Silage Microbiology

Dept of Animal
Nutrition, CoVSc & AH,
Jabalpur

Silage

- A preserved feed prepared with
  - high moisture forages
  - fermented with controlled microbial activity to achieve lower pH
  - under anaerobic conditions
  - restricting the growth of undesirable microbes

Silage Characteristics

	Good quality	Medium quality	Poor quality
pH	<4.2	4.2-4.8	>4.8
Volatile-N (%, N)	<10	10-15	>15
Butyrate, %	<0.2	0.3 - 0.5	>0.5
Smell	Good	Satisfactory	Bad
Fungal growth	(-)	(±)	(+)

Haylage

- Silage prepared from high DM forage
- Microbial activity is lower than that during ensiling due to lower water activity.
- DM in haylage varies between 40-60%
- Compression in silo is not complete due to high DM
- Large amount of air is entrapped in the silo while filling.
- Entrapped air facilitates growth of aerobic microbes, which may spoil haylage.

Advantages of Silage Making

- Availability of forage is more than requirement in peak season and lower in lean season. This variation in availability can be rectified by preservation.
- In rainy season, hay making is not possible, ensiling is preferred.
- Thick stems of mature forage are softened and may increase palatability.
- The germination power of weeds is destroyed due to ensiling.
- Green forages can be stored for very long periods without further losses of nutrients.
- Acids produced during ensiling are used as energy source in the rumen.
- Animal organic wastes can be used as one of the ingredients.

Disadvantages of Ensiling

- Permanent structure (silo) is essentially required.
- Effluent formation in high moisture silages results in nutrient losses.
- Poorly prepared silage results in :
  - High loss of nutrients
  - Poor acceptability by the animals

Disadvantages of Ensiling

- Permanent structure (silo) is essentially required.
- Effluent formation in high moisture silages results in nutrient losses.
- Poorly prepared silage results in :
  - High loss of nutrients
  - Poor acceptability by the animals

Characteristics of forage crops for ensiling

- **Water soluble carbohydrates**
  - Essentially required for lactic acid production
  - Soluble sugars sufficient in non-leguminous forages, but poor in leguminous forages
  - Non availability of sugars delays fermentation process & result in increased fermentation losses.
  - Such crops should be mixed with other forages and then ensiled.
- **Dry matter**
  - High moisture crops result in effluent losses.
  - Low moisture crops have low microbial activity.
  - DM should vary between 30-50% for optimum fermentation.
  - DM can be adjusted by mixing with dry roughages or wilting of forages

Ensiling of Leguminous Forages

- Ensiling process depends upon :
  - Moisture content
  - Lactic acid bacterial count
  - Water soluble carbohydrates
  - Buffering capacity
- Leguminous crops have :
  - High buffering capacity
  - Low soluble sugars
  - High moisture

Ensiling of Leguminous Forages

- **Process :**
  - Slow acid production
  - Extensive degradation of forage proteins
  - High ammonia production
- **Can be ensiled :**
  - By mixing with high sugar forage crop
  - By adding soluble carbohydrate like molasses
  - By inhibiting proteolysis during ensiling

Ensiling Process

- **Silo**
  - Structure or container used for ensiling
  - May be made of bricks, concrete, stainless steel, kucha pit lined with plastric sheet.
- **Site Selection for silo**
  - Easily approachable from shed and crop field
  - Chaff cutter should by near by.
  - Area should not be low lying, so that there is no water logging in the area.
  - In areas of high water table, silo should be erected on soil, so that there is no water seepage into the silo.

Ensiling Process

- **Phase I**
  - **Respiration continues till the silo is closed**
  - **Air entrapped with forage supports respiration and growth of aerobic microbes like *Escherichia, Bacillus, Klebsiella, Aerobacter* etc.**
  - **Acid production starts and anaerobic conditions are achieved.**
- **Phase II**
  - ***Streptococcus, Lactobacillus, Leuconostoc* and *Pediococcus* become active**
  - **pH drops below 4.5**
- **Phase III**
  - ***Lactobacillus* and some acid tolerant bacteria survive**
  - **Other bacteria are either killed or their activity is temporarily stopped.**

Ensiling Process

- Phase IV
  - At high moisture >80%, protein degrading clostridia are active and are responsible for reversion of ensiling process, generating basic ions in the silage.
  - pH starts rising and other microbes become active in the ensiling process.
  - Silage produced under these conditions has :
    - High pH
    - High volatile nitrogen
    - Low organic acids
    - High butyric acid

