All glories come from daring to begin ..........

Ready Reference Pattern
(Scripts and Trump Cards)
An Innovative Method Designed,
Blended and Executed By
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.....When the going gets tough, the tough gets going

Highlights

- More organized, well planned research way of studying.
- Collection of perfect, precise, point-wise and paragraphic information from various text books, research journals, reference books, encyclopedia, websites etc.
- Screening of target information with maximum illustrations.

Benefits

- Develops curiosity of learning.
- Enables the students to believe and remember principles, scientific laws, facts etc. established by others.
- Inculcate values of learning process.
- Builds up proper base for professional & higher educational courses.
- Allows students to interact with others during learning process.
- Increases confidence required to face the academic challenges.
- Facilitates ones understanding process and makes it easier for learners to remember.
- Encourages learners to quote references systematically inculcating values of acknowledging the work of others.

.....All’s well that ends well
GOVT. COLLEGE OF ARTS AND SCIENCE
AURANGABAD.

READY REFERENCE PATTERN

SCRIPTS
MICROBIOLOGY

NAME : Ms. Harinee Himadri Dey

CLASS : B. Sc. P. yr. R. NO. 69

SCRIPTS ENCLODED:
- Fermentation & fermentation media
- 
- 
- 

2003-2004
Fermenters & Fermentation media

Script no. 1

Holmee Dey
BSc II yr.

2003-2004
<table>
<thead>
<tr>
<th>Sl No</th>
<th>Chapters</th>
<th>Page No</th>
</tr>
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<tbody>
<tr>
<td>1.1</td>
<td>Introduction</td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>Fermenters</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>Fermentation media</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>Conclusion</td>
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<td>1.5</td>
<td>Glossary</td>
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</tr>
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<td>1.7</td>
<td>Notes</td>
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</tbody>
</table>
Introduction:

Fermentation technology is a widely applied stream of biology. It shares the same pedestal with other upcoming streams.

The technology finds its relevance in all types of bio-industries such as beverage industries, refineries, etc.

Proper working of these, hence, depends on a planned and scheduled work layout.

Fermenters, being an important part of the system, is designed according to specific requirements of every reaction. The considered issue ranges from economic condition, environmental condition to the many facets of biosynthesis.

Even the nutrient media is created in order to suffice all the required criterion for maximum and healthy yield and minimal cost rates.

The following chapter will enable us to commemorate every single detail regarding:

“Fermenters and Fermentation media.”
Overview of a biotechnological process.
Selection of appropriate strain of a particular species of micro-organism: Determines the product yielding phase of growth, temperature, pH range, degree of required aero-biosis, and the effect of contamination.

Selection of appropriate fermentor-configuration

Determination of fermentor dimension: Volume and diameter, operating variables, concentration, temperature, and pH; process line for batch fermentation vs. continuous fermentation.

Extent of heat transfer surface and mixing devices

Power and aeration required

Mechanical design: Selection of constructing media and maintenance of aseptic condition.

Monitoring and control

Safety factors

Fig. 12: Flowchart representing fermentor designing.
Fermenters:

- Fermenters are specially designed vessels loaded with a particular type of nutritive media used for growing microbes in fermentation industries. They are complicated in design, since they must provide for the control and observation of many facets of microbial growth and biosynthesis. The design of fermenters depends on the purpose for which it is utilized. Some specifically designed fermenters are the submerged used in laboratory, semi pilot plant & pilot plant scale.

12-1

Fermenter designing:

- The fermenters must be properly and specifically designed for each purpose. Fig. 1.2.

12-1.1

Factors influencing fermenter design:

- According to Lortz and Welte (1971), the two basics of fermenter designing are:
  - Selection of best reactor for a particular type of reaction.
  - Determination of best operating condition.

12-1.2

Objectives of design:

- Describe the effect of operating condition on performance a bioreactor.
- Comparison of alternative design with economic criteria.
<table>
<thead>
<tr>
<th>SL No.</th>
<th>CHARACTER</th>
<th>BIOCHEMICAL CONVENTY</th>
<th>CHEMICAL CONVENTY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>REACTANT MIXTURE</td>
<td>Complex</td>
<td>Relatively simple</td>
</tr>
<tr>
<td>1</td>
<td>MICROBIAL MASS</td>
<td>Increases with the accomplishment of reaction</td>
<td>No such phenomenon observed</td>
</tr>
<tr>
<td>2</td>
<td>CATALYST (ENZYMES)</td>
<td>Autosynthesised</td>
<td>External chemical catalyst required</td>
</tr>
<tr>
<td>3</td>
<td>TEMPERATURE</td>
<td>Relatively mild</td>
<td>Variable</td>
</tr>
<tr>
<td>4</td>
<td>pH STABILITY</td>
<td>Difficult in maintaiining</td>
<td>Varies with the nature of reaction</td>
</tr>
<tr>
<td>5</td>
<td>PHASE</td>
<td>Restricted to aqueous phase</td>
<td>No such restrictions</td>
</tr>
<tr>
<td>6</td>
<td>SUBSTRATE/PRODUCT CONCENTRATION</td>
<td>Relatively low</td>
<td>High</td>
</tr>
</tbody>
</table>

*Differences between biochemical & chemical process*
1.8. Transport process in aerobic fermentation

1. Oxygen absorbed by microorganisms
2. Oxygen absorbed through a filter
3. Oxygen is transported through the microorganism
4. Oxygen is transported to the surface
5. Oxygen is transported to the nutrient media
6. Oxygen is transported to the air bubble

Nutrient Media
Air Bubble

Rate processes:

The overall rates are influenced both by the reactants of the products (autocatalysis). This leads to rather unusual optimisation problems.

- Essentially all configurations of microbiological reactors consist of microbes dispersed in aqueous nutrient media.
- In aerobic reactions an additional dispersed phase consisting of air bubbles (Fig.) is present. The overall rate of reaction depends upon the absorption of oxygen.
- Secondary metabolites include carbon dioxide and other products.

Operational considerations:

- In a particular system all the flowing molecules neither have same residence time, nor the same history of temperature & concentration. Even in case of time invariant state of system, transients are equally important.
- Calculation of minimisation of time required for start-up procedure.
- Investigation of product time at the approach of start-up.
- Calculation of fluctuation speed at the inlet, outlet or any intermediate point.

Local conditions:

For any reaction the conversion depends on the
The Batch Fermentor.

- Varying range for aerator etc.
- Head space
- pH control: acid-alkali addition
- Sterilization: hot water jacket
- Used in pilot plant 15-100 gallons
- Max: 2000 gallons (max 100,000 gallons)
- Aeration: volume of air
- Chemical batch fermenter: 10,000 - 8,000,000 gallons

(Batch fermentor is left vacant for aeration etc. - Head space.

- pH control: acid-alkali addition
- Sterilization: hot water jacket
- Used in pilot plant 15-100 gallons
- Max: 2000 gallons (max 100,000 gallons)
- Aeration: volume of air
- Chemical batch fermenter: 10,000 - 8,000,000 gallons

The Continuous Stirred Tank Fermentor.

- Relative smaller than batch fermentor
- Aeration feature: In C.S.T.F.
- Flowchart of C.S.T.F.

- Flowchart of C.S.T.F.
- Flowchart of C.S.T.F.
The Tubular Fermentor:

- State: The microorganisms exist in a laminar flow, making the flow predictable.
- Temperature control: Easier to control due to the flow pattern.
- Positional variation: Substrate and product concentrations vary from inlet to outlet.
- Environmental history: Changes as they travel through the fermentor.
- Industrial importance: Requires constant feed, difficult to scale up.

Applications: Waste water treatment, vinegar production.

The Fluidized Bed Fermentor:

- State: The microorganisms exist in a fluidized state, resembling a suspension in the fluid.
- Temperature control: Used if only required.
- Positional variation: Substrate concentration is controlled by the fluid flow.
- Environmental history: Uniform as they travel through the fermentor.
- Industrial importance: Requires constant feed, difficult to scale up.


