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Thailand-Japan SSH Exchange Program

Biology Experiment

What is "Saccarification" and "Fermentation"?

Name（ ）

Group Member（ ）

Experiment 1　Observation of *Aspergillus oryzae*

What is *Aspergillus oryzae*?

Aspergillus is classified in sac fungus, it is generic name of useful mold contained in "Koji (malted rice)". *Aspergillus oryzae* is used most for Japanese Koji to make "Sake (rice wine)" and "Miso (bean paste)". *Aspergillus oryzae* produce and release various enzymes from the tip of the hypha, starch (is included in rice) is disassembled into glucose by enzymes, *Aspergillus oryzae* multiplies glucose as a nourishment source. This process is called "saccharification". "Multiple parallel fermentation" is a type of fermentation in the process to produce brew, meaning that saccharification and alcohol fermentation occur simultaneously in the same container. "Sake" is brewed in "moromi" made by "Multiple parallel fermentation".

Fig.1 "*A.oryzae*" of surface on the rice

Fig.2 "*A.oryzae*" through a microscope

(1) Sprinkle seed malt (powdered) on steaming rice, put it in incubator for 48 hours.
(2) After 48 hours, when you can find *Aspergillus oryzae* such as white hair on surface of rice, put a piece of rice on the slide glass.
(3) Observe it with a microscope by 40 or 100 times.

Experiment 2　Measurement of production of CO_2 and fermentation kinetics

What is Fermentation?

The phenomenon of fermentation is a chemical reaction by the enzyme in a microbe. Alcohol has been made from ancient times using the fermentation by the yeast.

Although it is based on work of a microbe, and we had passed through the historic change before this phenomenon was elucidated scientifically. In 1789, Lavoisier, a French scientist, showed that alcohol fermentation was a reaction which happens when sugar is disassembled into C_2H_5OH and CO_2. In 1857, Pasteur, a French scientist, discovered lactic acid bacteria and butyric acid bacteria which make things taste unpleasant during the process of alcohol fermentation, and in 1860, he found out that the speed of glucose consumption by yeast increased as oxygen partial pressure got lower on the occasion of alcohol fermentation (Pasteur Effect). In 1896, Buchner, a Germany scientist, discovered that the non-cell fluid extracted from beer yeast caused alcohol fermentation. Thereby, it became clear that the enzyme in a microbe causes a reaction. In 1930s, enzyme came to be taken out as a pure crystal, and it became clear that generally the true character of enzyme is protein.

Enzyme is protein and it works as a catalyst of a vital reaction. Protein is a single stranded high molecular compound which has connected to amino acid. When amino acid residue forms hydrogen bonds (N-H--O etc.), alpha-spiral structure, beta-sheet structure, etc. arise. When they are folded up, characteristic three-dimensional structure is formed. When specific substrate is taken in into the hollow pocket, the enzyme itself does not change, although a reaction is made to cause. Generally, the enzyme reacts to only specific substrate or the molecules similar to it, and does not take

in other substrates. This is called “substrate specificity”. Moreover, the speed of an enzyme reaction is influenced by temperature. If temperature is too low, reaction velocity will fall like the usual chemical reaction. If high temperature is used, the enzyme as it is protein, will denature with heat, and activity will fall above about 70[℃] . In an enzyme reaction, the temperature at which reaction velocity becomes the highest is called “optimal temperature”.

If the substrate [S] reacts to the enzyme [E], the complex [ES] will be formed, and products [P] will be made, but the enzyme [E] does not change.

$$E + S \rightarrow ES \rightarrow E + P$$

This reaction velocity is proportional to [S], when the concentration of [S] is low enough. Moreover, in order for enzyme to work efficiently, temperature and pH must be restricted. The optimal temperature of the alcohol fermentation by yeast is about 45[℃], and optimal pH is around 7. In addition, the reaction velocity of the fermentation using yeast becomes as higher as the concentration of yeast gets higher.

If yeast is put into the state of insufficient oxygen, glucose will be decomposed into C_2H_5OH and CO_2 through pyruvic acid ($C_3H_4O_3$) by anaerobic respiration.

$$C_6H_{12}O_6 \rightarrow (2C_3H_4O_3) \rightarrow (2CH_3CHO) \rightarrow 2C_2H_5OH + 2CO_2$$

Glucose is decomposed by action of “Zymase”. “Zymase” is 14 kinds of enzyme contained in yeast, and becomes pyruvic acid first. (This reaction path is called “Glycolysis” and about 10 kinds of enzyme works in it.) After that, CO_2 is deprived by the decarboxylation in the action of decarboxylase, and pyruvic acid becomes acetaldehyde (CH_3CHO). Furthermore, hydrogen is received from NADH in the action of alcohol dehydrogenase, which is reduction, and it becomes C_2H_5OH. It means that 1-molecule of glucose became 2-molecules CO_2 and 2-molecule C_2H_5OH in this process.

