"World Halal Summit"

7th WORLD HALAL SUMMIT

Cysteine Manufacturing from a Halal Perspective

In association with;

SMIIC
THE STANDARDS AND METROLOGY INSTITUTE FOR ISLAMIC COUNTRIES

New Era & New Normals:
Necessity of Halal Production and Consumption

Organized by;

DISCOVER EVENTS

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Confidence through Integrity.

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OUTLINE

Structure and properties of cysteine

Applications of cysteine:

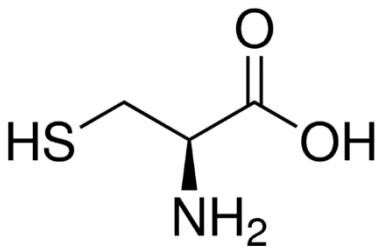
- Food
- Pharmaceutical products
- Cosmetics
- Animal feed

Production methods of cysteine:

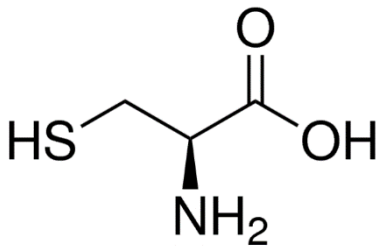
- Keratin Hydrolysate
- Fermentation
- Enzymatic Bioconversion

Structure and Properties

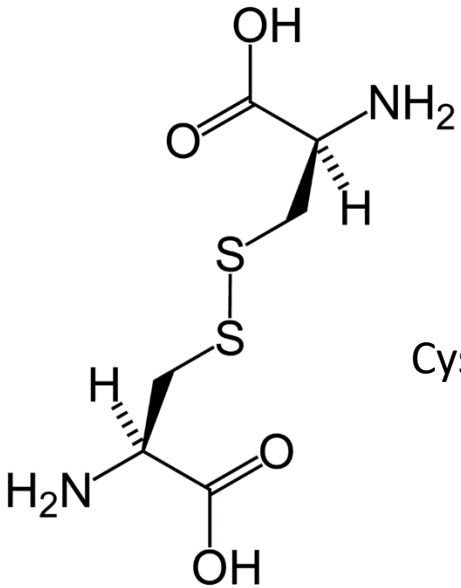
Cysteine is a naturally occurring, sulfur-containing amino acid that is found in most proteins, although only in small quantities. Cysteine is unique amongst the amino acids as it contains a thiol group. Cysteine can react with itself to form an oxidized dimer by formation of a disulfide bond.