Fermentation of sugars

1. Cellulose → Glucose

2. Hemicellulose → Xylose + Arabinose

3. Starch → Glucose

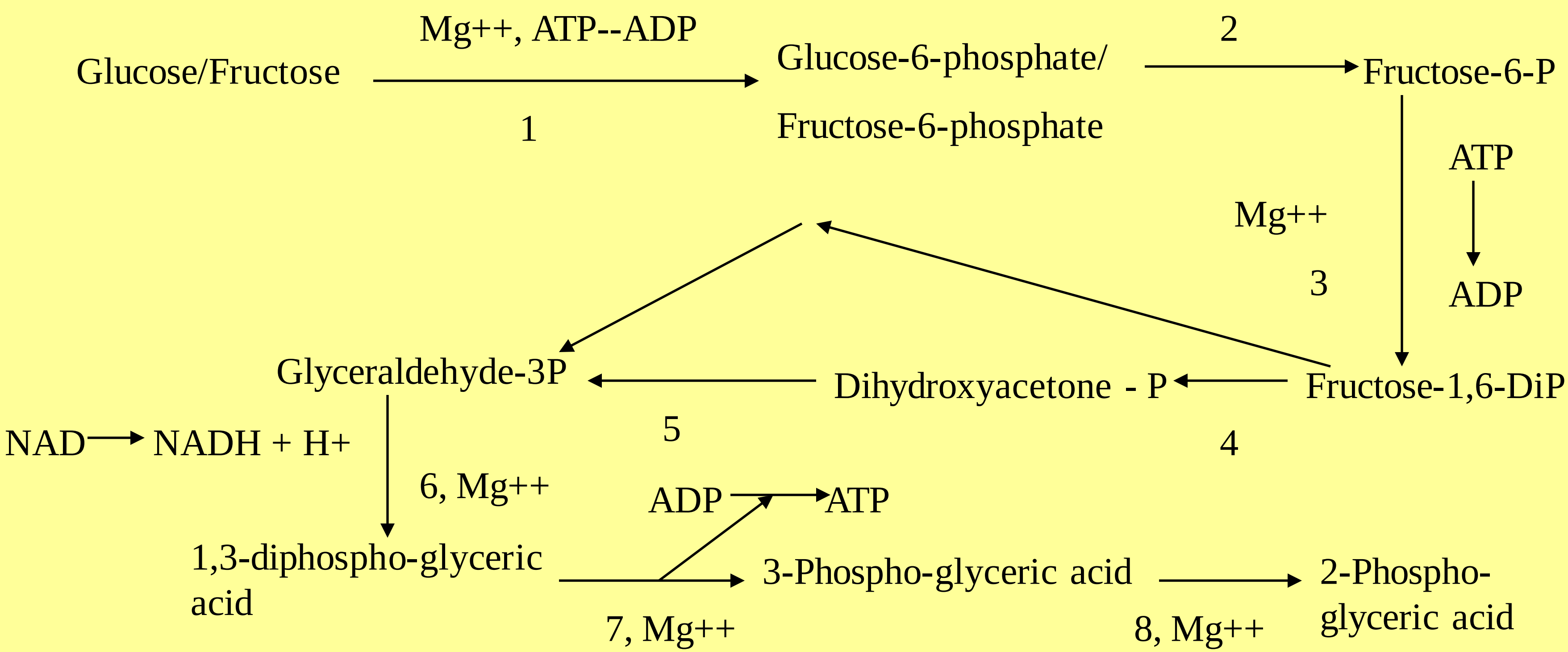
4. Sucrose → Glucose + Fructose

1. Cellulase enzyme complex, 2 - Hemicellulases, 3- Amylase, 4- Invertase

Lactate producing bacteria

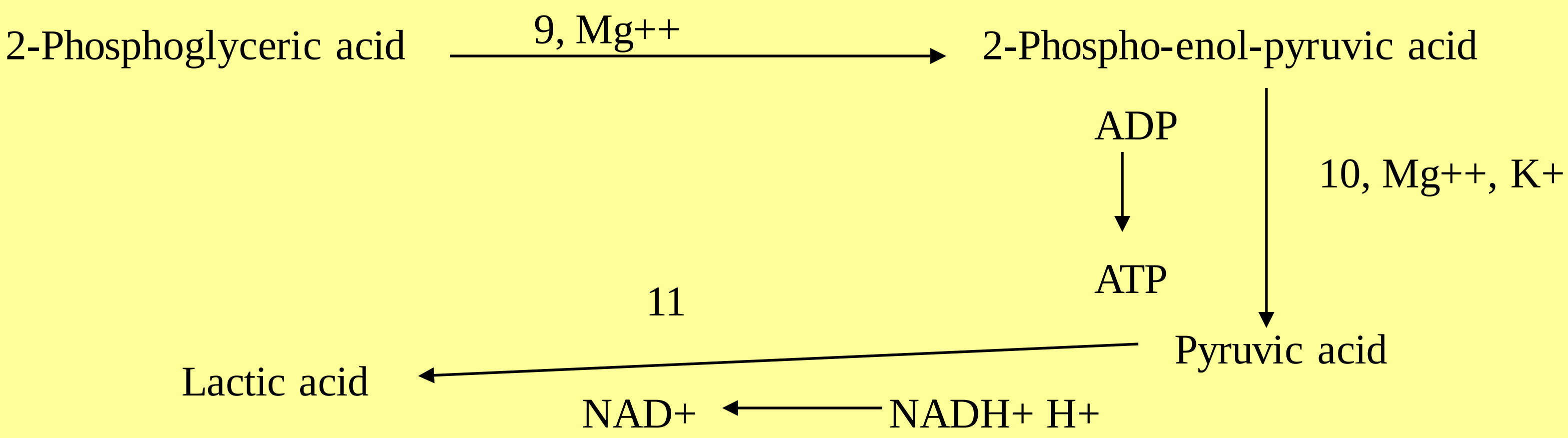
- **Homofermentative**
  - Covert each mole of glucose/fructose quantitatively to two moles of lactic acid
  - Minimum loss of energy during ensiling
- **Heterofermentative**
