THE UNIVERSITY OF MELBOURNE

FOOD20006

Food Microbiology and Safety

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Intended learning outcome

Explain how and why microbial enzymes are used in food production

Microbial products for food production: Enzymes

Enzymes are the large biomolecules that are required for the numerous chemical interconversions that sustain life.

They accelerate all the metabolic processes in the body and carry out specific tasks.

Enzymes are highly efficient, which can increase reaction rates by 100 million to 10 billion times faster than any normal chemical reaction.

Microbial enzymes are currently acquiring much attention with rapid development of enzyme technology.

Microbial enzymes are often preferred over live microorganisms due to :

- their economic feasibility
- high yields
- consistency
- ease of product modification and optimization
- stability
- high catalytic activity
- rapid growth of microbes on inexpensive media
- regular supply due to absence of seasonal fluctuations
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Some uses of microbial enzymes in food processing

pectinase for treatment of fruit juice (fresh juice or wine industry)

pectinase in chocolate fermentation

lactase to make low lactose foods

amylases for the conversion of starch into dextrins or glucose

proteases in cheese manufacture

proteases in soybean products (miso, soy sauce or tempeh)

glucose isomerase for the production of fructose-rich corn syrup.

Pectinase

Major uses

- treatment of fruit juices to reduce the cloudiness, viscosity and bitterness of fresh fruit juices
- break down grape pulp for winemaking extraction of tomato pulp
- to remove the mucilaginous coat from coffee beans in coffee fermentation

Pectinase

Pectinase is a general term for enzymes commonly referred to as pectic enzymes.

Pectic enzymes is a collective term that includes

- pectin lyase
- pectin methylesterase
- polygalacturonase

These break down pectin, a polysaccharide substrate that is found in the cell walls of plants

Pectins are high molecular weight acid polysaccharides, primarily made up of α-(1→4) linked D-galacturonic acid residues with a small number of rhamnose residues in the main chain and arabinose, galactose and xylose in the side chain. Different pectinases cleave different bonds in pectin

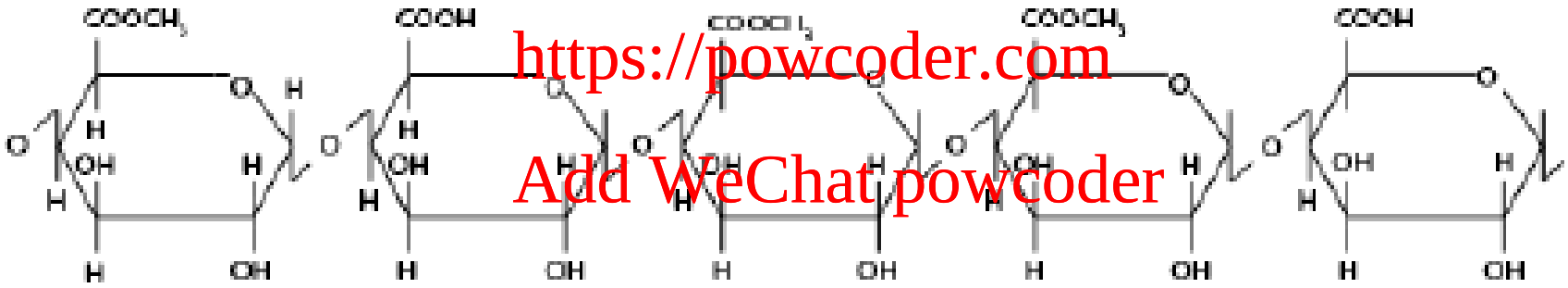


Figure 6: Chemical structure of pectin.

Pectin in fruit juice processing

During the early stages of fruit juice production, the fruit pulp has a lot of associated pectin giving the juice a gelatinous and viscous texture

Pectin impacts on the sensory properties of the juice and its colour, but also importantly impacts on the juice extraction process.

Pectin, a major component of juice cloud, is thought to play an important role in juice destabilization.

