-related algal blooms in surface waters around the globe, and simultaneously enabled consumers to get their clothes cleaner with lower wash water temperatures and concomitant energy savings.

Industrial biotechnology involves working with nature to maximize and optimize existing biochemical pathways that can be used in manufacturing. The industrial biotechnology revolution rides on a series of related developments in three fields of study of detailed information derived from the cell: genomics, proteomics, and bioinformatics. As a result, scientists can apply new techniques to a large number of microorganisms ranging from bacteria, yeasts, and fungi to marine diatoms and protozoa. Industrial biotechnology companies use many specialized techniques to find and improve nature's enzymes. Information from genomic studies on microorganisms is helping researchers capitalize on the wealth of genetic diversity in microbial populations. Researchers first search for enzyme-producing microorganisms in the natural environment and then use DNA probes to search at the molecular level for genes that produce enzymes with specific bio-catalytic capabilities. Once isolated, such enzymes can be identified and characterized for their ability to function in specific industrial processes. If necessary, they can be improved with biotechnology techniques.

This book Industrial Biotechnology is an attempt to deal with the use of living organisms or their products in large-scale industrial processes. It is an old field that has been rejuvenated in recent years due to the development of genetic engineering techniques. This comprehensive textbook is essential reading for all students of biotechnology and applied microbiology, and for researchers in biotechnology industries.

—Editor

Chapter 1

Introduction

The bioconversion of biomass to mixed alcohol fuels can be accomplished using the MixAlco process. Through bioconversion of biomass to a mixed alcohol fuel, more energy from the biomass will end up as liquid fuels than in converting biomass to ethanol by yeast fermentation. The process involves a biological/chemical method for converting any biodegradable material (e.g., urban wastes, such as municipal solid waste, biodegradable waste, and sewage sludge, agricultural residues such as corn stover, sugarcane bagasse, cotton gin trash, manure) into useful chemicals, such as carboxylic acids (e.g., acetic, propionic, butyric acid), ketones (e.g., acetone, methyl ethyl ketone, diethyl ketone) and biofuels, such as a mixture of primary alcohols (e.g., ethanol, propanol, *n*-butanol) and/or a mixture of secondary alcohols (e.g., isopropanol, 2-butanol, 3-pentanol). Because of the many products that can be economically produced, this process is a true biorefinery.

The process uses a mixed culture of naturally occurring microorganisms found in natural habitats such as the rumen of cattle, termite guts, and marine and terrestrial swamps to anaerobically digest biomass into a mixture of carboxylic acids produced during the acidogenic and acetogenic stages of anaerobic digestion, however with the inhibition of the methanogenic final stage. The more popular methods for production of ethanol and cellulosic ethanol use enzymes that must be isolated first to be added to the biomass and thus convert the starch or cellulose into simple sugars, followed then by yeast fermentation into ethanol. This process does not need the addition of such enzymes as these microorganisms make their own . As the microoganisms anaerobically digest the biomass and convert it into a mixture of carboxylic acids, the pH must be controlled. This is done by the addition of a buffering agent (e.g., ammonium bicarbonate, calcium carbonate), thus yielding a mixture of carboxylate salts. Methanogenesis, being the natural final stage of anaerobic digestion, is inhibited by the presence of the ammonium ions or by the addition of an inhibitor (e.g., iodoform). The resulting fermentation broth contains the produced carboxylate salts that must be dewatered. This is achieved efficiently by vapour-compression evaporation. Further chemical refining of the dewatered fermentation broth may then take place depending on the final chemical or biofuel product desired.

The condensed distilled water from the vapour-compression evaporation system is recycled back to the fermentation. On the other hand, if raw sewage or other waste water with high BOD in need of treatment is used as the water for the fermentation, the condensed distilled water from the evaporation can be recycled back to the city or to the original source of the high-BOD waste water. Thus, this process can also serve as a water treatment facility, while producing valuable chemicals or biofuels. Because the system uses a mixed culture of microorganisms, besides not needing any enzyme addition, the fermentation requires no sterility or aseptic conditions, making this front step in the process more economical than in more popular methods for the production of cellulosic ethanol. These savings in the front end of the process, where volumes are large, allows flexibility for further chemical transformations after dewatering, where volumes are small.

Carboxylic Acids

Carboxylic acids can be regenerated from the carboxylate salts using a process known as “acid springing”. This process makes use of a high-molecular-weight tertiary amine (e.g., trioctylamine), which is switched with the cation (e.g., ammonium or calcium). The resulting amine carboxylate can then be thermally decomposed into the amine itself, which is recycled, and the corresponding carboxylic acid. In this way, theoretically, no chemicals are consumed or wastes produced during this step.