Bed features:
- Increase in porosity from bottom to top.
- Decreased particle movement compared to beds of constant size particles.
- Inadequate gas phase particle distribution is common.

Temperature control: Used only if and when required.

Time dependence: Independent.

Positional variation: Substrate and product concentrations vary from inlet to outlet.

Environmental history: Largely constant, with some movement of particles taking place as different parts are exposed to different conditions.

Industrial importance: Produces a high-quality product.

Applications: Production of alcohols such as beer and cider, sugar fermentation. Lower fermentor used for continuous production of biodiesel.
following factors:
- Residence time distribution.
- Concentration distribution.
- Temperature distribution.

### Classification of Fermentation processes:
- Fermentors of these fermentation processes are classified into four types:
  - Batch fermentor
  - Continuous stirred tank fermentor
  - Tubular fermentor
  - Fluidised bed fermentor

### Other parts of fermentor system:
- In a bioreactor or fermentor, production of metabolite must be accomplished with maximum emphasis on reliability for the process and minimum capital investment.
- The reactors are hence designed specifically for special processes. E.g. "Gas distribution".
- In case of aerobic processes gas distribution is of four types depending upon requirement:
  - Gas distribution by stirring
  - Gas distribution through pumps
  - Gas distribution by means of pressurized air

The distribution phase is illustrated if explained in...
1. Stirred vessels are flexible and widely used.
2. Loop reactors are in mass production.
4. Modified air lift system - independent distribution.
5. Direct mixing by water jet is much efficient.
6. Pressure cycle fermenter - first model of this kind - used for single cell protein production. Others mostly have no movable parts.
8. Surface reactor - grow on semi-solid nutrient medium.
9. Blade wheel reactor - growth on blades
10. Paste water treatment, vinegar, citric acid production. etc.
Fig. 1-5 Mechanical foam separator (Fundalom, Chemap). a) Foam entrance, b) Gas exit, c) Lubrication, d) Double seal, e) Packing, f) Drive, g) Intermediate flange, h) Rotating plate.

Fig. 1-6 Types of Stirrer

- Disc stirrer
- Turbine stirrer

MIG Stirrer
INTERMIG Stirrer
For industrial use in pharmaceuticals, most versatile bioreactors are the "Simple Stirred Aerated Fermentor". However, no single design which adequately meets the needs of all biological systems can be constructed. Laboratory fermenters are made of glass and are of 20 l volume. For larger ones, the vessel extends to 3000 l and are made of stainless steel. The height-width scale varies between 2:1 to 6:1 if the stirrer may be top or bottom driven.

In order to bring about turbulence to the fermentor wall "baffles" are used. Four baffles are commonly used with a width of 1:10 or 1:12 of the fermenter diameter. In large fermenters, where heat dissipation is a problem even as much as 12 baffles may be installed. They also help in reducing vortex.

Foaming is a critical problem in large scale aerated systems. Simplest is the one with baffles mounted on the stirrer. In Sring's system and Fundalpa's system "foam separation" is done by centrifugal force.

The types of stirrers used in microbial reactors are as below:

- Disc stirrer: 4-8 blades project out beyond the disc edge, requires 50% less air for same yield & energy consumption.
- MIG stirrer: 25% less energy consumed.
- INTERMIC: 40% less energy consumed.
Uses of Cane and Beet molasses.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>20</td>
</tr>
<tr>
<td>Ash</td>
<td>8.0</td>
</tr>
<tr>
<td>Total sugar</td>
<td>40-60</td>
</tr>
<tr>
<td>Total nitrogen bodies</td>
<td>3.0</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>0.5</td>
</tr>
<tr>
<td>Gums</td>
<td>2.0</td>
</tr>
<tr>
<td>Free acid</td>
<td>2.0</td>
</tr>
<tr>
<td>Combined acids</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Patel (1985) 4547
<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Predominant Organism</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>Lactobacillus mesenteroides</td>
<td>Sauerkraut</td>
</tr>
<tr>
<td>Cucumber, tomatoes, lemon, cauliflower etc</td>
<td>Le. mesenteroides, Le. plantarum, Streptococcus faecalis, Pediococcus cerevisiae</td>
<td>Pickle</td>
</tr>
<tr>
<td>Rice &amp; black grams</td>
<td>Le. mesenteroides</td>
<td></td>
</tr>
<tr>
<td>Soybean, wheat and rice</td>
<td>Aspergillus oryzae, Flanensis, Saccharomyces</td>
<td>Soy sauce</td>
</tr>
<tr>
<td>Rice</td>
<td>Monascus purpureus</td>
<td>Ragi</td>
</tr>
<tr>
<td>Soybean</td>
<td>Rhizopus oligores</td>
<td>Ing-kak</td>
</tr>
<tr>
<td>Soybean, rice cereals etc</td>
<td>Bacillus subtilis</td>
<td>Temph</td>
</tr>
<tr>
<td>Beef/pork</td>
<td>Aspergillus oryzae, Saccharomyces, P. cerevisiae, P. acidilactici</td>
<td>Natto, Miso</td>
</tr>
</tbody>
</table>
Fermentation media:

Classification of Raw materials:

- Many different raw materials are used in fermentation industries. Mostly industrial products are used as raw materials, but industrial wastes are of more biological importance as:
  - Produced in huge quantity.
  - Have high B.O.D and hence are hazardous polluting agents.
  - Store high amount of usable nutrients and help meet the increasing protein demand.
  - Calorific value can be regained as biogas and ethanol.
  - Renewable, thereby causing no substrate shortage.
  - Less expensive recovery.
  - Not a part of human food chain.

Saccharine Material:

Molasses: By product of sugar cane and beet sugar.
- Palm:
- Blackstrap molasses:

Fermentable xage = 95%

Vitamins present:
- Biotin, pyridoxine, thiamine, pantetheine acid, and inositol.

Fermented product:
- Spirit (ethanol alcohol),
- Country liquor, rum, brandy, gin, whiskey.
### Chemical composition of beet molasses (dry wt):

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>48-60</td>
</tr>
<tr>
<td>Raffinose</td>
<td>1</td>
</tr>
<tr>
<td>Glucose + Fructose</td>
<td>1</td>
</tr>
<tr>
<td>Nitrogen compounds</td>
<td>12-13</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3.5</td>
</tr>
<tr>
<td>Other amino acids</td>
<td>5.5</td>
</tr>
<tr>
<td>(Asparagine, aspartic acid, glycine, alanine)</td>
<td>3.4 - 3.25</td>
</tr>
<tr>
<td>Betaine</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>11 - 12</td>
</tr>
</tbody>
</table>

### Approximate chemical composition of beet molasses ash:

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₂O</td>
<td>45</td>
</tr>
<tr>
<td>Na₂O</td>
<td>15</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.7</td>
</tr>
<tr>
<td>CaO</td>
<td>3</td>
</tr>
<tr>
<td>MgO</td>
<td>1.8</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>6.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>18</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>0.2</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>0.8</td>
</tr>
<tr>
<td>SiO₂</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### Fruit juices:

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/S sugar</td>
<td>17%</td>
</tr>
<tr>
<td>Acid (D-tartaric &amp; malic acid)</td>
<td>1%</td>
</tr>
<tr>
<td>Ash (mainly P₂O₅ and K₂O)</td>
<td>0.3%</td>
</tr>
</tbody>
</table>
Fruit juices: Source - Fruits (grapes, apple etc.)
Provide - Carbon source for fermentation industries
FERMENTED PRODUCT - Grape wine, Apple juice etc.