  - One mole of glucose converted to lactate, ethanol and CO_2
  - Fructose is converted to lactate, acetate and mannitol (further bioconversion of mannitol is very low under ensiling conditions)

Bioconversion of hexoses by homofermentative lactic acid bacteria



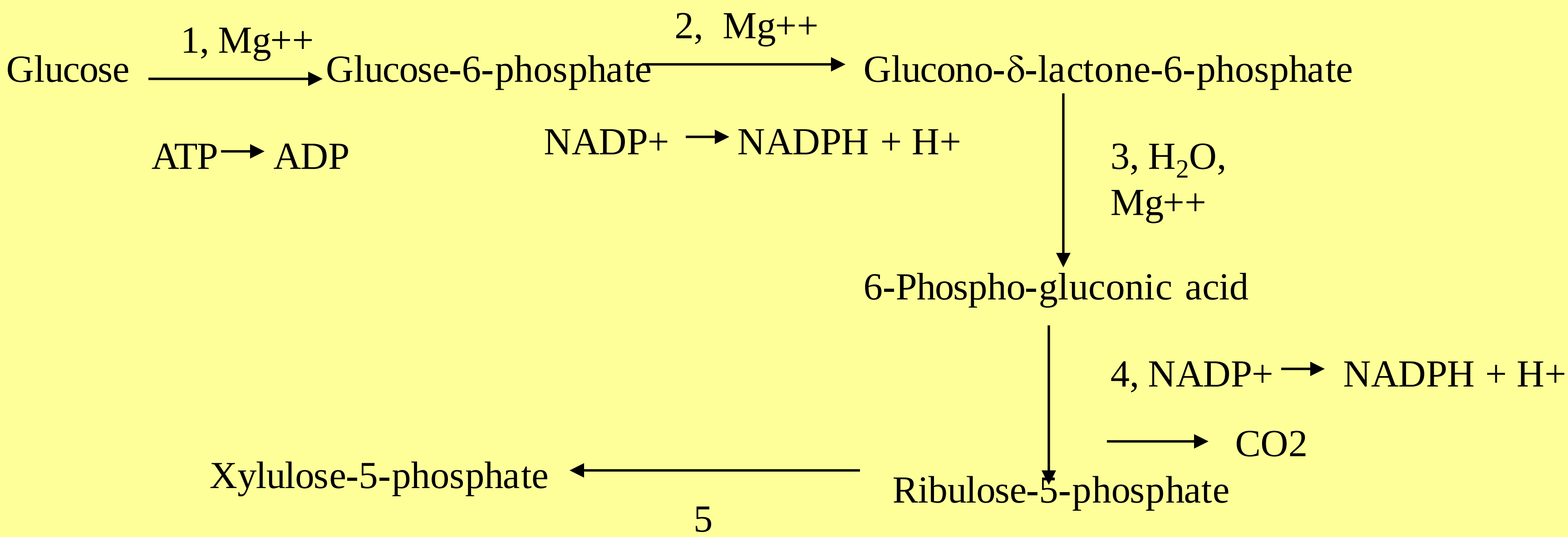
1-Hexokinase, 2-Phospho-hexo-isomerase, 3-Phospho-fructokinase, 4- Aldolase, 5 - Triose-phosphate isomerase, 6-Glyceraldehyde-3-phosphate dehydrogenase, 7-Phosphoglycerokinase, 8-Phosphoglyceromutase