Pectin forms calcium pectate complexes and causes the precipitation of cloud particles

Pectinase in fruit juice processing

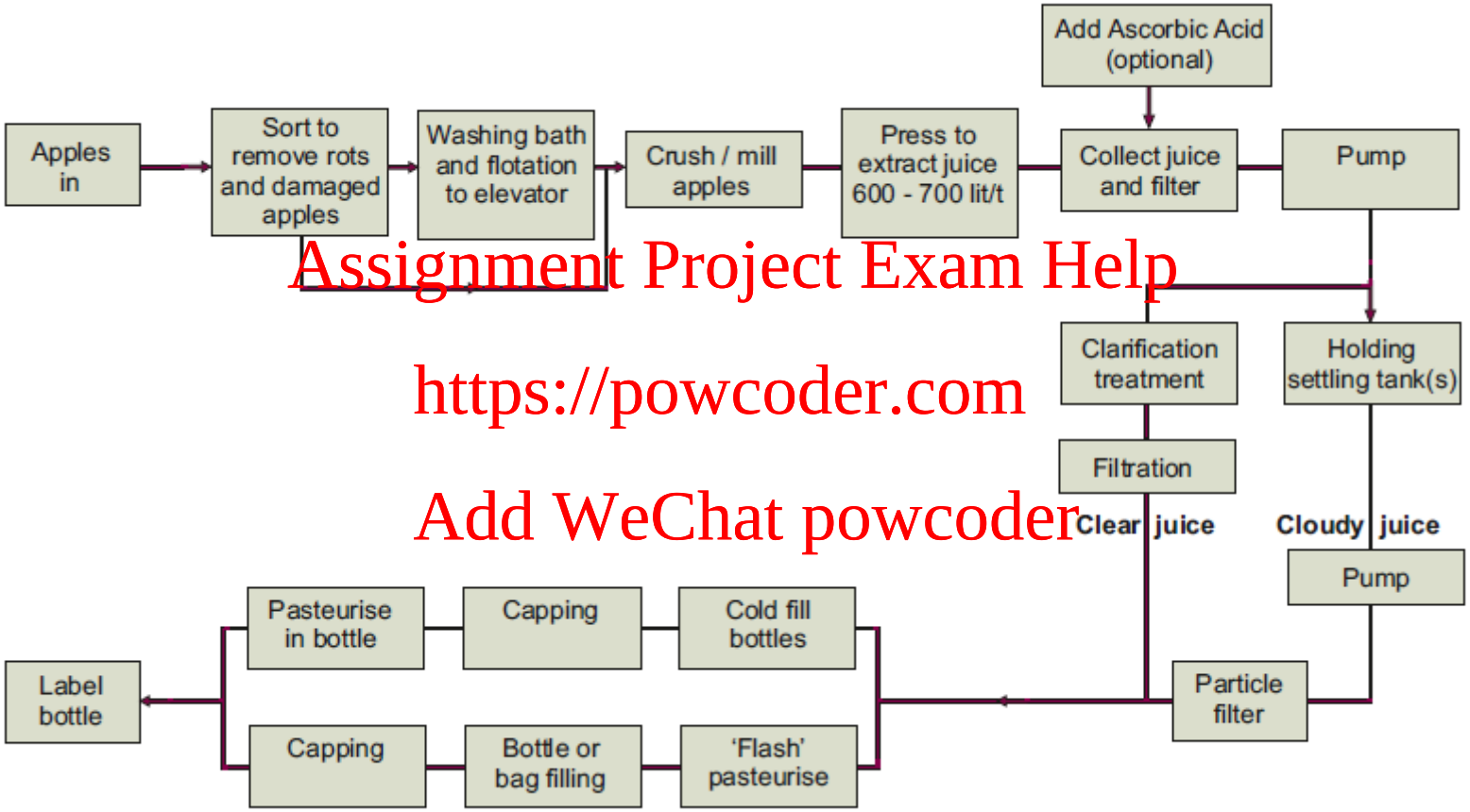
Pectinase is used commercially to aid in extracting juice from fruit.