Cheese whey: By-product of - Cheese
FERMENTED PRODUCT - Lactic acid, SCP, lactose
Vitamin (probiotic)

Chemical composition of codder whey (Berry):

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>6.6 - 7.1</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.83 - 0.95</td>
</tr>
<tr>
<td>Fat</td>
<td>0.12 - 0.36</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4.62 - 5.81</td>
</tr>
<tr>
<td>Ash</td>
<td>0.366 - 0.649</td>
</tr>
<tr>
<td>K</td>
<td>0.135</td>
</tr>
<tr>
<td>Ca</td>
<td>0.047</td>
</tr>
<tr>
<td>Mg</td>
<td>0.010</td>
</tr>
<tr>
<td>pD</td>
<td>0.160</td>
</tr>
</tbody>
</table>

Starchy Material:
SOURCE - Cereals (e.g. wheat, rice, maize etc.)
Roots and tubers (e.g. potatoes, tapioca etc.)

METHOD OF CONVERSION - Use intended and availability of hydrolytic agents and relative cost

Approximate chemical composition of cereal grain:

<table>
<thead>
<tr>
<th>Cereals</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Starch (%)</th>
<th>Glu. Res. (%)</th>
<th>Crude Fiber</th>
<th>Mineral Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (Enriched)</td>
<td>10.5</td>
<td>2.6</td>
<td>78.6</td>
<td>1.5</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Maize (Sweet)</td>
<td>12.1</td>
<td>9.1</td>
<td>74.5</td>
<td>2.2</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>12.4</td>
<td>3.4</td>
<td>79.7</td>
<td>2.7</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Millet</td>
<td>13.6</td>
<td>5.4</td>
<td>77.9</td>
<td>1.3</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Rye</td>
<td>13.8</td>
<td>1.4</td>
<td>79.7</td>
<td>2.6</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>11.8</td>
<td>1.8</td>
<td>78.1</td>
<td>5.3</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Rice (Brown)</td>
<td>11.0</td>
<td>2.7</td>
<td>83.2</td>
<td>1.2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Oats (Whole)</td>
<td>11.6</td>
<td>5.2</td>
<td>69.8</td>
<td>10.4</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>
### Chemical composition of spent sulphite liquor

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (% by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignosulphonic acid</td>
<td>43.0</td>
</tr>
<tr>
<td>Ammonium compounds</td>
<td>12.1</td>
</tr>
<tr>
<td>Sequentially hydrolysed hemicellulose of xylan</td>
<td>7.7</td>
</tr>
<tr>
<td>Monoferulic acid (Total)</td>
<td>2.2</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>2.6</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>1.0</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>2.9</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>0.9</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1.6</td>
</tr>
<tr>
<td>Aldehydic acid and other substances</td>
<td>10.0</td>
</tr>
</tbody>
</table>

[Press and Paasivaara (1978)].

### Chemical composition of wood molasses (% by weight)

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt</td>
<td>52.0-60</td>
</tr>
<tr>
<td>Reducing sugars (as glucose)</td>
<td>1.4-15</td>
</tr>
<tr>
<td>Other cellulose polymers</td>
<td>6.0-9.0</td>
</tr>
<tr>
<td>Nitrates</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Ash</td>
<td>0.065-1.0</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1.0-2.0</td>
</tr>
<tr>
<td>Volatile Organic Acids</td>
<td></td>
</tr>
</tbody>
</table>

[Patel (1985), 57].

### Chemical composition of rice straw

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>4.5</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1.5</td>
</tr>
<tr>
<td>Ash</td>
<td>3.0</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.5</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>34.0</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>48.0</td>
</tr>
<tr>
<td>Total digestible nutrients added</td>
<td>8.0</td>
</tr>
<tr>
<td>N</td>
<td>1.0</td>
</tr>
<tr>
<td>K</td>
<td>0.1</td>
</tr>
<tr>
<td>Mg</td>
<td>0.1</td>
</tr>
<tr>
<td>P</td>
<td>0.1</td>
</tr>
</tbody>
</table>

[Patel (1985), 57].
**Cellulosic Material:**

β-glucose  
β-Cellobiose

Cellulose chain (approx. 1000-10,000 units)

**CELLULOSIC MATERIAL**

**Sulphite Waste Liquor**  **RICE STRAW**

**Wood molasses**:

- Source: Wood cellulose (hydrolysis), sludge dust
- Involved MoS: Candida utilis → Fructose  
  Trichoderma viride → Enzyme preparation  
  Cellulomonas → Hydrolysis

Rice straw and other agricultural bio-products are widely used in diets but it is a poor quality animal feed due to lack of protein, poor palatability and bulkiness.
## Chemical components of some typical commercial vegetable oils:

<table>
<thead>
<tr>
<th>Oil</th>
<th>Saponification value</th>
<th>Iodine value</th>
<th>% unsaturation</th>
<th>Chief component acids (X% w/w)</th>
<th>Sterol acid</th>
<th>Phospholipids</th>
<th>Glucosinolates</th>
<th>Phytate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive</td>
<td>189-193</td>
<td>80-85</td>
<td>9-20</td>
<td>65-84</td>
<td>56-65</td>
<td>17-26</td>
<td>4-9</td>
<td></td>
</tr>
<tr>
<td>Groundnut</td>
<td>189-196</td>
<td>85-88</td>
<td>18</td>
<td>56-65</td>
<td>17-26</td>
<td>4-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>188-193</td>
<td>112-130</td>
<td>12</td>
<td>48-94</td>
<td>40-42</td>
<td>45-50</td>
<td>95-98</td>
<td></td>
</tr>
<tr>
<td>Sunflower</td>
<td>186-194</td>
<td>127-136</td>
<td>7-10</td>
<td>30-25</td>
<td>55-65</td>
<td>65-70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton seed</td>
<td>191-196</td>
<td>103-111</td>
<td>28</td>
<td>25-30</td>
<td>45-50</td>
<td>65-70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linseed</td>
<td>185-196</td>
<td>130-185</td>
<td>10-15</td>
<td>15-25</td>
<td>15-20</td>
<td>45-50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soyabean</td>
<td>190-193</td>
<td>124-183</td>
<td>12-19</td>
<td>25-30</td>
<td>30-55</td>
<td>5-8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Analysis of corn-methanol samples taken:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids</td>
<td>40-60</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>12-27</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>7.4-7.8</td>
</tr>
<tr>
<td>Amino nitrogen</td>
<td>2.6-3.3</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>1.5-14</td>
</tr>
<tr>
<td>Ash</td>
<td>18-20</td>
</tr>
</tbody>
</table>

### Pharmametics:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>56</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>24</td>
</tr>
<tr>
<td>Oil</td>
<td>5</td>
</tr>
<tr>
<td>Ash (Ca, Fe, Cl, P, S)</td>
<td>15</td>
</tr>
</tbody>
</table>
Hydrocarbon & Vegetable oils:
- less purified once used as raw materials are relatively cheap
- are able to produce single cell protein (SCP)
- result in huge yeast biomass production
- Upto 97% pure n-paraffin is achieved

Vegetable oil:
- Types used: Oleic acid (non-drying type) olive and groundnut oil
- Linoleic acid (semi-drying) maize, cotton, sunflower oils
- Linolenic acid (drying) linseed, soya bean

Nitrogenous Material:
- Corn steep liquor
- By product of: Formed during manufacture of starch gluten and oblic corn products and is formally known as steep water (soil steeping of corn)
- Fermented products: Many fungal and biohich main penicillin

Soya bean meal:
- By product of: Formed during decaking of soya bean seed
- Nitrogen: 10.8% approximately
- Products: Streptomyein

Pharmamedia:
- Produced from: Rendered embryo of cotton seed
Distillers solubles: Produced from residue of distilled alcohol (using grains or maize)  
Name: Evaporated syrup.

Others:
- Ground nut meal
- Fish meal
- Beef spleen
- Difco yeast extract

Pre-treatment given to media:-
- The media is thoroughly checked for its carbon, nitrogen, growth factor content.
- Precursors are added to certain medium (e.g., Penicillin fermentation) which require it for better yield.
- Optimum pH is maintained for proper yield. Buffers such as CaCO₃ help in pH control. Proteins are self-buffers in neutrality range. Phosphates also have buffering capacity.
- Defoamers are added to media to avoid foaming (e.g., Lord oil mixed with octadecanol used for penicillin fermentation).
- The medium is made toxin-free.
- Consistency of the medium is checked according to the necessity of the required fermentation.
- It is also checked of contaminants.
- Availability of raw material and its composition play an important role.
- Thus, the media is treated accordingly and specifically for maximum yield and utility.
Advantage of batch cooer is that it saves time as the fermenter is unoccupied between two runs.