Bioconversion of hexoses by homofermentative lactic acid bacteria-2



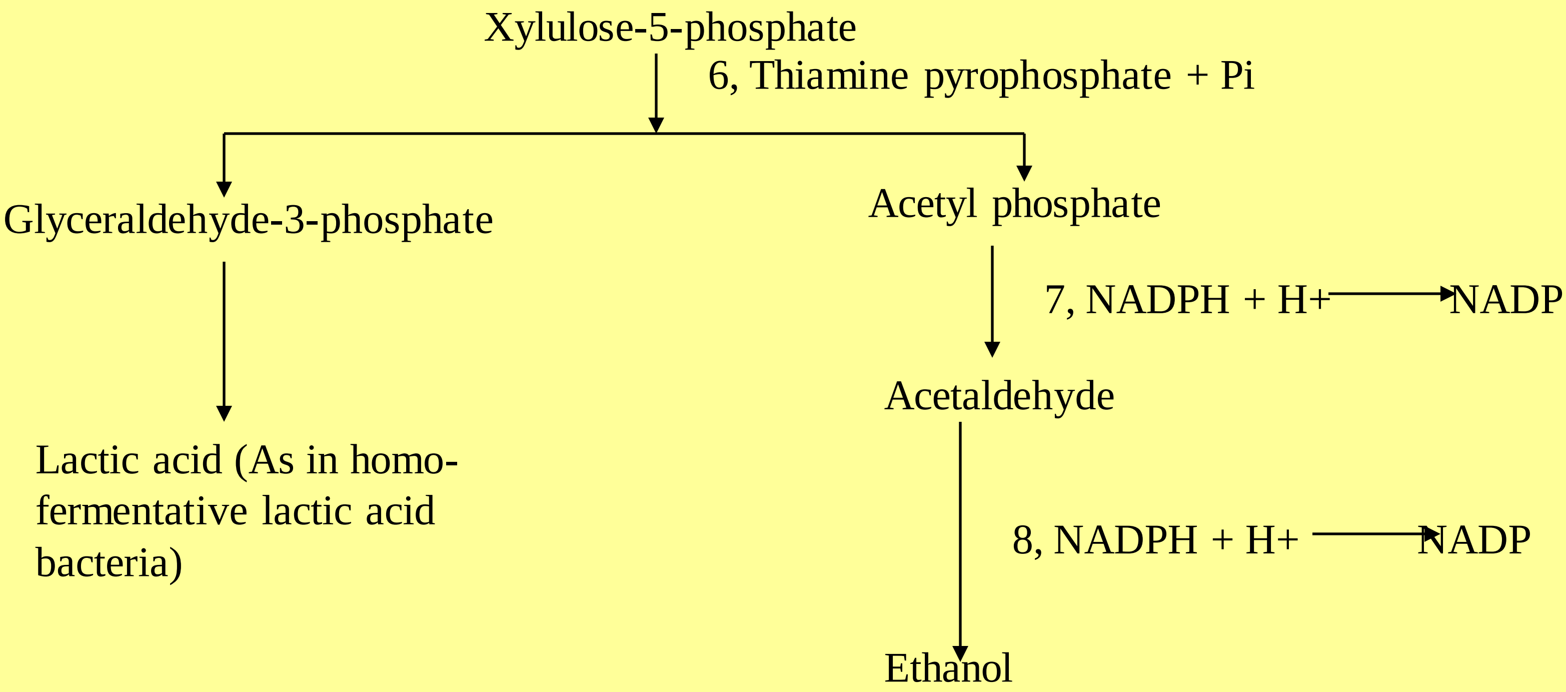
9 - Enolase, 10 - Pyruvic kinase, 11 - Lactic dehyrogenase