Ketones

There are two methods for making ketones. The first one consists on thermally converting calcium carboxylate salts into the corresponding ketones. This was a common method for making acetone from calcium acetate during World War I. The other method for making ketones consists on converting the vaporized carboxylic acids on a catalytic bed of zirconium oxide .

Alcohols

Primary Alcohols: The undigested residue from the fermentation may be used in gasification to make hydrogen (H_2). This H_2 can then be used to hydrogenolyze the esters over a catalyst (e.g., copper chromite), which are produced by esterifying either the ammonium carboxylate salts (e.g., ammonium acetate, propionate, butyrate) or the carboxylic acids (e.g., acetic, propionic, butyric acid) with a high-molecular-weight alcohol (e.g., hexanol, heptanol). From the hydrogenolysis, the final products are the high-molecular-weight alcohol, which is recycled back to the esterification, and the corresponding primary alcohols (e.g., ethanol, propanol, butanol).

Secondary Alcohols

The secondary alcohols (e.g., isopropanol, 2-butanol, 3-pentanol) are obtained by hydrogenating over a catalyst (e.g., Raney nickel) the corresponding ketones (e.g., acetone, methyl ethyl ketone, diethyl ketone).

Drop-in Biofuels

The primary or secondary alcohols obtained as described above may undergo conversion to drop-in biofuels, such as biogasoline, green diesel and bio-jet fuel. Such is done by subjecting the alcohols to dehydration followed by oligomerization using zeolite catalysts in a manner similar to the methanex process, which used to produce gasoline from methanol in New Zealand.

Acetic Acid Versus Ethanol

Cellulosic-ethanol -manufacturing plants are bound to be net exporters of electricity because a large portion of the lignocellulosic biomass, namely lignin, remains undigested and it must be burned, thus producing electricity for the plant and excess electricity for the grid. As the market grows and this technology becomes more widespread, coupling the liquid fuel and the electricity markets will become more and more difficult. Acetic acid, unlike ethanol, is biologically produced from simple sugars without the production of carbon dioxide:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$$

(Biological production of ethanol)

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH$$

(Biological production of acetic acid)

Because of this, on a mass basis, the yields will be higher than in ethanol fermentation. If then, the undigested residue (mostly lignin) is used to produce hydrogen by gasification, it is ensured that more energy from the biomass will end up as liquid fuels rather than excess heat/electricity .

$$3\ CH_3COOH + 6\ H_2 \rightarrow 3\ CH_3CH_2OH + 3\ H_2O$$

(Hydrogenation of acetic acid)

$$C_6H_{12}O_6 \text{ (from cellulose)} + 6\ H_2 \text{ (from lignin)} \rightarrow 3\ CH_3CH_2OH + 3\ H_2O$$

(Overall reaction)

A more comprehensive description of the economics of each of the fuels is given on the pages alcohol fuel and ethanol fuel, more information about the economics of various systems can be found on the central page biofuel.

Stage of Development

The system has been in development since 1991, moving from the laboratory scale (10 g/day) to the pilot scale (200 lb/day) in 2001. A small demonstration-scale plant (5 ton/day) has been constructed as is under operation and a 220 ton/day demonstration plant is expected in 2012.

Composition

Table: *Typical composition of biogas*

Compound	*Chem*	*%*
Methane	CH_4	50–75
Carbon dioxide	CO_2	25–50

Nitrogen	N_2	0–10
Hydrogen	H_2	0–1
Hydrogen sulfide	H_2S	0–3
Oxygen	O_0	0–0

The composition of biogas varies depending upon the origin of the anaerobic digestion process. Landfill gas typically has methane concentrations around 50%. Advanced waste treatment technologies can produce biogas with 55–75% CH_4 or higher using in situ purification techniques As-produced, biogas also contains water vapour, with the fractional water vapour volume a function of biogas temperature; correction of measured volume for water vapour content and thermal expansion is easily done via algorithm.

In some cases biogas contains siloxanes. These siloxanes are formed from the anaerobic decomposition of materials commonly found in soaps and detergents. During combustion of biogas containing siloxanes, silicon is released and can combine with free oxygen or various other elements in the combustion gas. Deposits are formed containing mostly silica (SiO_2) or silicates (Si_xO_y) and can also contain calcium, sulfur, zinc, phosphorus. Such white mineral deposits accumulate to a surface thickness of several millimetres and must be removed by chemical or mechanical means. Practical and cost-effective technologies to remove siloxanes and other biogas contaminants are currently available.