Limitations:
1. Occupies increased plant space
2. Higher cost of additional equipment
3. Increased steam usage

Parameters involved in continuous sterilization:

<table>
<thead>
<tr>
<th>Details</th>
<th>Steam Injection</th>
<th>Heat Exchanger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holding Time</td>
<td>1-5 minutes</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Holding Temperature</td>
<td>140°C</td>
<td>120°C</td>
</tr>
<tr>
<td>Holding up/Cooling down</td>
<td>Negligible</td>
<td>1% contribution</td>
</tr>
</tbody>
</table>

Advantage:
1. Saves time and plant space
2. Improved medium quality
3. Economy of steam cost on application of heat exchange principle
4. Non-sterilization limit of holding period at low pH

(a) Continuous Sterilization: Plate Heat Exchanger

(b) Continuous Sterilization: Steam Injection, Flash Cooling

Fig. 1.7: Schematic diagram for continuous medium sterilization.
Sterilization of Production media:

Sterilization denotes the use of physical and chemical agents to eliminate all viable microbes from a material.

In case of production media sterilization is decided by the chemical composition of the medium. Like in medium containing both sugar and phosphate sterilization is done separately as they react on prolonged heating. Sterilization is generally done by 3 methods:

- by boiling
- by passing live steam
- by subjecting the medium to pressurized steam (autoclaving)

Overcooking should be avoided for proper yield. Steam sterilization may be done in two ways:

- batchwise in fermentor
- continuous sterilization

The temperature of the whole system is raised to 120°C and the steam is maintained inside for 20 minutes in batch cooker while in the later more flexibility of time and temperature is offered.

Cooling is carried out by flush cooling vacuum chamber. Some medium component are not subjectable to heat and are hence sterilized by filtration (eg: yeast filter). Certain medium do not require sterilization at all (eg: yeast fermentation conducted at a relatively low pH).

Thick sterilization should be conducted keeping in view the nature of the media.
Contamination and its control:

Contaminants are possible micro-organism or spoilage agents which when (accidentally) involved in fermentation process affects the same adversely and subsequently lowers the yield of fermentation products. They may be classified into:

- microorganisms.
- spoilage agents.

Amongst the most some are infectious in nature which would otherwise interfere with efficient fermentation processes while the others are pathogenic and devalue the fermentation processes.

Spoilage agents also might either be inhibitory or product devaluing in nature.

Some troublesome contaminants are:

- Lactobacilli act as a troublesome contaminant in acetone-butyl alcohol fermentation using moss such as Clostridium acetobutylicum, C. butyricum & C. butyricum.
- Heavy metals inhibit or sometimes lower the rate of certain fermentation processes such as wine production.

Contamination can be controlled by:

- Sterilization of production media, equipment and surrounding air
- Using antifoam reagents to stop foaming
- Proper drawing of samples and introduction of inoculum
- Analysis of contaminants — yeasts, proteozoa, bacteria, spores and phages.
<table>
<thead>
<tr>
<th>Scale up criterion</th>
<th>Designation</th>
<th>Small fermentor 80L</th>
<th>(4) Product fermentor 19,000L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy input</td>
<td>Pe</td>
<td>1.0</td>
<td>3.125</td>
</tr>
<tr>
<td>Energy input/volume</td>
<td>Pe/N</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Impeller rotation number</td>
<td>N</td>
<td>1.0</td>
<td>0.35</td>
</tr>
<tr>
<td>Impeller diameter</td>
<td>D</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Height of impeller</td>
<td>H</td>
<td>1.0</td>
<td>40.5</td>
</tr>
<tr>
<td>Number of impellers above</td>
<td>PN</td>
<td>1.0</td>
<td>0.34</td>
</tr>
<tr>
<td>Maximum impeller speed</td>
<td>N/D</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>(max achievable rate)</td>
<td>N/D/L</td>
<td>1.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Energy input: P
Reynolds number: N/D/L
Scale up of fermentation:

The conversion of laboratory procedure to an industrial process is termed scale up.

These conversions are generally poorly successful if applied blindly.

Scale up is necessary only in certain cases such as:

- A new process is implemented in plants.
- Mutants with 10-20% greater yield are to be introduced to large scale production as soon as possible.
- Construction of a completely new fermentation plant (rare occurrence)

Comparison of most parameters is an important part of fermentation scaling-up.

Most important methods of scaling-up are:

- Constant power consumption per unit of broth
- Constant volumetric oxygen transfer rate

In geometrically similar sized reactors scaling-up is not required.

Buffers and Antifoaming agents:

For proper fermentation and maximum yield at minimal cost, optimum pH must be maintained. Buffers are components of medium that control the pH. E.g.: Calcium carbonate. Proteins and amino-acids are natural buffers. Phosphate (mono & dihydrogen potassium and Sodium phosphate) act as good buffers.

Certain chemicals such as hard oil mixed with octadecanol in penicillin fermentation are used to reduce foam formation. Even many mechanical methods are employed for same.
Conclusion:

Much has been advocated by different authors regarding Fermenters & Fermentation media. The foregoing chapter gives a brief and well-formulated glimpse of all relevant topics and subtopics. The informations blended with illustrations make reading interesting and easy for others to emulate.

Glossary:
Microbial Nutrition

Hoimee Dey
B.Sc. 1st yr (Microbiology)
<table>
<thead>
<tr>
<th>SL No.</th>
<th>Chapter</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Study of Bacterial Nutrition</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>The Conclusion</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>Glossary</td>
<td>12</td>
</tr>
<tr>
<td>5.</td>
<td>Bibliography</td>
<td>13</td>
</tr>
</tbody>
</table>
"A sound beginning is ecstasy itself ......."

The Introduction

Nutrition: The word nutrition has been derived from the Greek word 'nourine', which means to nourish. It is the sum of all those activities which are concerned with ingestion, digestion, absorption of the digested food into blood or lymph; oxidation of simple food to produce energy for the growth, repair, synthesis of biomolecules and digestion.

The primary necessity of all living organisms is to obtain energy and matter. Energy is required for continuation of metabolic functions. The material required for living organisms to sustain their life is called nutrient.

The raw material from which the nutrients are derived in infinite form is known as food. Organic or inorganic substance which passes in solution through protoplasmic membrane is termed as nutrients. In order to encoch the nutritional value of the food it must be digested into simple molecules thus making it readily absorbable through the protoplasmic membrane.
<table>
<thead>
<tr>
<th>S.No</th>
<th>ELEMENTS</th>
<th>% OF DRY WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbon.</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Oxygen.</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Nitrogen.</td>
<td>14</td>
</tr>
<tr>
<td>4.</td>
<td>Hydrogen.</td>
<td>08</td>
</tr>
<tr>
<td>5.</td>
<td>Phosphorus.</td>
<td>03</td>
</tr>
<tr>
<td>6.</td>
<td>Sulfur.</td>
<td>01</td>
</tr>
<tr>
<td>7.</td>
<td>Potassium.</td>
<td>01</td>
</tr>
<tr>
<td>8.</td>
<td>Sodium.</td>
<td>01</td>
</tr>
<tr>
<td>9.</td>
<td>Calcium.</td>
<td>0.5</td>
</tr>
<tr>
<td>10.</td>
<td>Magnesium.</td>
<td>0.5</td>
</tr>
<tr>
<td>11.</td>
<td>Chlorine.</td>
<td>0.5</td>
</tr>
<tr>
<td>12.</td>
<td>Iron.</td>
<td>0.2</td>
</tr>
<tr>
<td>13.</td>
<td>All others.</td>
<td>~0.3</td>
</tr>
</tbody>
</table>

Shauck 1983
"Even the most imperceptible thing has an im-
perative entity ... ."