Bioconversion of glucose by heterofermentative lactic acid bacteria



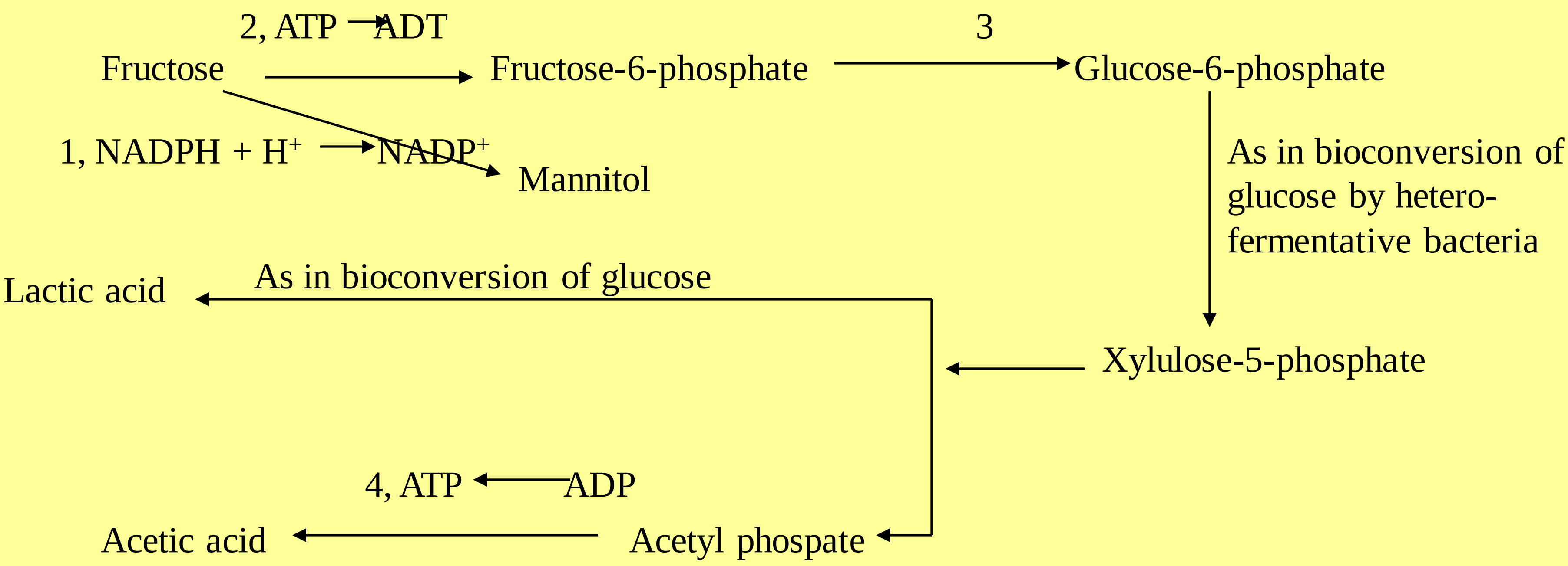
1 - Hexokinase, 2 - Glucose-6-phosphate dehydrogenase, 3 - 6-Phosphoglucono-lactonase, 4 - 6-Phosphogluconic dehydrogenase, 5 - Phospho-keto-pento-epimerase

Bioconversion of glucose by heterofermentative lactic acid bacteria-2



6 - Phosphoketolase, 7 - Acetaldehyde dehydrogenase, 8 - Alcohol dehydrogenase

Bioconversion of fructose by hetero-fermentative lactic acid bacteria



1 - Mannitol dehydrogenase, 2 - Fructose kinase, 3 - Phospho-hexo-isomerase, 4 - Acetokinase

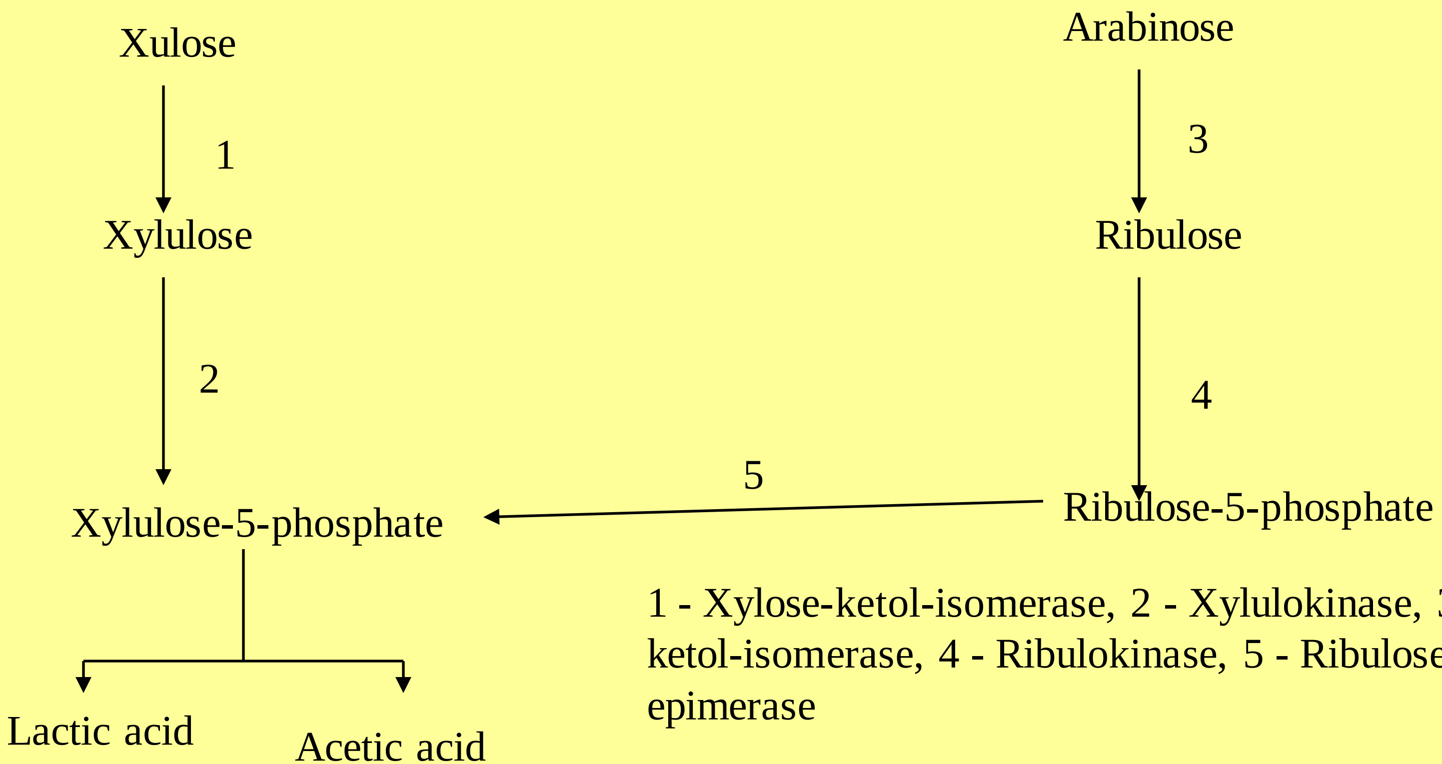
Fermentation of mannitol by *Lactobacillus plantarum*