Cysteine



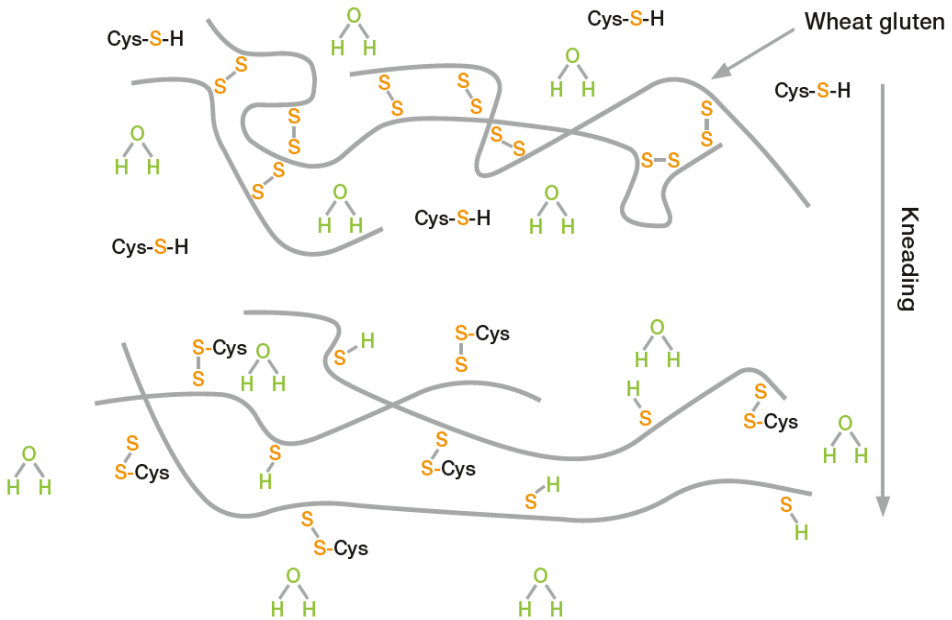
Oxidation



Cystine

Applications of Cysteine

In food industry, especially in bakery application, cysteine is used as flour additives to break up the gluten in flour, thus reducing its stickiness and facilitating the kneading of the dough.



Cysteine reduces the disulfide bonds (S-S) within the gluten network (1).

One of the largest applications of cysteine is the production of various flavors. For example, reacting cysteine with sugars in a Maillard reaction yields meat flavors.

Applications of Cysteine

In cosmetics area, cysteine is used in permanent hair wave preparation due to its ability to break disulfide bonds in keratin. The acetylated cysteine (N-acetylcysteine) is being used in the formulation of safe and effective products for antiaging and skin care products (2,3).

In pharmaceutical field, the cysteine derivative N-acetyl cysteine (NAC) is often used as a cough medicine.

Cysteine has been linked to aiding in the remedy of certain symptoms from drinking alcohol. It directly counteracts the poisonous effects of acetaldehyde, a particularly toxic by-product of alcohol in the human body.

Applications of Cysteine

As animal feed, cysteine is considered efficacious in partially meeting the requirements of Sulphur containing amino acids in animal (4).

Cysteine is required by sheep in order to produce wool. However, for sheep, it is an essential amino acid that cannot be synthesized by the sheep and must be taken in as food from grass. This means that during drought conditions, sheep produce less wool.

