Biotechnology and the manufacturing industry

Industrial biochemistry, microbiology and biotechnology involve the use of microorganisms and other micro and macro-molecules purposely to achieve specific goals. Basically, it involves:

(i) Production of new products with monetary, economy and/or social values.

(ii) Also for purpose of improving the standard of living and that of the environment under general acceptability.

Industrial biotechnology focuses on production of products such as foods, drinks, pharmaceuticals and medical compounds e.g. antibiotics, hormones, solvents, organic acids and enzymes that have direct economic values.

In industrial processes microorganisms are often used simply because:

(i) They are easier to handle.

(ii) Easier to cultivate.

(iii) Easier to manipulate.

Most microbes employed are usually isolated from nature and then modified using classical mutation and/or selection procedures.

Analysis of the microbial cell composition have revealed that over 90% of the cells dry weight is made up of both micro and macro elements which includes C, O, N, H, S, K, Zn, Mg and Ni.

The C, O, N, H, S and P for instance are important component of CHO, lipids, proteins and nucleic acids.

The K is required for activity of some enzymes while Mg could serve as cofactor for many enzymes.

The S is needed for the synthesis of amino acids such as cysteine and methionine

The Fe is usually part of cytochrome.

Ca^{2+} ion contributes to the heat resistance properties of some bacteria endospores.

The Zn^{2+} ion is usually present at the active site of some enzymes.

The N is required for the synthesis of amino acids and NH_3.

Apart from these elements, microorganisms also required other source(s) for growth, in order reflect some special nature of their morphology. Other compounds such as vitamins are also utilized by microbes such as, biotin, folic acid and thiamine (vit. B1).

Enzymes have been known to excel in their ability to alter chemical components of foodstuffs.

For instance, the protein, lipid and cellulose presence in food are ready made substrate for protease, lipases and cellulase respectively. Furthermore, most natural products have been known to be biodegradable due to the action of enzymes inherent in environmental microorganisms. Thus, production of substances of good economic, social and commercial

values in the industrial processes have been made possible by combining the right organism and inexpensive substrate (like some waste product e.g. mollases, saw-dust) and proper environment for both the enzyme and organisms involved in the industrial process. For instance, in fermentation, transformation of organic raw materials (substrate) by microorganism is usually carried out in a controlled favorable environment (created by fermentor) in order to form desired end product(s).

Choice of enzyme and its control

Substantial biochemical efforts are needed in order to understand the choice of enzyme in the context of the process or products desired. This required fundamental research efforts into classical parameters of enzyme kinetics under working condition such as pH and temperature optimum, K_m, V_{max}, presence or absence of inhibitor(s) and/or activator. Also, the structure and nature of enzyme in relation to its mode of catalytic functions needs to be understood. In addition, in most cases, before an enzyme is added or used in food or food related processes, its level of toxicity is normally assessed. It should be noted that the task of regulatory machinery is a bit complex and the pathway must be regulated and co-coordinated effectively such that all the cell components and other required materials would be present in precisely or relatively correct amount. Furthermore, a microbial cell is expected to respond to the environmental changes by using the nutrients present at a particular moment. It therefore meant that it must possess ability to synthesis as well as alter biosynthetic activity in response to changes in nutrient availability.

Note that regulation is necessary for the cell to

(i) Conserve microbial energy and materials.

(ii) Maintain metabolic balance.

(iii) Prevent over/under production of materials.

(iv) Save cost.

(v) Avoid production of toxic substances. e.t.c.

The principal uses of enzymes in industries involves among others:

-for alcoholic beverages production, bread making, cheese making, meat tenderizing, as sweeteners, clarification of beer, wine and fruit juice, production of detergent and medical application e.g. use of trypsin as an anti-inflammatory agent and wound cleanser; streptokinase from *streptococcus haemolyticus* is used to relief peripheral thrombosis.