$$\text{Mannitol} \xrightarrow[]{1,\ ATP \rightarrow ADP} \text{Mannitol-1-phosphate}$$

$$\text{Mannitol-1-phosphate} \xrightarrow[]{2,\ NAD^{+} \rightarrow NAD^{+} + H^{+}} \text{Fructose-6-phosphate}$$

1, Mannitol kinase, 2 - D-mannitol-1-phosphate dehydrogenase

Bioconversion of pentoses by hetero- and homo- fermentative lactic acid bacteria



1 - Xylose-ketol-isomerase, 2 - Xylulokinase, 3 - Arabinose-ketol-isomerase, 4 - Ribulokinase, 5 - Ribulose-5-phosphate epimerase

Fermentation of different sugars with lactic acid bacteria

Bacteria	Substrate	End products
Homo-fermentative	Glucose/fructose	Lactic acid
Homo-fermentative	Pentose	Lactic + Acetic
Hetero-ferment.	Glucose	Lactic + Ethanol + CO_2
Hetero-ferment.	Fructose	Lactic + Acetic + CO_2 + Mannitol
Hetero-ferment.	Pentose	Lactic + Acetic

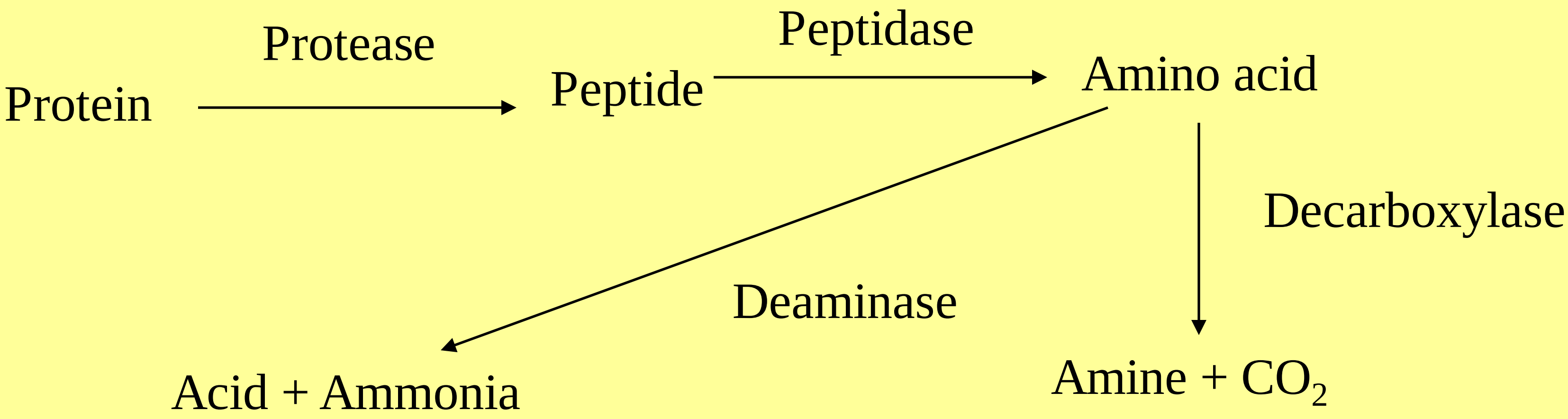
Fate of nitrogen during ensiling

- Green forages have :
  - True protein 80-90%
  - Non protein nitrogen (10-20%) including AA, amines, amides, nucleotides, chlorophill, nitrates, ammonia etc.
  - Green forages and silage made from these have similar AA composition.
  - No selective AA degradation, but protein turn over is very high (sometimes more than 50%)