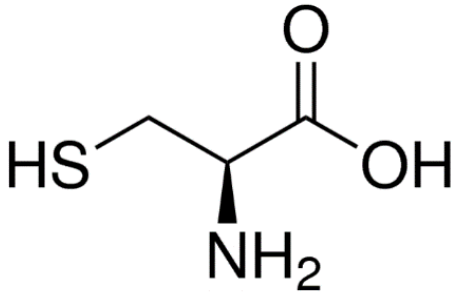
Production Methods of Cysteine

- Keratin Hydrolysate
- Fermentation
- Enzymatic Bioconversion

1. Keratin Hydrolysate

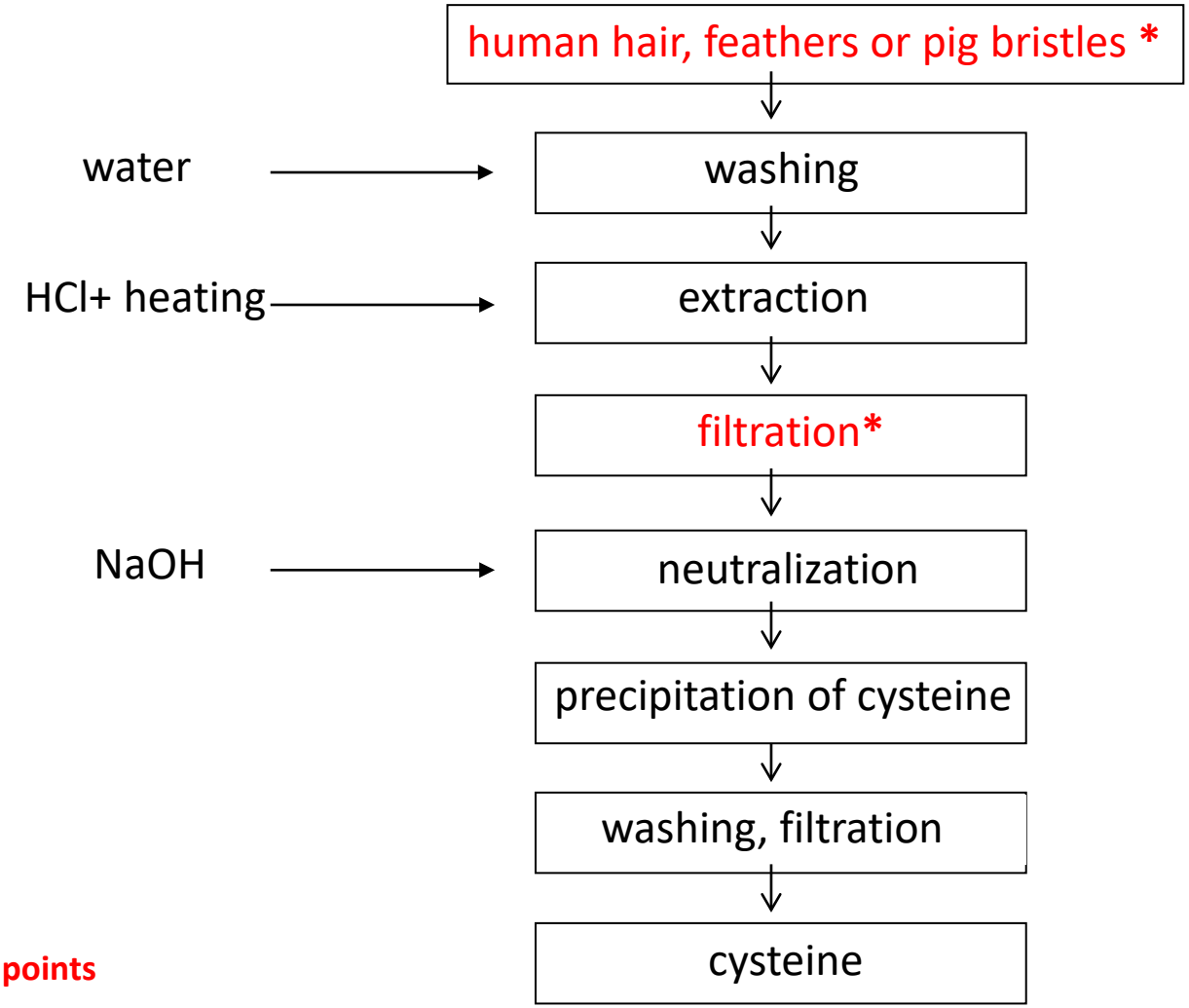
Pig bristle

Human hair



Feathers

Keratin Hydrolysate



* Critical points

Keratin Hydrolysate

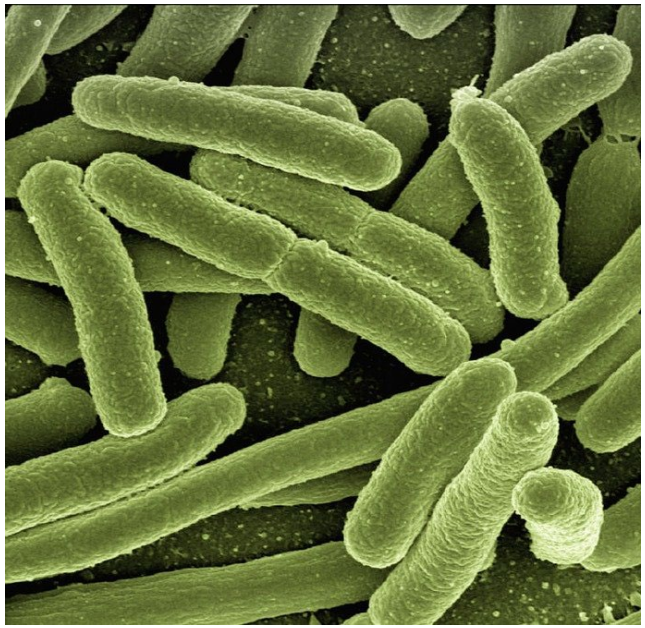
Advantage:

- Cheap and natural source, one ton of hair yields 100 kg of cysteine.