By enzymatically breaking down the cell wall, pectinase releases the juice from within the cells.

Pectinases can be used to clarify cloudy juices such as apple juice

Cloudy juices are processed with some pectinases, particularly polygalacturonases, but with the purpose of stabilising the cloud of the juice

Apple juicing process



Product Description

Fructozym® P is a liquid, highly concentrated pectolytic enzyme preparation for a fast and complete pectin degradation in fruit mash and fruit juice.

rapid breakdown of pectic substances in fruit mash for **enhanced pressability** and liberation of valuable fruit ingredients.

complete pectin degradation in juice, precondition for good **clarification and filtrability**.

rapid degradation of pectin results in a drastic reduction of mash viscosity, thus good pressability, high juice yield a

In the juice, Fructozym® P breaks down the pectin skeletal structure which has a stabilizing effect on sediments, thus creates the prerequisite for **good clarification and filtrability**.

http://www.vigoltd.com/files//b647b152-f5db-4a0c-9938-a182009548fe/94371%20Fructozym_P.pdf

Microbial pectinase

Bacteria:	*Bacillus, Erwinia, Pseudomonas*
Yeasts:	*Kluyveromyces,*
Moulds:	*Aspergillus, Rhizopus, Penicillium* and *Fusarium* are good producers of pectinases

Filamentous fungi, such as *Aspergillus niger* and *Aspergillus carbonarius* and *Lentinus edodes*, are preferred in industries since approximately 90% of produced enzymes may be secreted into the culture medium

Aspergillus niger

Aspergillus is particularly useful industrially

The fungal metabolites produced by *Aspergillus* are penicillin, citric acid, koji acid, L_malic acid, amylase, catalase, cellulase, galactosidase, glucanase, glucosidase, hemicellulase, lipase, pectinase and protease

It is 'Generally Recognised as Safe'

Aspergillus niger produces more than one different type of pectinase

Grown in solid state or submerged fermentation systems (more later)

Lactase

Lactase is used in the manufacture of low lactose dairy products

Lactase (β-D-galactosidase) hydrolyzes milk lactose into its constituent monosaccharides, glucose and galactose.

Chemical and physical changes that occur as a result of lactose hydrolysis

The principal changes are

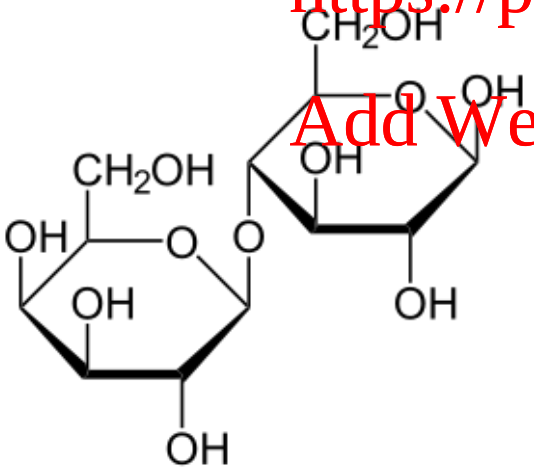
- reduced lactose content
- increased carbohydrate solubility

- increased sweetness
- higher osmotic pressure
- reduced viscosities
- more readily fermentable sugar.

Enzymatic hydrolysis of lactose in dairy foods improves product quality and provide low-lactose products for the lactose intolerant people

Lactase

Beta-galactosidase is highly important in the dairy industry, in the hydrolysis of lactose into glucose and galactose with an improvement in the solubility and digestibility of milk and dairy products.



Lactase

Natural sources include

- animal organs such as the intestine, the brain and skin
- peaches, almonds and certain species of wild roses

Bacteria: *Escherichia coli*, *Lactobacillus bulgaricus*, *Streptococcus lactis* and *Bacillus* sp

Yeasts: *Kluyveromyces lactis*, *K. fragilis* and *Candida pseudotropicalis*

Filamentous fungi: *Aspergillus foetidus*, *A. niger*, *A. oryzae* and *A. phoenecia*.