The summary of some enzymes with their corresponding industrial applications is given below:

INDUSTRIES	ENZYMES	USES
Baking	fungal protease	soften dough
	Fungal x-amylase	general improvement
	Lipoxidase	whitens bread
Brewing	bacterial x-amylase	liquefaction cereal
	Bacterial protease	adjuncts
	Fungal amyloglucosidase	removal of dextran from wort or beer
	Papain	chill haze stability
	b-glucanase	lowers viscocity of wort or beer
Syrups	bacterial x-amylase	solbilise starch at high temperature
	Fungal amyloglucosidase	to complete the conversion of starch
	Inveetase	to convert sucrose to invert sugar
Cheese	rennin	curds milk by precipitation
	Catalase	removes H202 from milk
Ice cream	lactose	to remove crystallised lactose
Fruit	pectinase	general improvement
Vegetable	cellulose	softening and flavour promotion
Egg	glucose oxidase	removes glucose.
Meat	papain	tenderization
Textile	bacterial x-amylase	desizing
Paper	bacterial x-amylase	dextran adhesive
Laundering	bacterial proteases	protein stain removal
Medical	various uses	

Bacterial growth curve (See previous lecture)

The generation time for some bacteria under optimum condition s is given below:

Bacterium generation time

BACTERIUM	MEDIUM	GENERATION TIME
Escherichia coli	glucose-salts	17 minutes
Streptococcus lactis	milk	26 minutes
Bacillus megaterium	sucrose-salt	25 minutes
Streptococcus lactis	lactose broth	48 minutes
Rhizodium paponicum	mannitol-salt	344 minutes
Treponema pallidum	rabbit testis	1980 minutes

The importance of these phases is that it will enables one to have ideas as to the appropriate time to culture, harvest and manipulate the microorganism concern in relation to the desired enzyme(s) and/or other product(s).

Control of microbial growth

The control of microbial growth could be affected basically by the use of either chemical or physical agents. And this could involve the use of inhibitory agents or by physical killing agents. Agents that kill cells are called 'cidal'agents while those that inhibit growth of cell without killing are called 'static' agents.

Definition of terms:

Sterilization: This is the process by which living cells, viable spores and viruses are either destroy or remove from an object or habitat in such a way that the object will be totally free from any form of viable microorganism. Chemical agents used to sterilization are called sterilant

Disinfection: This is the killing, inhibition or removal of micro-organism that may cause disease and the chemical agent used for that are called disinfectant. Note that a disinfectant does not necessarily sterilize an object because

(i) viable spores and a few micro-organisms may remain and

(ii) disinfectants are normally used on inanimate objects

Antiseptics: These are chemical agents commonly applied to tissues to prevent infection by killing or inhibiting the growth of such pathogen. Antiseptics are generally not as toxic as disinfectants since they do not, in most cases, destroy the host tissues.

Physical methods of control includes among others;

Application of heat: Use of moist or dry heat has been known to be able to kill viruses, bacteria and fungi. Exposure to boiling water for about 10 minutes is enough to destroy vegetative cells but may not be high enough to destroy bacteria endospores. Most food processing industries used to combine the two methods of moist and dry heat applications in order to eliminate completely the risk of contamination. Dry heat has the advantages; it can be used to sterilize compounds like powder and oil. Also, used on glass ware and metal without corrosion.

Refrigeration and/or freezing: Low temperature is only good for short term storage of food and other items since it only slows down microbial growth. However, freezing at about -20^{o}C or lower would stop microbial growth completely. Thus, freezing is a very good method for long term storage, if properly carried out.

Filtration: This is an excellent way of reducing microbial population especially in solution of heat sensitive materials. Two types are known namely:

(i) Depth filters which consist of fibrous or granular materials. (ii) Membranes filter which consist of porous membrane made of cellulose acetate, cellulose nitrate or other synthetic materials. It is commonly used for oil, antibiotics and other heat sensitive materials or solutions.

Radiation: These are of two types:

(i) Ultra-violent which is used in only a few specific situations because it burn skin and could damage the eye? Though, commercial UV unit is often used in water purification.

(ii) Ionizing radiation which could penetrate and sterilize subjects by destroying the cell nature be it the endospore or the vegetative cells. Gamma radiation from cobalt-60 source is used in cold sterilization of antibiotics, hormones and plastic disposable substances such as syringes.

Use of chemical agents

Although, chemicals agent meant to be used should be toxic to the pathogen, it should not be toxic to the people. Effective and continuous use of some chemical agents has led to frequency of antibiotic resistance in some cases. Examples of chemical often used are phenolic and hexachlorophene. Some of these agents act by denaturing proteins. However, they have disadvantages in having offensive odor and in being able to cause skin irritation and brain damage.