Disadvantage:

- The hair method requires 27 Kg of hydrochloric acid to produce just one kilogram of cysteine (5).
- Unpleasant odor.
- For pharmaceutical companies, it is crucial to eliminate the possibility of dangerous contaminations, such as the pathogens responsible for BSE, SARS or bird flu.
- Non-Halal raw materials can be used in the production.

2. Fermentation



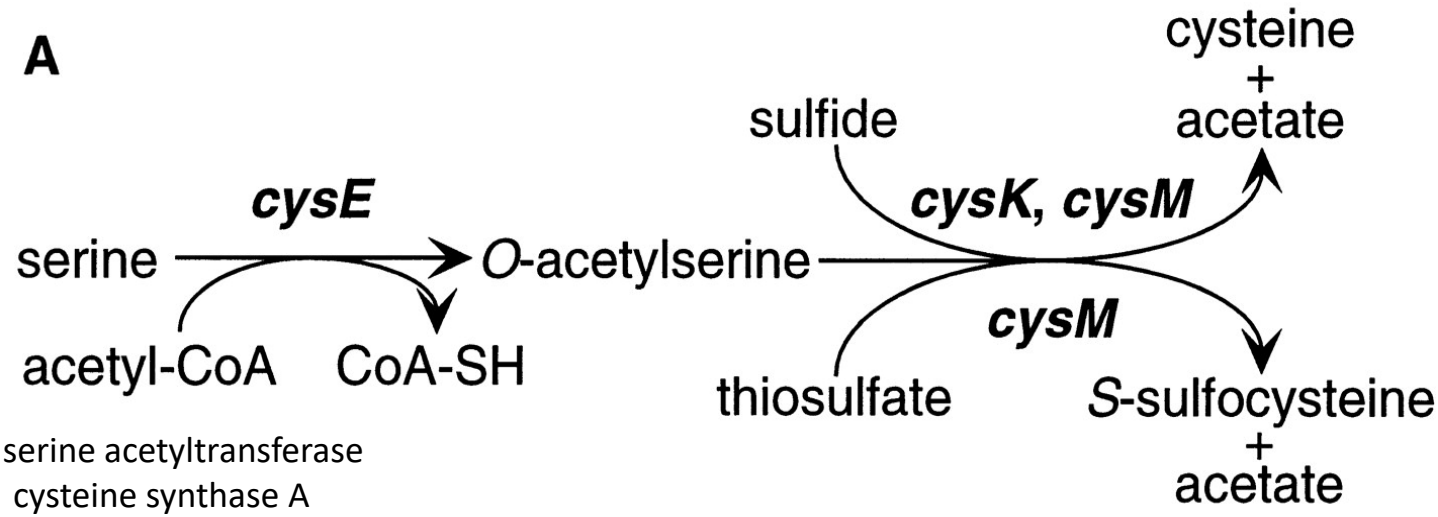
Advance technology is used to ferment the improved strain-producing cysteine that will produce high yields. New approach is used by optimizing the existing metabolism of the bacterium's native metabolism (*E.coli*).

This enables large quantity of cysteine to be released by the bacterium into the fermentation media, as 90 percent of pure cysteine ends up in the final product which fulfills the quality standards for foods and pharmaceutical industries (6).

Fermentation

E. coli cells synthesize L-cysteine using a simple two-step pathway.

- L-serine, the starting molecule for the pathway, is either acquired from the cell surroundings or made from other cell precursors.
- Acetyl-CoA is used to activate the L-serine to prepare it for incorporation of an atom of sulfur.