List of basic nutritional requirements
- Carbon source: from carbohydrates
- Nitrogen source: from protein & ammonia
- Certain inorganic salts
- Essential metabolites: from vitamins & possible amino acids
- Water

Scope of required nutrients
- Carbon: Bacteria can generally use all sources of carbon except some synthetic
  plastics for synthesis of protoplasm. Even materials such as wood, asphalt-
  and gasoline are used up. Pathogenic bacteria obtain carbon from metabolism of carbo-
  hydrates and proteins. Strictly hetero-
  trophic bacteria must have organic com-
  pounds as carbon source while a facultative
  bacteria can utilize both organic as well
  as inorganic sources. Variation in carbon
  source in accordance with specific require-
  ments accounts for the flora of specific milieu.
  Compounds other than carbohydrates used
  for this purpose are malic acid, succinic
  acid, citric acid, lactic acid & monoalcohols.
Pathway of tryptophan biosynthesis.

Salley 1985
The formation of lipids by bacteria is dependent upon the nature of the carbon compounds added to media. Stephenson and Whetham; 1922, reported that in a medium containing acetate, lactate and glucose, singly or in various combinations, Mycobacterium phlei synthesised sufficient lipids to become acid-fast whereas in the same medium without the carbon source the cells stain non-acid-fast.

Jardon & Jardon; 1922, found that lipid synthesis occurs only if the organism uses carbon source without fermentation.

andonda; 1963a, b, reported the synthesis of two straight chain fatty acids by the B. subtilis taken grown on nutrient medium.

Nitrogen: The strict autotrophic bacteria are able to utilize inorganic ammonium salt as the only source of nitrogen. They cannot utilize exogenous organic compounds. The strict heterotrophic bacteria do not utilize ammonium salts but must have organic nitrogen such as is present in amino acids. The facultative bacteria can exploit both the sources of nitrogen equally. Bacteria shows wide difference in their amino acid requirements.
Hunt and Pittillo, 1968, employed a chemically defined medium to determine the nitrogen requirements of a single cell of E. coli. Ammonium chloride and glucose were added to a nutritionally deficient culture of organisms. Immediately thereafter, samples were removed at 3-hrin interval and counts of viable cells made. Appropriate calculations revealed the approximate $10^{-19}$ of ammonium chloride was required for cell.

Fildes et al.; 1933, found that the amino acid, tryptophan, is one of the indispensable constituents of protoblasts.

Curro; 1948, produced tryptophan independent mutants of Salmonella typhosa, capable of synthesizing its own essential amino acids.

Carlton; 1967, proposed a scheme for production of tryptophan in E. coli, Salmonella typhimurium.

- Inorganic ions: The bacterial cells sometimes require numerous other inorganic ions. The phosphorus is used for stock of energy, sulphur containing amino acids. Some other ions such as Mg$^{2+}$, K$^+$, and Ca$^{2+}$ ions act as cofactors. The other inorganic ions can be retrieved from mineralized tap water itself.
Vitamin B₁: Thiamine hydrochloride.

(Salley 1985)
Some bacteria secrete aerobophores. These substances which solubilize iron. The host iron is tightly bound to the iron transporting protein. The aerobophores compete along with iron transporting protein for growth of the bacteria. The major difference between virulent and avirulent strains is the competency of aerobophores. Virulent organism are more competent with the host iron due to presence of aerobophores (mycobacterin).

Vitamins in bacteria as growth factor.
Growth factor or vitamins are substances which when added even in minute quantity, produce a stimulatory effect. Williams coined the term nutritive which means the same.

Growth of an organism in the absence of certain vitamin does not necessarily mean that the factor is not required. Rather it reveals the fact that autotrophic and facultative bacteria can synthesize at least some of the essential amino acids. The important vitamins required by bacteria are explained below:

- Vitamin B (Thiamine hydrochloride)
  Uses: - Growth factor for all bacteria.
  It serves as a precursor for co-carbo-
Molecular structure of Biotin.

Carboxylation using Biotin.

Molecular structure of Pantothenic acid.

Molecular structure of D-Riboflavin.

(Salley 1985)
which intimately participate in decarboxylation of α-keto acids with formation of aldehydes and carbon dioxide

**Vitamin B7 or Vitamin H** (2'-keto-3'-4-imido-3-letrahydrothiophene-4-valeric acid)

*Uses:* Very important part for bacterial growth. A spore as small as one in 50 billions can produce hundred percent increase in the yeast growth.

May sometimes participate in denitration of adenosine, thiamine and coenzyme A.

- Carboxylation of pyruvate, adenine and guanine.
- Decarboxylation of oxaloacetate & succinate.
- Oxidation of pyruvate and lactate.

It is capable of forming intramolecular hydrogen bonds.

**Vitamin B3 (Pantothenic acid)**

*Uses:* Acetylation of aromatic amines and choline.

Utilization of other vitamins.

**Vitamin B2 or Riboflavin** (6,7-dimethyl-5-(β-ribofuranosyl)isoalloxazine)

*Uses:* It is a component of several en-
Pyridoxin is the co-occurring substances and the functionally active co-enzymes.

Niacin and niacinamide (molecular st.)

Molecular structure of folic acid.

β-Aminobenzoic acid  Inositol

(Salley 1988)
enzyme known as flavoprotein such as cytochrome oxidoreductase; liponamide oxidoreductase etc.

- **Vitamin B6 (Pyrdoxin)**
  Uses: It functions as transaminase for synthesis of amino acids from their 5′ analogs.

- **Vitamin B5 (Nicolic acid)**
  Uses: Nicolic acid and its amides are necessary for all living cells. It is a component of NAD and NADP.

- **β-Amino benzoic acid (PABA)**
  Uses: PABA is highly active in reversing the bacteriostatic action of sulfonamides.

- **Vitamin B9 (Folic acid)**
  Uses: It synthetizes certain amino acids which involve the incorporation of single carbon fragments.

- **Inositol**
  Uses: It acts both as vitamin as well as energy yielding nutrient. In bacteria it acts both as enzyme and coenzyme.

- **Vitamin B12**
  Uses: It produces growth in suitable culture medium. It helps in protein biosynthesis.
Graphical representation of vitamin assay by bacterium. (Yolk 1984)

Common nitrogen bases.
**Vitamin assay by bacterium.**

Bacteria have proved useful tools in the assay of small amounts of growth factors. Since for those organisms that require an essential metabolite, the quantity of growth is limited by concentration of available growth factor, a medium can be set up which supplies all the requirements of a given species except the essential metabolite or vitamins. The sterile material to be assayed for the vitamins is then added to the medium in measured quantities. The resulting growth gives a quantitation of the amount of growth factor in the material assayed.

**Purines and pyrimidines as growth factors.**

The purines, adenine and guanine and the pyrimidines, thymine and cytosine and uracil are required by most bacteria. They are necessary for the synthesis of nucleic acids and related compounds. The folic acid has been reported to act as a precursor of the pyrimidines. Since folic acid contains a purine-like component, probably small amounts of purines are utilized in the synthesis of that vitamin. The structure of pyrimidines, purines and folic acid are given in adjacent page.
The 'Carbon fixation pathway.'

The Ribulose di-phosphate pathway group

RIBULOSE 5-PHOSPHATE → GAP → RIBULOSE-1,5-DIPHOSPHATE → CO₂, H₂O

The Ribulose mono-phosphate pathway group

RIBULOSE 5-PHOSPHATE → HCHO → METHANE OXIDATION → DHAP 4-FRUCTOSE-1,6-DIPHOSPHATE → PYRUVATE

The Serine pathway

GLYCINE → HCHO → SERINE → CO₂

GLYOXALATE → ACETYL COA → CITRATE

OXALOACETATE → SUCCINATE → ISO-CITRATE
Energy source

As noted, the cell needs energy capacity to do work, in order to carry out various of its life processes. The source of energy for bacteria is generally the chemical oxidation.