Applications

Biogas can be utilized for electricity production on sewage works, in a CHP gas engine, where the waste heat from the engine is conveniently used for heating the digester; cooking; space heating; water heating; and process heating. If compressed, it can replace compressed natural gas for use in vehicles, where it can fuel an internal combustion engine or fuel cells and is a much more effective displacer of carbon dioxide than the normal use in on-site CHP plants. Methane within biogas can be concentrated via a biogas upgrader to the same standards as fossil natural gas(which itself has had to go through a cleaning process), and becomes biomethane. If the local gas network allows for this, the producer of the biogas may utilize the local gas distribution networks. Gas must be very clean to reach pipeline quality, and must be of the correct composition for the local distribution network to accept. Carbon dioxide, water, hydrogen sulfide and particulates must be removed if present. If concentrated and compressed it can also be used in vehicle transportation. Compressed biogas is becoming widely used in Sweden, Switzerland, and Germany. A biogas-powered train has been in service in Sweden since 2005. Biogas has also powered automobiles. In 1974, a British documentary film entitled *Sweet as a Nut* detailed the biogas production process from pig manure, and how the biogas fueled a custom-adapted combustion engine.

Benefits

By using biogas, many advantages arise. In North America, utilization of biogas would generate enough electricity to meet up to three percent of the continent's electricity expenditure. In addition, biogas could potentially help reduce global climate change. Normally, manure that is left to decompose releases two main gases that cause global climate change: nitrous dioxide and methane. Nitrous dioxide warms the atmosphere 310 times more than carbon dioxide and methane 21 times more than carbon dioxide. By converting cow manure into methane biogas via anaerobic digestion, the millions of cows in the United States would be able to produce one hundred billion kilowatt hours of electricity, enough to power millions of homes across the United States. In fact, one cow can produce enough manure in one day to generate three kilowatt hours of electricity; only 2.4 kilowatt hours of electricity are needed to power a single one hundred watt light bulb for one day. Furthermore, by converting cow manure into methane biogas instead of letting it decompose, we would be able to reduce global warming gases by ninety-nine million metric tons or four percent.

The 30 million rural households in China that have biogas digesters enjoy 12 benefits: saving fossil fuels, saving time collecting firewood, protecting forests, using crop residues for animal fodder instead of fuel, saving money, saving cooking time, improving hygienic conditions, producing high-quality fertilizer, enabling local mechanization and electricity production, improving the rural standard of living, and reducing air and water pollution.

Biogas Upgrading

Raw biogas produced from digestion is roughly 60% methane and 29% CO_2 with trace elements of H_2S, and is not high quality enough if the owner was planning on selling this gas or using it as fuel gas for machinery. The corrosive nature of H_2S alone is enough to destroy the internals of an expensive plant. The solution is the use of a biogas upgrading or purification process whereby contaminants in the raw biogas stream are absorbed or scrubbed, leaving 98% methane per unit volume of gas. There are four main methods of biogas upgrading, these include water washing, pressure swing absorption, selexol absorption and chemical treatment. The most prevalent method is water washing where high pressure gas flows into a column where the carbon dioxide and other trace elements are scrubbed by cascading water running counter-flow to the gas. This arrangement can deliver 98% methane with manufacturers guaranteeing maximum 2% methane loss in the system. It takes roughly between 3-6% of the total energy output in gas to run a biogas upgrading system.

Biogas Gas-grid Injection

Gas-grid injection is the injection of biogas into the methane grid (natural gas grid). Injections includes biogas: until the breakthrough of micro combined heat and power two-thirds of all the energy produced by biogas power plants was lost (the heat), using the grid to transport the gas to customers, the electricity and the heat can be used for on-site generation resulting in a reduction of losses in the transportation of energy. Typical energy losses in natural gas transmission systems range from 1–2%. The current energy losses on a large electrical system range from 5–8%.

Legislation

The European Union presently has some of the strictest legislation regarding waste management and landfill sites called the Landfill Directive. The United States legislates against landfill gas as it contains VOCs. The United States Clean Air Act and Title 40 of the Code of Federal Regulations (CFR) requires landfill owners to estimate the quantity of non-methane organic compounds (NMOCs) emitted. If the estimated NMOC emissions exceeds 50 tonnes per year the landfill owner is required to collect the landfill gas and treat it to remove the entrained NMOCs. Treatment of the landfill gas is usually by combustion. Because of the remoteness of landfill sites it is sometimes not economically feasible to produce electricity from the gas. However, countries such as the United Kingdom and Germany now has legislation in force that provide farmers with long term revenue and energy security.