cysE serine acetyltransferase
cysK cysteine synthase A
cysM cysteine synthase B

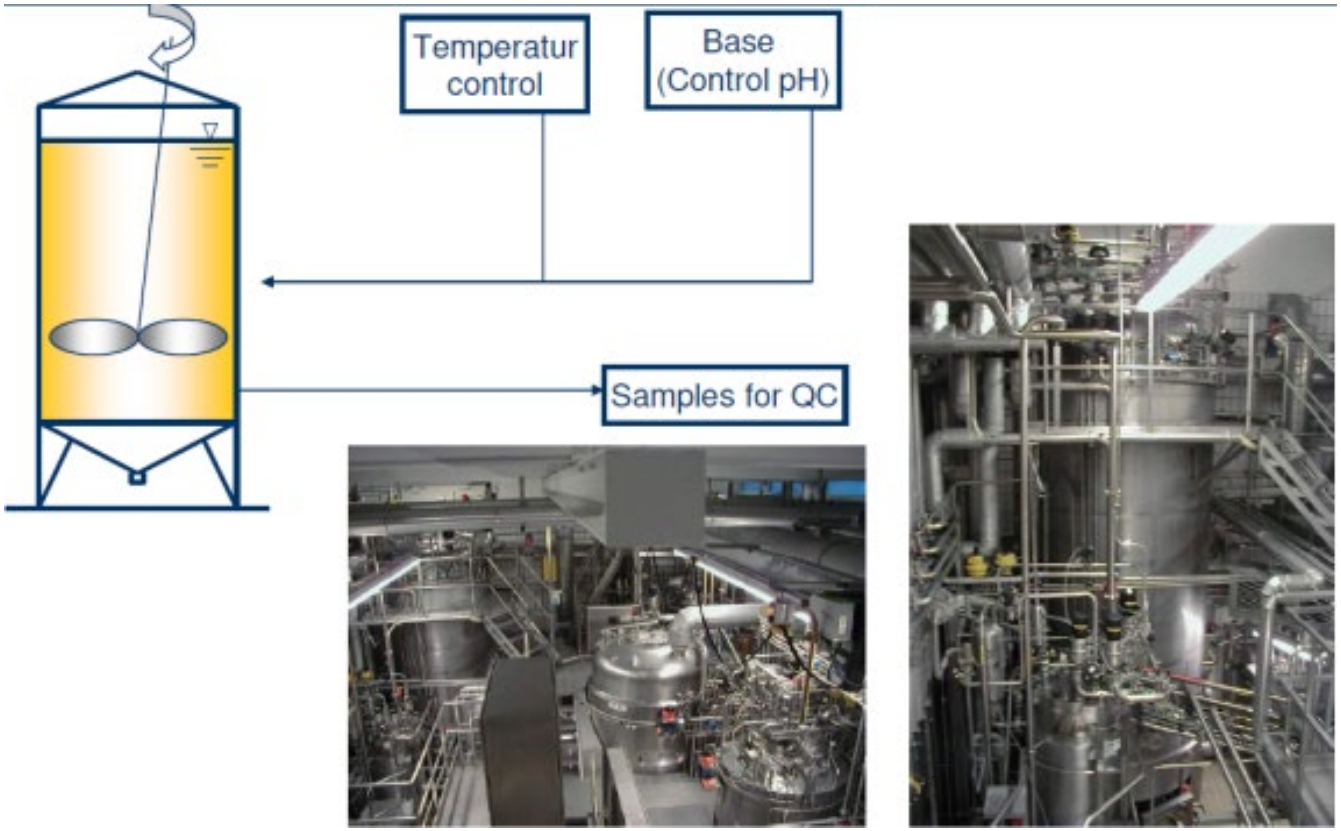
Cysteine biosynthesis in *E. coli* (7)

Fermentation

Preparation of growth media

Fermentation

Cultivation of bacteria (fermentation)



Fermentation

Downstream processing (filtration, centrifugation, precipitation and crystallization).