Lactase from different LAB fount in dairy fermentations

Table 3. Summary of lactase activity from different *Lactobacillus* bacteria.

Sources of Strains	*Lactobacillus sp.*	*Supernatant fractions (ml)*	*Protein conc. (mg/ml)*	*Glucose conc. (µg/ml)*	*Total activity (U/l)*	*Specific activity (U/mg)*
JGS	*L. lactis*	78	12.00	221.25	512.15	42.67
CM	*L. bulgaricus*	64	17.00	36.50	850.69	50.04
AD	*L. delbrueckii*	64	14.50	5.00	11.57	0.826
RM	*L. lactis*	65	17.25	131.25	303.819	17.612
MCS	*L. bulgaricus*	78	9.00	86.25	199.65	22.18

In this research paper, scientists compared different *Lactobacillus* bacteria to see which produced the most lactase and the highest activity lactase

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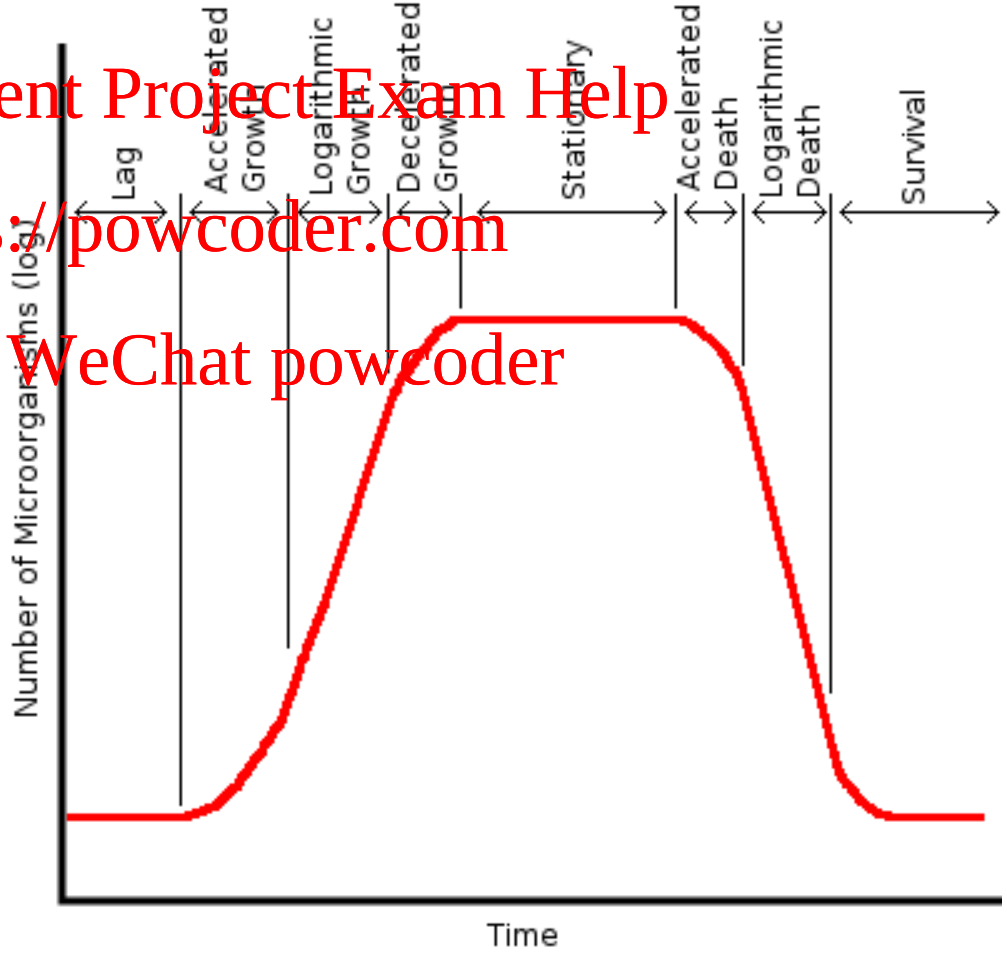
Harvesting microbial products from cultures: Batch culture