Development Around the World

In 2007 an estimated 12,000 vehicles were being fueled with upgraded biogas worldwide, mostly in Europe.

In the United States

With the many benefits of biogas, it is starting to become a popular source of energy and is starting to be utilized in the United States more. In 2003 the United States consumed 147 trillion BTU of energy from "landfill gas", about 0.6% of the total U.S. natural gas consumption. Methane biogas derived from cow manure is also being tested in the U.S. According to a 2008 study, collected by the *Science and Children* magazine, methane biogas from cow manure would be sufficient to produce 100 billion kilowatt hours enough to power millions of homes across America. Furthermore, methane biogas has been tested to prove that it can reduce 99 million metric tons of greenhouse gas emissions or about 4% of the greenhouse gases produced by the United States.

In Vermont, for example, biogas generated on dairy farms around the state is included in the CVPS Cow Power program. The Cow Power program is offered by Central Vermont Public Service Corporation as a voluntary tariff. Customers can elect to pay a premium on their electric bill, and that premium is passed directly to the farms in the program. In Sheldon, Vermont Green Mountain Dairy has provides renewable energy as part of the Cow Power program. It all started when the brothers who own the farm, Bill and Brian Rowell, wanted to address some of the manure management challenges faced by dairy farms, including manure odor, and nutrient availability for the crops they need to grow to feed the animals. They installed an anaerobic digester to process the cow and milking centre waste from their nine hundred and fifty cows to produce renewable energy, a bedding to replace sawdust, and a plant friendly fertilizer. The energy and environmental attributes are sold. On average the system run by the Rowell brothers produces enough electricity to power three hundred to three hundred fifty other homes. The generator capacity is about three hundred kiloWatts. In Hereford, Texas cow manure is being used to power an ethanol power plant. By switching to methane biogas, the ethanol power plant has saved one thousand barrels of oil a day. Overall, the power plant has reduced transportation costs and will be opening many more jobs for future power plants that will be relying on biogas.

In the United Kingdom

In the UK, sewage gas electricity production is tiny compared to overall power consumption - a mere 80 MW of generation, compared to 70 GW on the grid.. There are currently less than 50 non-sewage landfill plants in the UK.

In the Indian Subcontinent

In Pakistan and India biogas produced from the anaerobic digestion of manure in small-scale digestion facilities is called gobar gas; it is estimated that such facilities exist in over two million households in India and in hundreds of thousands in Pakistan, particularly North Punjab, due to the thriving population of lifestock . The digester is an airtight circular pit made of concrete with a pipe connection. The manure is directed to the pit, usually directly from the cattle shed. The pit is then filled with a required quantity of wastewater.

The gas pipe is connected to the kitchen fireplace through control valves. The combustion of this biogas has very little odour or smoke. Owing to simplicity in implementation and use of cheap raw materials in villages, it is one of the most environmentally sound energy sources for rural needs. One type of these system is the Sintex Digester. Some designs use vermiculture to further enhance the slurry produced by the biogas plant for use as compost. The Deenabandhu Model is a new biogas-production model popular in India. (*Deenabandhu* means “friend of the helpless.”) The unit usually has a capacity of 2 to 3 cubic metres. It is constructed using bricks or by a ferrocement mixture. The brick model costs approximately 18,000 rupees and the ferrocment model 14,000 rupees, however India's Ministry of Non-conventional Energy Sources offers a subsidy of up to 3,500 rupees per model constructed.

In Developing Nations

Domestic biogas plants convert livestock manure and night soil into biogas and slurry, the fermented manure. This technology is feasible for small holders with livestock producing 50 kg manure per day, an equivalent of about 6 pigs or 3 cows. This manure has to be collectable to mix it with water and feed it into the plant. Toilets can be connected. Another precondition is the temperature that affects the fermentation process. With an optimum at 36 C° the technology especially applies for those living in a (sub) tropical climate. This makes the technology for small holders in developing countries often suitable.

Depending on size and location, a typical brick made fixed dome biogas plant can be installed at the yard of a rural household with the investment between 300 to 500 US $ in Asian countries and up to 1400 US $ in the African context. A high quality biogas plant needs minimum maintenance costs and can produce gas for at least 15–20 years without major problems and re-investments. For the user, biogas provides clean cooking energy, reduces indoor air pollution and reduces the time needed for traditional biomass collection, especially for women and children. The slurry is a clean organic fertilizer that potentially increases agricultural productivity.