The conventional definition of autotrophs includes the concept that the organisms obtain energy from oxidation of reduced inorganic chemicals. Whittemore and Jelley, however, pointed out that autotrophs do not have any common mechanism of inorganic chemical oxidation. The different substrates like \( \text{NO}_2^- \), \( \text{NH}_4^+ \), reduced sulphur compounds, \( \text{H}^+ \) and Fe\(^{2+} \) are oxidized by different enzyme complexes and pathways. Moreover, certain organisms considered to be heterotrophs also oxidize inorganic substances. Thus, Desulfovibrio and Desulfothiobacillus species oxidize hydrogen and various pseudomonads oxidize thiosulphate to dichromate.

Process of obtaining nutrients

- Decomposition of matter containing the nutrients.
- Absorption of simple components obtained by dissociation of complex molecules.
- Synthesis of necessary macromolecules such as lipids, proteins, carbohydrates etc. within the cell.
Classification of bacteria.

Autotrophs: CO₂ is sole source of carbon. They require only inorganic salts, CO₂ + H₂O for growth. Different energy sources are used to fix the carbon as organic compounds.

Phototrophs: Photosynthetic autotrophs. Use of inorganic electron donor.

Chemolithotrophs: Chemoautotrophic. Growth depends on oxidation of inorganic compound.

Chemotrophs: Organic compound as a source of CO₂. The carbon must be supplied in organic form i.e. the ones formed by plant and animals.

Photolithotrophs: These are photosynthetic heterotrophs. Use of organic electron donor.

### Active & Passive absorption of nutrients

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characteristics</th>
<th>Passive Absorption</th>
<th>Active Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Direction of absorption</td>
<td>From higher concentration</td>
<td>From lower concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>to lower concentration</td>
<td>to higher concentration</td>
</tr>
<tr>
<td>2.</td>
<td>Energy</td>
<td>No dependency on energy</td>
<td>Depends greatly on energy</td>
</tr>
<tr>
<td>3.</td>
<td>Direction</td>
<td>Bi-directional</td>
<td>Unidirectional</td>
</tr>
<tr>
<td></td>
<td></td>
<td>process</td>
<td>process</td>
</tr>
<tr>
<td>5.</td>
<td>Speed</td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td>6.</td>
<td>Effect of temp.</td>
<td>Shows no effect on rate</td>
<td>Decrease in presence of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>decreases in presence of</td>
<td>oxygen absence of oxygen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cyanide</td>
<td>cyanide</td>
</tr>
<tr>
<td>7.</td>
<td>Carrier protein</td>
<td>Not dependent</td>
<td>Dependent</td>
</tr>
</tbody>
</table>

(Arova 2002)

---

**Osmosis & Osmotrophs**

Osmosis is the transport of water or any other solvent molecules from a region of lower concentration (or lower water/chemical potential) to a region of higher concentration (or lower water/chemical potential) through a semi-permeable membrane. It does not allow movement of solute particles. Osmotrophs take up all nutrients in dissolved form.
Pinocytosis in bacteria

(Avery, 2002)

Phagocytosis in bacteria

(Avery, 2002)
**Pinocytosis**

Greek: *Pnein* = to drink; *Kytos* = cell

The process involves intake of large sized liquid nutrients. It was first shown by Hess in 1931 A.D.

**Phagocytosis**

Greek: *Phagein* = to eat

The process involves intake of large sized solid particles including cellular debris and microbes. It was first observed by Metchnikoff in 1883 A.D.

**Symbiosis**

It is a type of mutualism involving the exchange of nutrients between two species. Many microorganisms synthesize vitamins and amino-acids in excess of their nutritional requirements. Others have a requirement for one or more of these nutrients. Still others synthesize certain nutrients in suboptimal amounts. Hence such combination of species will grow together but not apart when the nutrition level is low.
'All's well that ends well...'

THE CONCLUSION

Much has been advocated by different authors and scientists to enlighten up the study of Bacterial Nutrition. The foregoing chapters give all the modernized and well-formulated details of Bacterial Nutrition. The updated information blended with picturesque illustrations make reading interesting. Any updated information or opinion by readers are requested to be placed in the notes.

'el Dorado.....'

THE GLOSSARY

Assay: The qualitative or quantitative determination of the components of a material, such as a drug.

Oxidase: An enzyme that brings about oxidation.

Pseudopodium: A temporary projection in the proto plant of amoeboid cells in which the cytoplasm flows.


<table>
<thead>
<tr>
<th>SL. NO.</th>
<th>CHAPTERS</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Introduction</td>
<td>1.</td>
</tr>
<tr>
<td></td>
<td>(Formulation of medium, Criterion of good culture medium, Types of medium, pH of a medium, Buffers)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Conclusion</td>
<td>6.</td>
</tr>
</tbody>
</table>
The Introduction

Cultivation is the process involving the deliberate growth of microorganisms on or in the nutrient media. The different microbial species growing on the same kind of media may appear differently. The knowledge of the cultural characteristics of a microbial species is therefore useful in recognition of certain types of micro-organisms. In order to have a good cultivation of the micro-organisms it is necessary to have a good culture medium. The main aim of a culture medium is to obtain a balance in supplied nutrients to establish a well formulated colony of micro-organisms. A good culture medium has all the necessary components to maximum proximity. A good and maintained medium disallows any change in internal and external experimental milieu in order to obtain healthy microbea.
<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Medium for <em>Lactobacillus mesenteroides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WATER</strong></td>
<td>1 liter</td>
</tr>
<tr>
<td><strong>ENERGY SOURCE</strong></td>
<td><strong>Glucone</strong> 25 g</td>
</tr>
<tr>
<td><strong>NITROGEN SOURCE</strong></td>
<td><strong>NH₄Cl</strong> 3 g</td>
</tr>
<tr>
<td><strong>MINERALS</strong></td>
<td></td>
</tr>
<tr>
<td><strong>KH₂PO₄</strong></td>
<td>600 mg</td>
</tr>
<tr>
<td><strong>K₂HPO₄</strong></td>
<td>600 mg</td>
</tr>
<tr>
<td><strong>MgSO₄ · 7H₂O</strong></td>
<td>200 mg</td>
</tr>
<tr>
<td><strong>ORGANIC ACID</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sodium acetate</strong></td>
<td>20 g</td>
</tr>
<tr>
<td><strong>AMINO ACIDS</strong></td>
<td></td>
</tr>
<tr>
<td><strong>DL-β-Alanine</strong></td>
<td>200 mg</td>
</tr>
<tr>
<td><strong>L-Arginine</strong></td>
<td>242 mg</td>
</tr>
<tr>
<td><strong>L-Asparagine</strong></td>
<td>400 mg</td>
</tr>
<tr>
<td><strong>L-Aspartic acid</strong></td>
<td>100 mg</td>
</tr>
<tr>
<td><strong>L-Cysteine</strong></td>
<td>50 mg</td>
</tr>
<tr>
<td><strong>L-Glutamic acid</strong></td>
<td>300 mg</td>
</tr>
<tr>
<td><strong>L-Histidine · HCl</strong></td>
<td>62 mg</td>
</tr>
<tr>
<td><strong>DL-Isoleucine</strong></td>
<td>250 mg</td>
</tr>
<tr>
<td><strong>DL-Leucine</strong></td>
<td>250 mg</td>
</tr>
<tr>
<td><strong>PURINES AND PYRIMIDINES</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Adenine sulfate · H₂O</strong></td>
<td>10 mg</td>
</tr>
<tr>
<td><strong>Guanine · HCl · H₂O</strong></td>
<td>10 mg</td>
</tr>
<tr>
<td><strong>VITAMINS</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Thiamine · HCl</strong></td>
<td>0.5 mg</td>
</tr>
<tr>
<td><strong>Pyridoxine · HCl</strong></td>
<td>1.0 mg</td>
</tr>
<tr>
<td><strong>Pyridoxal · HCl</strong></td>
<td>0.3 mg</td>
</tr>
<tr>
<td><strong>Calcium pantothenate</strong></td>
<td>0.5 mg</td>
</tr>
</tbody>
</table>

*Note: Values are in milligrams.*

*(Stanier 1984)*
"When the going gets tough, the tough gets going..."