Cell density and medium composition change constantly throughout the growth cycle so it is impossible to choose and maintain a particular environment

Increasing:
cell numbers
Concentration of metabolic waste products

Decreasing:
Oxygen
Nutrients
pH

Constant:
Volume
Temperature
Mixing speed



Continuous culture

Continuous systems can achieve steady state conditions for a prolonged period

Continuous culture systems can take a number of forms

- Stirred reactors-mixing is assumed to be instantaneous and perfect and the growth rate of the population is constant

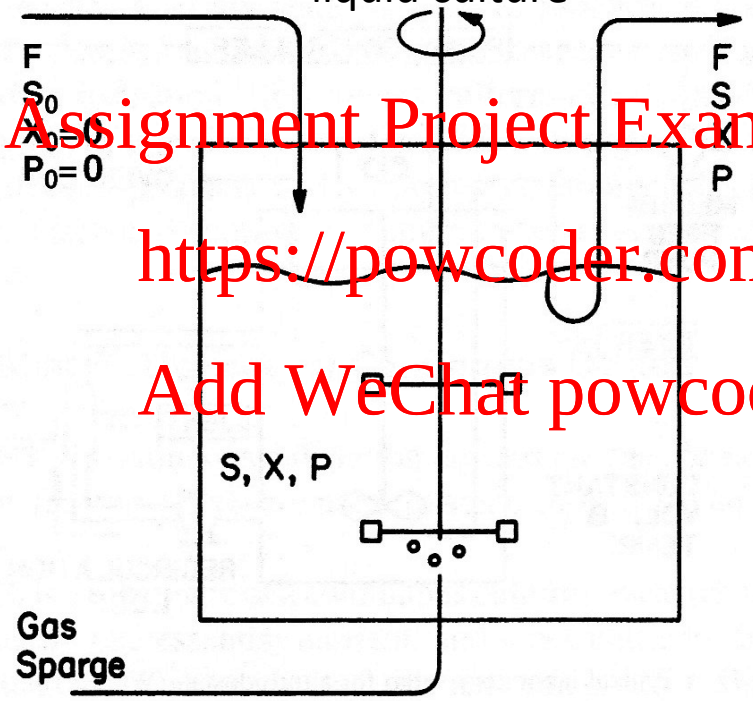
Harvesting microbial products from cultures: Continuous culture, submerged