Domestic biogas technology is a proven and established technology in many parts of the world, especially Asia. Several countries in this region have embarked on large-scale programmes on domestic biogas, such as China and India. The Netherlands Development Organisation, SNV, supports national programmes on domestic biogas that aim to establish commercial-viable domestic biogas sectors in which local companies market, install and service biogas plants for households. In Asia, SNV is working in Nepal, Vietnam, Bangladesh, Cambodia, Lao PDR, Pakistan and Indonesia, and in Africa in Rwanda, Senegal, Burkina Faso, Ethiopia, Tanzania, Uganda and Kenya.

Biodegradation

Biodegradation is the chemical breakdown of materials by environment. The term is often used in relation to ecology, waste management and natural environment (bioremediation). Organic material can be degraded aerobically with oxygen, or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process. Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms. Some microorganisms have a naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions. Products that contain biodegradable matter and non-biodegradable matter are often marketed as biodegradable.

Methods of Measuring Bio Degradation

Bio degradation can be measured in a number of ways. The activity of aerobic microbes can be measured by the amount of oxygen they consume or the amount of carbon dioxide they produce. It can be measured by anaerobic microbes and the amount of methane or alloy that they may be able to produce. In formal scientific literature, the process is termed bio-remediation.

Biodegradable Plastic

Biodegradable plastics are plastics that will decompose in natural aerobic (composting) and anaerobic (landfill) environments. Biodegradation of plastics can be achieved by enabling microorganisms in the environment to metabolize the molecular structure of plastic films to produce an inert humus-like material that is less harmful to the environment. They may be composed of either bioplastics, which are plastics whose components are derived from renewable raw materials, or petroleum-based plastics which utilize an additive. The use of bio-active compounds compounded with swelling agents ensures that, when combined with heat and moisture, they expand the plastic's molecular structure and allow the bio-active compounds to metabolize and neutralize the plastic.

Biodegradable plastics typically are produced in two forms: injection moulded (solid, 3D shapes), typically in the form of disposable food service items, and films, typically organic fruit packaging and collection bags for leaves and grass trimmings, and agricultural mulch.

Scientific Definitions of Biodegradable Plastic

In the United States, the FTCFederal Trade Commission is the authoritative body for biodegradable standards. ASTM International defines appropriate testing methods to test for biodegradable plastic, both anaerobically and aerobically as well as in marine environments. The specific subcommittee responsibility for overseeing these standards falls on the Committee D20.96 on Environmentally Degradable Plastics and Biobased Products . The current ASTM standards are defined as standard specifications and standard test methods. Standard specifications create a pass or fail scenario whereas standard test methods identify the specific testing parameters for facilitating specific time frames and toxicity of biodegradable tests on plastics.

Currently, there are three such ASTM standard specifications which mostly address biodegradable plastics in composting type environments, the ASTM D6400-04 Standard Specification for Compostable Plastics, ASTM D6868 - 03 Standard Specification for Biodegradable Plastics Used as Coatings on Paper and Other Compostable Substrates, and the ASTM D7081 - 05 Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment. The most accurate standard test method for anaerobic environments is the ASTM D5511 - 02 Standard Test Method for Determining Anaerobic

Biodegradation of Plastic Materials Under High-Solids Anaerobic-Digestion Conditions. Another standard test method for testing in anaerobic environments is the ASTM D5526 - 94(2002) Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions, this test has proven extremely difficult to perform. Both of these tests are used for the ISO DIS 15985 on determining anaerobic biodegradation of plastic materials.

Examples of Biodegradable Plastics

- While aromatic polyesters are almost totally resistant to microbial attack, most aliphatic polyesters are biodegradable due to their potentially hydrolysable ester bonds:
  - *Naturally Produced:* Polyhydroxyalkanoates (PHAs) like the poly-3-hydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and polyhydroxyhexanoate (PHH);
  - *Renewable Resource:* Polylactic acid (PLA);
  - *Synthetic:* Polybutylene succinate (PBS), polycaprolactone (PCL)...
- Polyanhydrides
- Polyvinyl alcohol
- Most of the starch derivatives
- Cellulose esters like cellulose acetate and nitrocellulose and their derivatives (celluloid).

Environmental Benefits of Biodegradable Plastics Depend upon Proper Disposal

Biodegradable plastics are not a panacea, however. Some critics claim that a potential environmental disadvantage of certified biodegradable plastics is that the carbon that is locked up in them is released into the atmosphere as a greenhouse gas. However, biodegradable plastics from natural materials, such as vegetable crop derivatives or animal products, sequester CO_2 during the phase when they're growing, only to release CO_2 when they're decomposing, so there is no net gain in carbon dioxide emissions. However, certified biodegradable plastics require a specific environment of moisture and oxygen to biodegrade, conditions found in professionally managed

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