What is the yeast?

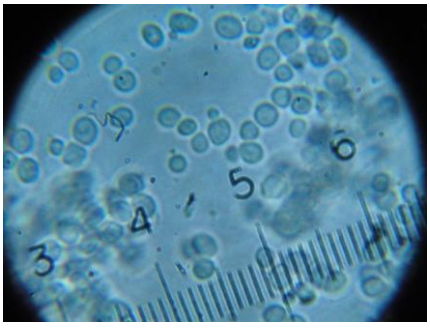


Fig.3 “*Yeast*” through a microscope

“Yeast” is not the term on a classification but originally, it is the name attached to the fungi which cause fermentation. It belongs to basidiomycetes, sac funguses and imperfect fungi. Yeast is unicellular, and it’s size is 2 to 20 micrometer (many are 3 to 5 micrometer) which is several times as large as ordinary bacteria. It is a globular form, an egg form and an elliptical globular form. It is the facultative anaerobic bacteria that ferments in an environment without oxygen, and when oxygen is supplied in, performs aerobic respiration and grows. They have a sheath film outside of a cell wall, and their multiplication is a style called “budding” which means a part of cell poked and comes. Thus, although yeast is a fungus, it is the expedient name conferred upon the bacillus group which has only a generation of unicellular and does not build a typical fungal thread like a filamentous bacterium. There are many kinds of yeast and 151 genera and 1312 species are contained in the 5th edition of “The Yeasts and A Taxonomic Study” (April, 2011). The *Saccharomyces cerevisiae* is representative in yeast performing alcohol fermentation. A pathogenic yeast has *Candida albicans*, and a marine yeast has *Rhodotorula glutinus*.

(1) Take fermentation liquid 50mL (15% glucose solution and dry yeast 3g) into Erlenmeyer flask, and react it while warming it to about 40[℃] by hot stirrer.

(2) Using the water substituting method, measure how long it takes 10[mL] of CO_2 to occur. Perform the measurement to 100[mL] and record the time every 10[mL].

※ Start the measurement after you see generating of a bubble from tip of the glass tube.

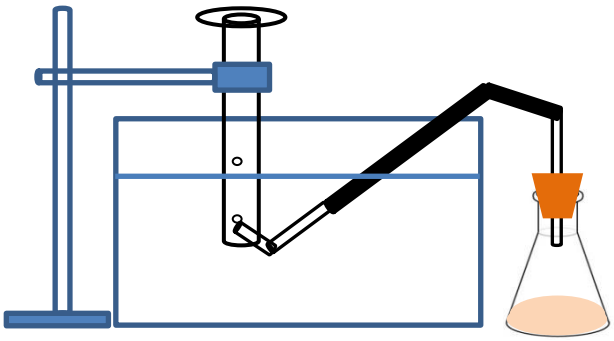
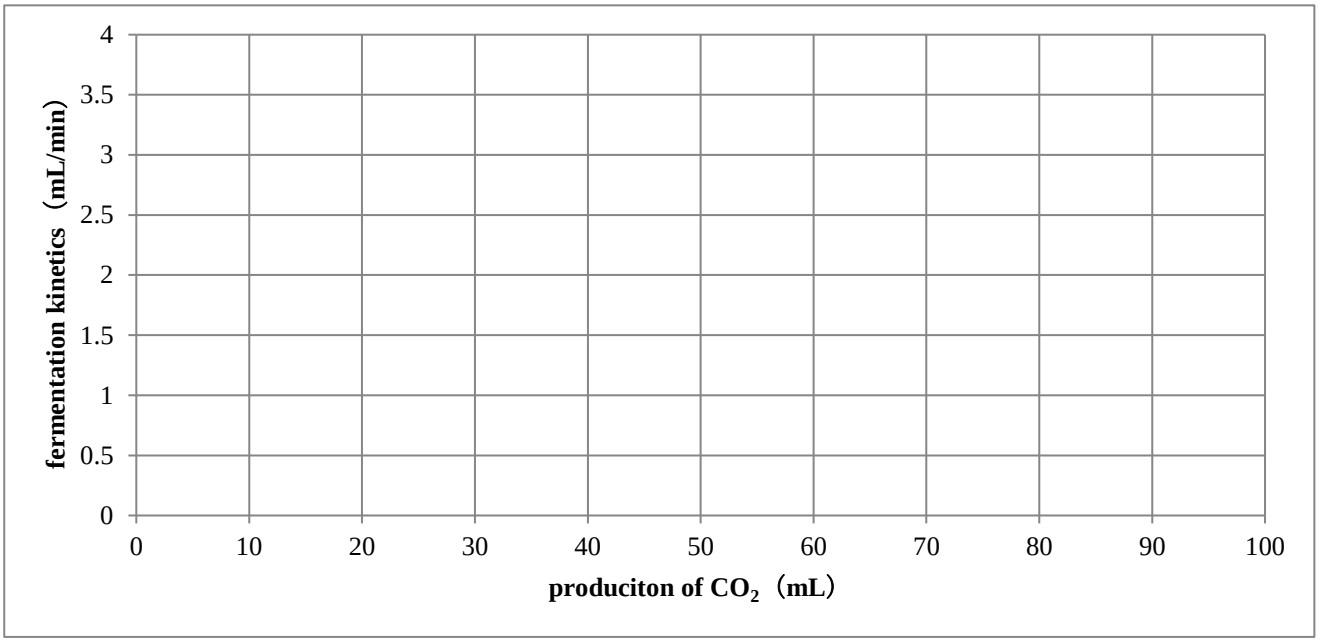