The summary of some chemicals often used in controlling microbial growth is given below:

CHEMICAL	ACTIONS	USES
Ethanol	dentures protein and solubilise lipids	antiseptic used on skin
Isopropanol	denatures protein and solubilise lipids	antiseptics used on skin
Formaldehyde	reacts with –NH2, -SH groups	disinfectant,kills endospore
Chlorine gas	oxidizing agent	disinfect drinking water
$HgCl_2$	inactivate protein	disinfectant on skin
NH_4 compd	disrupts cell membrane	skin antiseptics
Ethylene oxide	alkylating agent	disinfectant on rubber/plastics .

Commonly used preservatives are also summarized below:

PRESERVATIVES	CONCENTRATION	USES
Propionic acid	0.32%	antifugal agent in bread, cake
Sorbic acid	0.2%	antifugal agent in syrup, cheese
Benzoic acid	0.1%	antifugal in margarine, relishes
Lactic acid	0.1-10%	antimicrobial in yoghurt
Sulfur dioxide	200-300ppm	antimicrobial in dried fruits
Sodium nitrite	200 ppm	antimicrobial in meat and fish
Sodium chloride	unknown	prevents spoilage of fish, meat
Wood smoke	unknown	prevents spoilage of fish, meat.

Commonly used chemotherapeutic agents

CHEMICAL CLASS	EXAMPLE	MODE OF ACTION
B-lactams	penicillin, cephalothin	inhibits cell wall synthesis
Clavulanic acid	clavamox, amoxicillin	inhibits betalactomases
Aminoglycosidase	streptomyces	inhibits translation
Macrolides	erythromycin	inhibits translation
Rifamycins	rifampicin	inhibits transcription
Tetracyclines	tetracycline	inhibits translation
Chloramphenicol	chloramphenicol	inhibits translation.
Monobactams	aztreonam	inhibits cell wall synthesis

Production of antibiotics

Antibiotics are defined as the complex chemical substances in forms of secondary metabolites, which are produced by micro-organism purposely to act against other micro-organisms. Four major broad groups of antibiotics are most extensively used throughout the world and these are; **penicillins, tetracyclines, erythromycinsb** and **cephalosporin**. Outline for the commercial production of penicillin is given below:

This is usually carried out in a fermentor which is meant to provide optimum growth condition for *P. chrysogenum* for its maximum yield. The following steps are to be followed:

(i) inoculate 100ml medium in 500ml flask with spores of *P.chrysogenum* strain and incubate at 25^{o}C by keeping on a rotary shaker.

(ii) After 4 days transfer the content to another 4 litre flask and leave for another 4 days

(iii) Transfer to 800litre containing 500litre medium.

(iv) After 3 days, use the contents for inoculation of about 180,000 litre medium kept in a fermentor (250,000 litre capacity).

(v) Filter the content of fermentor after 6 days of inoculation.

(vi) The filtrate containing penicillin is then extracted with amyl or butyl-acetate

(vii) From this, transfer the penicillin into aqueous solvent by extracting with phosphate buffer.

(viii) Crystallize the penicillin out of the mixture. The major steps are:

1. Preparation of the inoculum.
2. Preparation and sterilization of medium.
3. Inoculation of the medium in the fermentor.
4. Forced aeration with sterile air during incubation.
5. Removal of the mold mycelium after fermentation.
6. Extraction and purification of the penicillin.

Production of Ethanol from Cassava

Cassava (peeling, washing) → milling→ cassava flour (add water and α-amylase) → liquefaction (90-95ºC, pH 4-4.5, at 400 rpm) → saccharification→ cooling→ fermentation→ filtration→ distillation→ ethanol.

Production of cheese from milk

Milk→ acidification→ coloring→ coagulation by renin→ separation of curd from whey→ addition of flavor to curd→ compression of curd→ aging→ finished cheese.

Amino acids derivatives e.g monosodium glutamate (MSG)

The first amino acid to be produced and commercialized is L-glutamate in which *Corynebacterium glutamicum* is used for successful production.

The biochemical pathway for the production of L-glutamate is shown below:

Glucose→phosphoenolpyruvate→pyruvate→acetylcoA→citrate→isocitrate→ x-ketoglutarate →L-glutamate.

The flow chart for industrial/commercial production:

Sugar tank→continous stirrer→buffer tank→seed fermentor→NH4, pH control unit→batch sterilizer→production fermentor→harvesting tank.

Reference:

Industrial Biochemistry course, Dr. Oluseyi Adeboye. http://www.unaab.edu.ng