In ports supply fresh medium containing the substrate

The impeller mixes the liquid culture

Out port

Out port is where the culture products are collected. Cells may be recycled

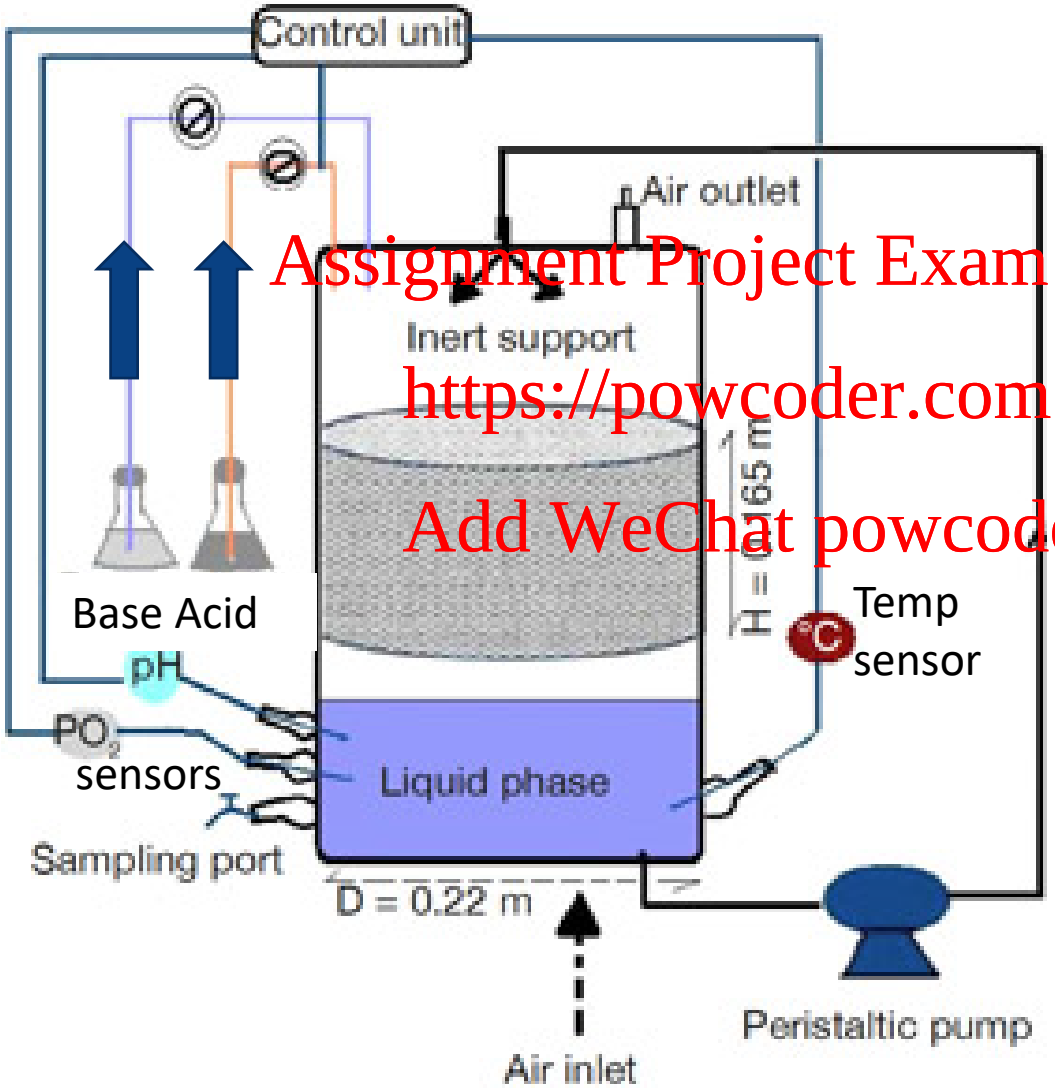


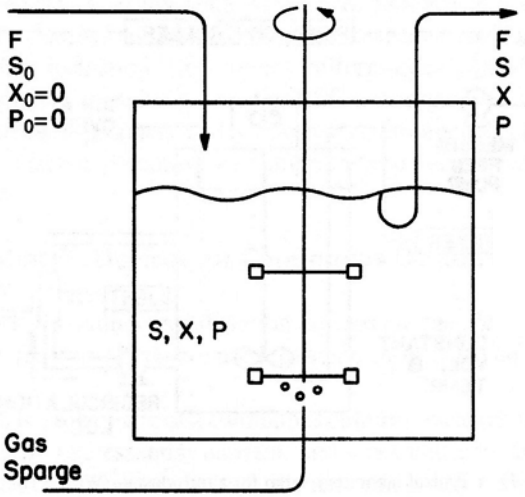
The culture volume remains constant and the flow rate is set to match the growth rate so the cell density remain constant

Gas supply constant O_2.

F = flow rate
S = substrate
X = cells
P = product

Harvesting microbial products from cultures: Solid state





Harvesting microbial products from cultures: Solid state

Support colonized by microorganism

Metallic support before colonization by microorganism

Tank without wall growth

Summary: Microbial enzymes

Enzymes can be produced from cultures for use in food processing

Methods of culture include batch culture, continuous culture, submerged fermentation and solid state fermentation

Examples: Pectinases, Lactase