Fig.4 Experimental device

The time to spend for production of CO_2 10 mL

CO_2(mL)	0	10	20	30	40	50
Time(m,s)	0,00					
CO_2(mL)	50	60	70	80	90	100
Time(m,s)						

Fermentation kinetics (mL/min)



Chemical reaction formula of fermentation (anaerobic respiration)

	$C_6H_{12}O_6$	$2C_2H_5OH$	$2CO_2$
Number of moles [g/mol]	1 [180]	2 [2×46]	2 [2×44]
When room temperature is 20[℃]		1mL=1.917×10^{-3}[g] (46g/24000mL)	1mL=1.833×10^{-3}[g] (44g/24000mL)
Glucose using this reaction [g]	7.5 [g]		
Number of moles [mol]	[mol]	[mol]	[mol]
Ethanol and CO_2 producing in this reaction [g]		[g]	[g]
Max of production of CO_2 per a minute in this reaction [mL/min]			[mL/min]
Production of CO_2 and ethanol per a minute [g/min]		[g/min]	[g/min]
How long it takes all ethanol to produce in this reaction? [hour]		[hour]	
Production of ethanol for 100 mL CO_2 [g]		[g]	
Concentration of ethanol per 50 mL liquid for 100 mL CO_2 [%]		[%]	

(Calculation)

Experiment 3　Making of Simple Bioreactor

What is Bioreactor?

Bioreactor is the reactor which performs composition and decomposition of a substance using living things (such as an animal and plant cell and a microbe) as the biological catalysts. For example, a bioreactor carries out purification processing of the organic matter under drainage using the work of a microbe which preys on an underwater organic matter as food and it decomposes into sludge, in a sewage disposal plant.

A reactor is equipment which performs composition and decomposition of a substance using the characteristic of the enzyme, and it is applied in various fields. For example, it is used for production of useful substance by cultivation of a microbe or a cell, generating of energy, sick diagnosis, and decomposition of environmental pollutant.

Fig.5　"Yeast beads"

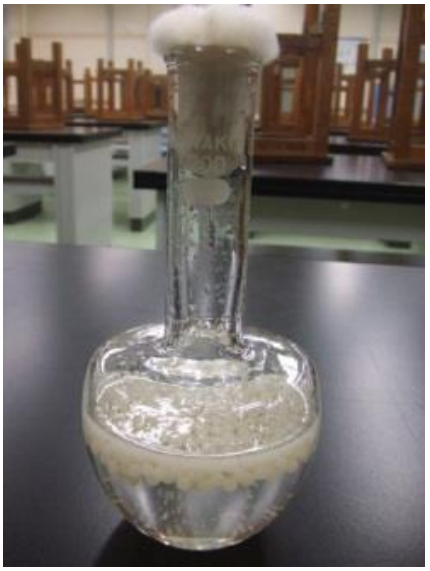


Fig.6　Simple "Bioreactor"

(1) Make yeast liquid (add water 20mL to dry yeast 3g)
(2) Mix (1) with 1.5% sodium alginate solution 100mL.
(3) Make 1.0% calcium chloride solution 200mL.
(4) Using 5 mL pipette, drop one drop of liquid [100mL] of (2) into (3), and make beads of the same size.
(5) Take out and wash only yeast beads with a tea strainer, and remove a calcium chloride.
　※ Alginate is a polysaccharide contained in brown algae. It plays a role of the ingredient of a cell wall, and quality of packing between cells.
(6) Put fixed yeast into 15% glucose solution 150mL (figure.2).
(7) Cork (6) with cotton wool and let it ferment for 24 hours within water bath (45[℃]).
(8) After 24 hours, check whether the alcohol is made.