Fermentation

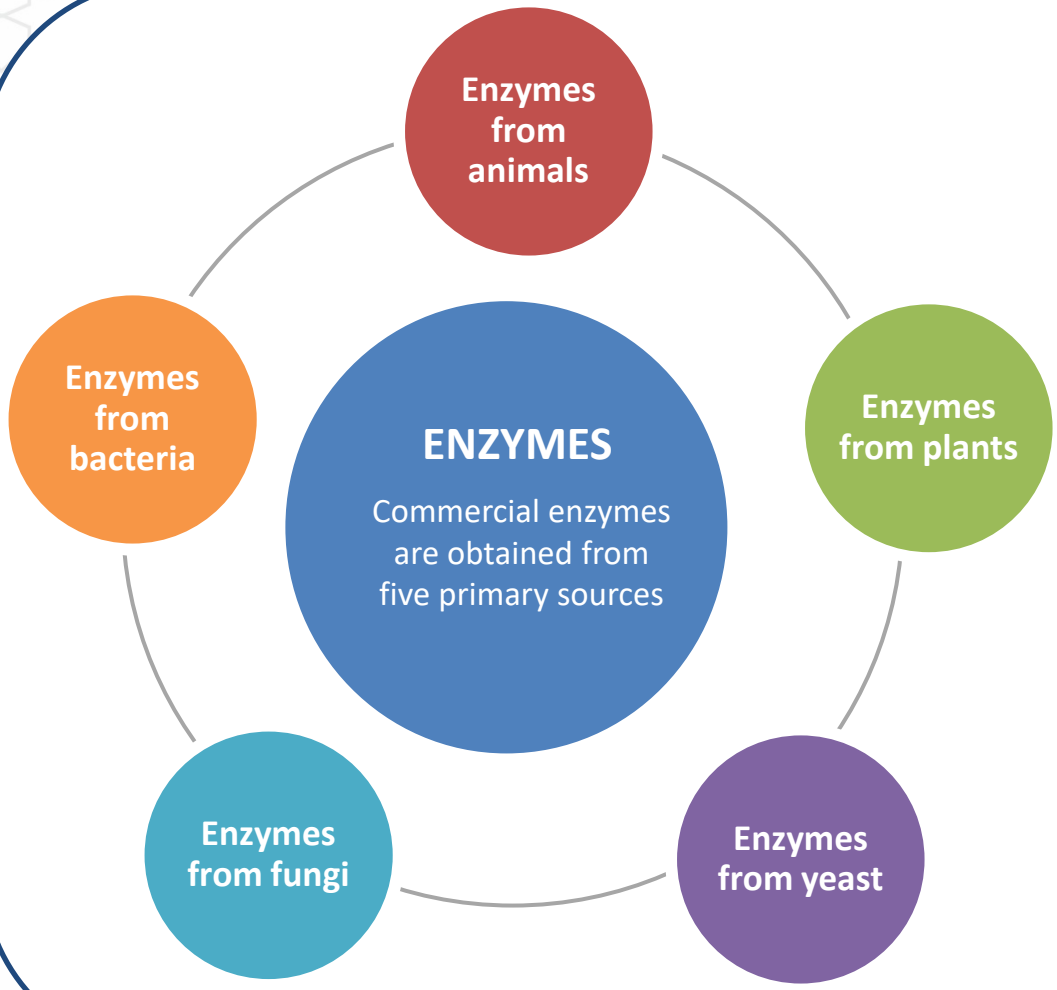
Critical Points

- Growth Media
- Enzyme as processing aids in media preparation
- Processing aids
- GM Microorganisms

Growth Media

No	Material	Source
1	Beef extract	Extracted from beef
2	Peptone	Hydrolysis of protein from meat, casein, or gelatin using acid of enzymes
	a. Acid-hydrolyzed casein	Hydrolysed protein from casein using acid for hydrolysis example: casamino acids (Difco); Hy-case (Sheffield)
	b. Enzymatic digest of casein	Hydrolysed casein using enzymes for hydrolysis Example: Casitone (Difco); Tryptone; trypticase peptone
	c. Enzymatic digest of meat protein	Hydrolysed protein from meat using enzymes for hydrolysis. Bactopeptone proteos pepton
	d. Enzymatic digest of plant (soy) protein	Hydrolysed protein from plant (soyben) using enzymes for hydrolysis. Example: Soyapeptone
3	Mixed hydrolisate	Biosate (yeast extract and casein digest); Trypton (mixed-enzymatic source
4	Yeast extract	Extracted from yeast
5	Agar	Carbohydrate from sea weed

Enzymes as Processing Aids in Media Preparation



Enzymes from bacteria
α-amylase, β-amylase, glucose isomerase, protease, pullulanase

Enzymes from animals
Catalase, lipase, rennet.