The formulation of Culture Medium.

Much of microbiology depends upon the ability to grow and maintain micro-organisms in the laboratory and this is possible only if suitable culture media are available. In addition, specialized media are essential in isolation & identification of cells, the testing of antibiotic sensitivities, water & food analysis, industrial microbiology and other activities. Even though all microorganisms need sources of energy, carbon, nitrogen, oxygen etc., the precise composition of a satisfactory medium will depend upon the species to be cultivated. Proper selection of the microorganism with respect to the milieu enables quick and steady of micro-organisms.

Criterion for a good culture medium:

- Adequate amount of necessary nutrients
- Addition of supplementary mineral base
- Control of pH of the medium
- Avoidance of mineral precipitation
- Control of oxygen concentration
- Avoidance of exposure to air (as molecular oxygen is inhibitory in action)
- Regular provision of carbon dioxide
- Regular provision of light
<table>
<thead>
<tr>
<th>Organic substrates, no illumination</th>
<th>Preferably nonfermentable substrate</th>
<th>Preferably nonfermentable substrate</th>
<th>Fermentable substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>N\textsubscript{2} as sole nitrogen source</td>
<td>Combined nitrogen present</td>
<td>(Azotobacter group)</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{3} as electron acceptor</td>
<td>CO\textsubscript{2} as electron acceptor</td>
<td>(Aerobes, e.g., Pseudomonas, Acinetobacter)</td>
</tr>
<tr>
<td></td>
<td>SO\textsubscript{4}^{2-} as electron acceptor</td>
<td>N\textsubscript{2} as sole nitrogen source</td>
<td>(Denitrifying bacteria)</td>
</tr>
<tr>
<td></td>
<td>Combined nitrogen present</td>
<td>Combined nitrogen present</td>
<td>(Sulfate reducers)</td>
</tr>
<tr>
<td>Anaerobic</td>
<td></td>
<td></td>
<td>(Methanogenic bacteria)</td>
</tr>
<tr>
<td>Fermentable substrate</td>
<td></td>
<td></td>
<td>(Clostridium pasteurianum and related species)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Fermentative bacteria, e.g., Enterobacter)</td>
</tr>
</tbody>
</table>

*Stanier (1987)*

**TABLE 1**
Primary Environmental Factors That Determine the Outcome of Enrichment Procedures for Chemoautotrophic Bacteria with the Use of Synthetic Media
Synthetic or Defined Medium.

- Some microorganisms particularly photolithotrophic autotrophs such as cyanobacteria and eutrophic algae, can be grown on relatively simple media containing CO₂ as carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as nitrogen source, sulphur, phosphorus and a variety of minerals are added.
- Such a medium in which all components are known is called synthetic or defined medium.
- A number of chemosynthetic or can be grown in defined medium. This type of media are widely used in research.

Complex media.
- The media that contain some ingredients of unknown chemical composition are complex media.
- Such media are very useful as a single complex medium may be sufficiently rich and complete to meet the nutritional requirements of many different microbes. In addition these are often needed because the nutritional requirements of a particular microorganism are unknown and thus a defined medium cannot be constituted. Three commonly used complex media are:
  - Nutrient broth
  - Trypsin soy broth
  - Cooked meat medium
- The medium is required is solidified using 1.5% agar.
<table>
<thead>
<tr>
<th>TABLE *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Environmental Factors That Determine the Outcome of Enrichment Procedures for Some Chemoautotrophic Bacteria</td>
</tr>
<tr>
<td>Absence of organic compounds in medium</td>
</tr>
<tr>
<td>Anaerobic (as electron acceptor)</td>
</tr>
<tr>
<td>NH₄⁺ as oxidizable substrate</td>
</tr>
<tr>
<td>NO₂⁻ as oxidizable substrate</td>
</tr>
<tr>
<td>H₂ as oxidizable substrate</td>
</tr>
<tr>
<td>S or S₂O₃⁻ as oxidizable substrate</td>
</tr>
<tr>
<td>(Ammonia oxidizing bacteria, e.g. Nitrosomonas)</td>
</tr>
<tr>
<td>(Nitrite oxidizing bacteria, e.g. Nitrobacter)</td>
</tr>
<tr>
<td>(Hydrogen bacteria)</td>
</tr>
<tr>
<td>(Thiobacillus)</td>
</tr>
<tr>
<td>Anaerobic (CO₂ as electron acceptor)</td>
</tr>
<tr>
<td>H₂ as oxidizable substrate</td>
</tr>
<tr>
<td>(Methanogenic bacteria)</td>
</tr>
</tbody>
</table>

* (Stanier 1987) |

<table>
<thead>
<tr>
<th>TABLE *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Environmental Factors That Determine the Outcome of Enrichment Procedures for Photosynthetic Microorganisms</td>
</tr>
<tr>
<td>Light as source of energy</td>
</tr>
<tr>
<td>Absence of organic compounds</td>
</tr>
<tr>
<td>Presence of organic compounds</td>
</tr>
<tr>
<td>Absence of sulfide</td>
</tr>
<tr>
<td>Presence of sulfide, anaerobic conditions</td>
</tr>
<tr>
<td>Anaerobic conditions</td>
</tr>
<tr>
<td>N₂ as sole nitrogen source</td>
</tr>
<tr>
<td>Presence of combined nitrogen</td>
</tr>
<tr>
<td>High sulfide concentration</td>
</tr>
<tr>
<td>Low sulfide concentration</td>
</tr>
<tr>
<td>(Cyanobacteria)</td>
</tr>
<tr>
<td>(Algae)</td>
</tr>
<tr>
<td>(Green sulfur bacteria)</td>
</tr>
<tr>
<td>(Purple sulfur bacteria)</td>
</tr>
<tr>
<td>(Purple or green nonsulfur bacteria)</td>
</tr>
</tbody>
</table>

* (Stanier 1987)
General purpose media.
- Media like tryptic soy broth are known as the general purpose media as they support the growth of many bacteria.

Enriched media.
- Some fastidious heterotrophs require special types of nutrients to support their growth. These specially fortified media are called enriched media.

Selective media.
- Selective media favor the growth of particular microorganisms. The use of dyes like basic fuchsin and crystal violet favors the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria without affecting gram-negative bacteria. Endo-agar and eosin-methylene blue agar, two media widely used for the detection of E. coli and related bacteria in water supplies, contain dyes that suppress gram-positive bacterial growth. Bacteria can also be selected by incubation with specifically utilisable nutrients. For cellulose digesting bacteria medium containing cellulose is used.
- Thus several possible selections can be made for different species of bacteria.

Differential media.
- Differential media are media that distinguish between different groups of bacteria and even.
permit tentative identification of microorganisms on the basis of their biological characteristics.

- Blood agar is a differential medium as well as enriched one. It distinguishes between hemolytic and non-hemolytic bacteria. Enriched agar is both differential and selective since it contains lactose and a dye, lactose fermenting colonies appear pink to red in colour and are easily distinguished from colonies of non-fermenters.

- Assay media:
  - Media of prescribed compositions are used for the assay of vitamins, amino acids and antibiotics.
  - Media of a special composition are also available for testing disinfectants.

- Media for enumeration of bacteria:
  - Specific kinds of media are used for determining the bacterial content of such materials as milk and water. Their composition must adhere to prescribed specifications.