Fate of nitrogen during ensiling

- Proteases of plant origin are active in the cut crop. Their activities can be stopped/lowered by :
  - Reducing the pH
  - Increasing dry matter by wilting or high DM fodder.
  - By creating anaerobiosis at an early stage.

Conversion of protein by plant enzymes



Deaminases and decarboxylases of lactic acid bacteria

Deaminases

Serine $\longrightarrow$ Pyruvic acid + NH_3

Arginine $\longrightarrow$ Ornithine + NH_3

Glutamine $\longrightarrow$ Glutamic acid + NH_3

Decarboxylases

Tyrosine $\longrightarrow$ Tyramine + CO_2

Lysine $\longrightarrow$ Cadaverine + CO_2

Ornithine $\longrightarrow$ Putrescine + CO_2

AA degradation by clostridia

Stickland's Reaction (Coupled oxidation/reduction of AA)

$$\text{Glycine} + 2H \longrightarrow \text{Acetic acid} + NH_3$$

$$\text{Alanine} + 2H_2O \xrightarrow{\nearrow 4H} \text{Acetic acid} + NH_3 + CO_2$$

AA degradation by clostridia

Deaminases

Lysine ⟶ Acetic acid + Butyric acid + $2NH_3$

Phenyl-alanine ⟶ Phenyl-propionic acid + NH_3

Threonine ⟶ α-ketoglutaric acid + NH_3

Decarboxylases

Tryptophan ⟶ Tryptamine + CO_2

Histidine ⟶ Histamine + CO_2

Effect of ensiling on nitrate

- **High use of nitrogen fertilizer results in high content of nitrate in forages (Sometimes >10% of TN).**
- ***L. plantarum, Enterococcus sp., Clostridium tyrobutyricum, C. sporogenes*** **are able to reduce nitrate to ammonia which can further be incorporated in AA.**
- ***L. brevis, S. faecalis, Pediococcus, C. butyricum*** **and plant enzymes are not able to reduce nitrate to ammonia.**

Chemical Additives

- **Mineral Acids**
  - Mineral acids (first used by A.I. Virtanen in 1933) thus named as "AIV process" of forage preservation.
  - Acids are added to bring the pH down to 3.5-4.0 to inhibit most of the microbial activity.
  - Forages with low soluble carbohydrates are preserved better with acids
  - Difficult to use due to corrosive nature of acids

Chemical Additives

- **Organic acids**
  - **Formic acid (2.5-3.0%) is recommended.**
  - **Complete inhibition of bacterial growth does not take place.**
  - **Yeast is tolerant to formic acid, thus yeast count is higher.**
  - **Yeast leads to formation of alcohol and results in dry matter loss during ensiling.**

Chemical Additives

- Formaldehyde
  - **Bacterial growth inhibited, but clostridia are more resistant.**
  - **Very high concentration of formaldehyde needed to inhibit clostridia completely.**
  - **A combination of formaldehyde and formic acid is more effective for preservation.**
  - **This treatment protects protein and reduces its hydrolysis and deamination during ensiling.**

Animal Wastes as Supplement

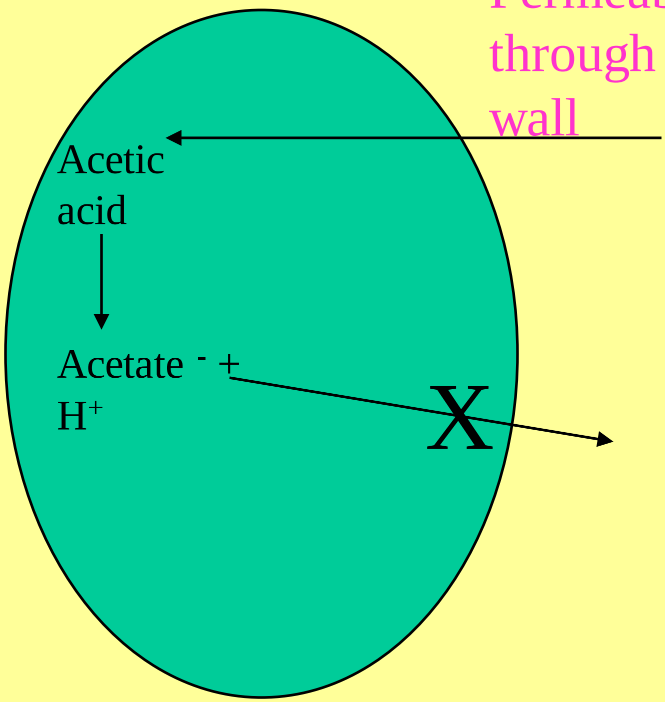
- Help in increasing CP of silage (made from non-leguminous forages)
- Type of wastes
  - Poultry excreta (25-30% CP)
  - Pig faeces (15-18%)
  - Excreta of ruminants fed high concentrate diet (10-16%)