Enzymes from fungi
Amylase, catalase, dextranase, lactase, lipase, pectinase, protease.

Enzymes from yeast
Invertase, lactase, lipase, raffinase

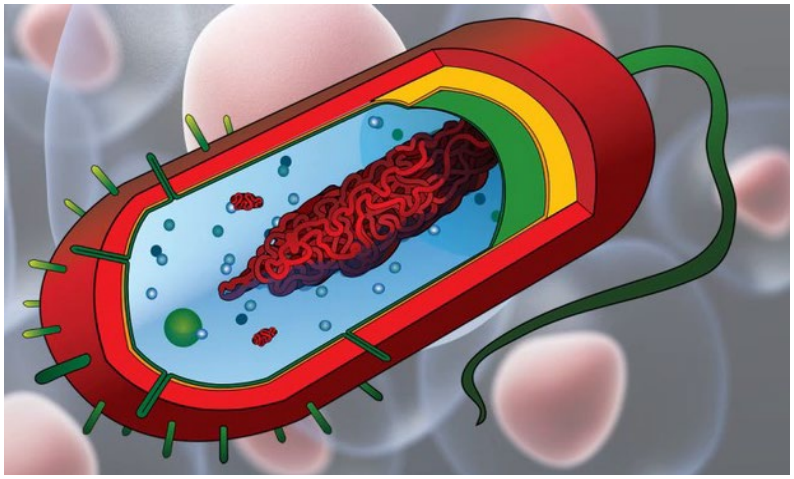
Processing Aids

Antifoam
Normally people use silicon, sulphonates, esters, and animal oil (lard) or fatty acids from animal oil.

Activated carbon
Activated carbon is usually obtained from wood, coconut shell, or animal bones.

GM Microorganism

L-serine is the precursor for L-cysteine; however it is very difficult to produce L-serine by direct fermentation. Thus, it requires metabolic engineering approach to produce L-cysteine by combining the overexpression of genes encoding for serinebiosynthetic enzymes and deletion of gene encoding for serine-degrading enzyme (8-9).



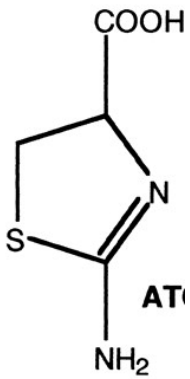
3. Enzymatic Bioconversion

Some bacteria, particularly in the genus of *Pseudomonas*, can hydrolyze DL-2-amino-Δ2- thiazoline-4-carboxylic acid (DL-ATC) to L-cysteine (10,11).

DL-ATC is a desirable substrate for product L-cysteine due to simplicity of the process for chemical synthesis of DL-ATC.

This method has the potential to produce high yield of cysteine with low energy requirement during production.

Enzymatic Bioconversion

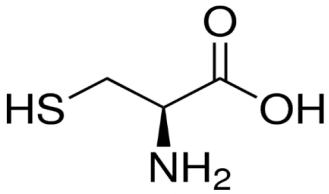


DL-2-amino-A2-thiazoline-4-carboxylic acid (DL-ATC)

↓

Bioconversion by *Pseudomonas sp*.