Ensiling with wastes

- High buffering capacity of animal wastes
- High microbial load
- Presence of pathogenic microbes and parasites (These must be eliminated during ensiling)

Mechanism of killing of pathogenic bacteria at low pH in the presence of organic acids

Permeable through cell wall



pH > pKa

Acetic acid $\rightleftharpoons$ Acetate^{-} + H^{+}

pH < pKa

Cannot come out of cell and thus accumulates in the cell

pKa value of lactic, acetic, propionic and butyric acids vary between 3.7-4.8

Organic acids at pH 7.0 are not toxic, but at pH 4.0 are toxic

Microflora of fresh forages

- **The numbers not very high**
- **Mostly aerobic and not required in ensiling**
- **Micro-aerophillic like *Escherichia, Klebsiella, Streptococcus* are acid producers, help in creating anaerobic conditions**
- ***Lactobacillus, Pediococcus, Leuconostoc* (numbers < 100 cells/g) are lactic acid producers.**
- ***L. plantarum, L. cellobiosus, Streptococcus lactis***
- **Number of lactics increases abruptly by growing on cell sap of forages.**

Characteristics of a microbial inoculum

- Must be homofermentative lactic acid producer.
- Should be tolerant to low pH and high concentration of organic acids.
- Should have high saprophytic competitive ability
- Should be active in a large pH range (4-7)
- Protease negative
- Non acid utilizer
- Able to grow at low water activity.
- Preferably cellulase positive
- Antagonistic activity against undesirable microbes

Effect of inoculum on fermentation

- A combination of *L. plantarum* and *S. faecalis*
- Rapid fall in pH and Low pH
- Increased lactate:total acids ratio
- Lower ammonia concentration
- Silage stable to aerobic deterioration
- High water soluble carbohydrates
- Elimination of coliform bacteria, listeria, clostridia if present in the premix.
- Lower alcohol in silage
- Better acceptability by the animals

Aerobic stability of silage

- **On exposure to air :**
  - pH starts rising
  - The level of organic acids decreases
  - ME and nitrogen contents decrease
  - Ammonia nitrogen increases
  - Aerobic fungal growth speeds up deterioration

Aerobic deterioration of silage

- **On exposure to air, aerobic bacteria (*Bacillus* and *Aectobacter*), yeast, molds (lactate utilizing) present in dormant stage begin to flourish and respire away energy sources.**
- **Aerobic losses may accounts for as high as 70% of total losses**

Losses during ensiling

- **Pre-ensiling losses**
  - On cutting forage crop, aerobic fermentation continues till the DM is high or pH is low enough to inhibit
  - In first 24h, net losses are negligible as photosynthesis compensates for the fermentation losses.
  - On further wilting the losses are proportional to time.
  - Silo must be filled within 24h of the harvest of forage to avoid pre-ensiling losses.
  - These losses may account for 5-10% of total losses.

Losses during ensiling

- **Ensiling losses**
  - **Aerobic losses**
    - **Respiration continues till oxygen is available**
    - **Heat is generated which raises the temp. of silo (sometimes 30-40C higher than ambient)**
    - **Depends upon the compactness of silo**
    - **Losses can be minimized if properly packed**

Losses during ensiling

- **Ensiling losses**
  - **Anaerobic losses**
    - **Due to formation of volatile compounds like CO_2, alcohol, ammonia etc.**
    - **Due to higher number of hetero-fermentative lactic acid producing bacteria**
    - **Losses may account for 4-6% of total losses**

Losses during ensiling

- **Effluent losses**
  - **Due to high moisture in forage**
  - **No loss of nutrients at DM of 32.7%**
  - **D=17.614 - 0.534X**
  - **where**
    - **D=% DM in the effluent**
    - **X=% DM in forage crop**

Listeria Infection in Silage fed Animals

- **Listeriosis**
  - **Inferior quality silage is the main source of listeria infection in ruminants.**
  - **In good quality silage listeria is eliminated (at pH lower than 5.6)**
  - **The chances of infection are high at high pH (7-8) silage i.e. big bale silage in which large air pockets are left.**