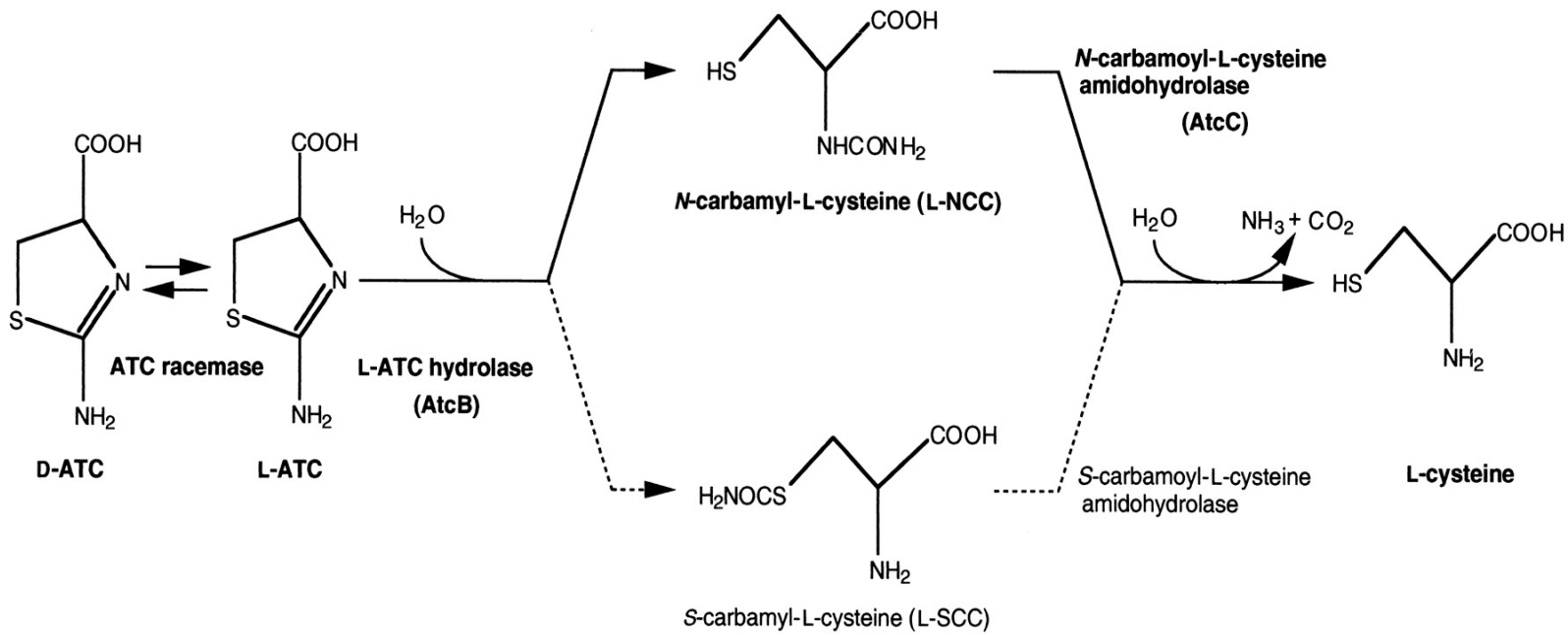
↓



Cysteine

Enzymatic Bioconversion

The conversion of DL-ATC to cysteine can be described in the following successive steps: (i) enzymatic reclamation of D-ATC to L ATC; (ii) a ring-opening reaction of L-ATC to Ncarbamyl-L-cysteine (L-NCC) as intermediate; and (iii) hydrolysis of L-NCC to L-cysteine (2, 14).



Enzymatic Bioconversion

Selection medium

DL-ATC, glycerol, KH_2PO_4, $MgSO_4$ $7H_2O$, $FeSO_4$ $7H_2O$, $MnSO_4.4H_2O$, agar and water (11).

Fermentation medium

DL-ATC, yeast extract, peptone, meat extract, NaCl, glycerol and distilled water (12).

Fermentation medium

DL-ATC, glycerol, beef extrat, yeast extract , peptone, NaCl, $MnSO_4 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$. (12).

Conclusion

The pharmaceutical, cosmetic and food industries worldwide require up to 4,750 tons of cysteine per year.

Until recently, it was one of the few amino acids that had to be obtained from animal or human raw materials, for example human hair, feathers, pig bristles and hooves. With improvements in biotech, it has been possible to engineer a bacteria that produces excess cysteine.

Owing to the fact that many Haram materials can be used in the manufacturing process, cysteine is considered as a Halal critical material.

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