- Maintenance media:
  - Satisfactory maintenance of the viability and physiological characteristics of a culture over time may require a medium different from that which is optimum for growth.
<table>
<thead>
<tr>
<th>No.</th>
<th>Indicator Name</th>
<th>pH Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10 11 12 13 14</td>
</tr>
<tr>
<td>010</td>
<td>Methyl Red</td>
<td></td>
</tr>
<tr>
<td>025</td>
<td>Phenolphthalein</td>
<td></td>
</tr>
<tr>
<td>030</td>
<td>Brom Cresol Purple</td>
<td></td>
</tr>
<tr>
<td>035</td>
<td>Brom Phenol Red</td>
<td></td>
</tr>
<tr>
<td>040</td>
<td>Phenol Red</td>
<td></td>
</tr>
<tr>
<td>045</td>
<td>Brom Thymol Blue</td>
<td></td>
</tr>
<tr>
<td>050</td>
<td>Cresol Red</td>
<td></td>
</tr>
<tr>
<td>055</td>
<td>Cresol Green</td>
<td></td>
</tr>
<tr>
<td>060</td>
<td>Cresolphthalein</td>
<td></td>
</tr>
</tbody>
</table>

*Stanier 1984*
Buffers:
- The salts of weak acids have the power of preventing pronounced changes in the reactions of solution on addition of acids and alkalies. Substances of these nature are called buffers.
- The important salts added to nutrient media is replaced by weak basic phosphate.
- A good nutrient besides being well supplied with nutrients must also be well buffered.

pH of the medium:
- To select for acid tolerant bacteria, a low pH medium can be used.
- For example, to select for lactobacilli present in cheddar cheese, the pH is maintained at 5.36.
- To select for alkali tolerant bacteria, a high pH is required.
- For example, to select Vibrio cholerae bacterium from stool sample, a pH of 8.5 is required.

"All's well that ends well."

The conclusion:
Much has been advocated by different authors and scientists to enlighten the study on Bacterial Cultivation. The script has been formulated in such a fashion so as to inculcate as many relevant details as possible.
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<table>
<thead>
<tr>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Their Mini Definitions</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Blood Grouping</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
</tbody>
</table>

Saswat Chakravarty
B.Sc 3rd Yr.
Roll: 17
REFERENCES


I. Blood Staining:

Diseases & Their Mini Definitions:

**Neutrophils**
- Increase in number → 1. Septic Endocarditis
- Pus Formation

- **Septic Endocarditis** → (kahr'-di-tis)
  → Exudative and proliferative inflammation alteration of the endocardium.
  → Usually characterized by the presence of vegetations on the surface of the endocardium (within the heart) or in the endocardium itself, and most commonly involving heart valve, but also affecting the inner lining of the cardiac chambers or the endocardium elsewhere.
  → Causal organisms: Streptococci, Staphylococci, Enterococci, Gonococci & Gram negative bacilli.

- Pus Formation: →
  → A protein rich liquid inflammation product made up of cells (leukocytes) a thin fluid (liquefied pus) & cellular debris.
  → Causal organism: Strept pyogens, Staph aureus etc.
**SCARLET FEVER**

- An acute disease caused by Group A β-hemolytic streptococci, marked by pharyngitis, fever, and a skin rash caused by an erythrogenic toxin produced by the organism.
- The rash is a diffuse, bright red erythema and desquamation; the skin begins as fine scaling with eventual peeling of the palms and soles.

**LEUKOPENIA**

Reduction in the number of leukocytes in the blood below about 5000 per cubic mm. Basophilic Leukopenia pertains to Basophilia.

**RICKETTSIAL DISEASE**

- Caused by Rickettsia.
Rocky Mountain Spotted Fever

- Infection with *Rickettsia rickettsii*
- Transmitted by ticks, marked by fever, muscle pain, & weakness, followed by a macular petechial (red spot due to escape of a small amount of blood) eruption that begins on the hands & feet & spreads to the trunk and face, with other symptoms in the CNS & elsewhere.
BLOOD GROUPING

The Basis of Human ABO Isoantigens and Blood Types.

The existence of human blood types was first demonstrated by an Austrian pathologist, Karl Landsteiner, in 1904.

While studying incompatibilities in blood transfusions, he found that the serum of one person could clump the red blood cells of another.

Landsteiner identified four distinct types, subsequently called the ABO blood groups.

Like the MHC antigens on white blood cells, the ABO isoantigen markers on red blood cells are genetically determined and composed of glycoproteins.

These ABO antigens are inherited as two (one from each parent) of three alternative alleles: A, B, or O.

A and B alleles are dominant over O and codominant with each other.

This mode of inheritance gives rise to four blood types (phenotypes), depending on the particular combination of genes:

- A blood type: genotype AA or AO
- B blood type: genotype BB or BO
- AB blood type: genotype AB
- O blood type: genotype OO

Important Points about Blood Types:

They are named for the dominant antigen.

The RBCs of type O persons have antigens, but not A & B antigens.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype A, B* CRBC Antigen</th>
<th>Prevalence in Population **</th>
<th>Serum Content of Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>Neither</td>
<td>Most common</td>
<td>Both anti A &amp; anti-B</td>
</tr>
<tr>
<td>AA, AO</td>
<td>A</td>
<td>Second most common</td>
<td>Anti-B</td>
</tr>
<tr>
<td>BB, BO</td>
<td>B</td>
<td>Third most common</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>Least common</td>
<td>Neither antibody</td>
</tr>
</tbody>
</table>

Legend: * Capital letters generally denote antigen; lowercase denotes antibody. ** True of most large population of mixed racial & ethnic groups.

![Agglutination Reactions Controlled by the ABO Blood Type Locus in Humans](image.png)
Tissues other than RBC's carry A & B antigens.

**GENETIC BASIS:** ABO BLOOD TYPE ALLELES IN HUMANS

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>PHENOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I^A I^B</td>
<td>A</td>
</tr>
<tr>
<td>I^A I^0</td>
<td>B</td>
</tr>
<tr>
<td>I^B I^0</td>
<td>AB</td>
</tr>
<tr>
<td>I^0 I^0</td>
<td>O</td>
</tr>
</tbody>
</table>

One of the most firmly established series of multiple alleles in humans involves the genetic locus controlling the blood types A, B, AB & O.

The ABO locus has three common alleles: I^A, I^B & I^0.

- I^A & I^B are codominant (I^A I^B)
- I^0 is recessive (I^0 I^0)

Individuals with these genotypes produce a red cell surface glycosyltransferase that adds A or B antigens to the terminal sugars of the cell surface carbohydrates. Heterozygotes have both A & B antigens, respectively, on their RBC's.

The ABO locus controls the type of glycolipids found on the surface of erythrocytes and appears to be responsible for the synthesis of glycosyltransferases (enzymes catalyzing the synthesis of complex carbohydrates) synthesized in the RBC's.

The specific types of glycolipids on the red cell surface provide the antigenic determinants that react with specific antibodies present in the blood serum.

Humans, like all other mammals, produce antibodies to foreign substances. Fortunately, no antibodies are synthesized in normal individuals.
# Table 3: Blood Transfusion Compatibilities for the ABO Blood Groups

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Terminal Sugars of Ags Present</th>
<th>Antibodies Present</th>
<th>Red Cell Types Agglutinated</th>
<th>Transfusions Accepted From</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A (galactosamine)</td>
<td>Anti-B</td>
<td>B, AB</td>
<td>A or O</td>
</tr>
<tr>
<td>B</td>
<td>B (galactose)</td>
<td>Anti-A</td>
<td>A, AB</td>
<td>B or O</td>
</tr>
<tr>
<td>AB</td>
<td>A (galactosamine)</td>
<td>None</td>
<td>None</td>
<td>A, B, AB and O</td>
</tr>
<tr>
<td>O</td>
<td>None</td>
<td>Anti-A &amp; Anti-B</td>
<td>A, B and AB</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig 2.**

**Legend:** In this test, a drop of blood is mixed with a specially prepared antiserum known to contain antibodies against the A, B, and RH antigens. If a particular ag is not present, the RBCs in that droplet do not agglutinate and form an even suspension.
that react with antigens present on the individual's own cells. However, when type A blood & type B blood are mixed, the anti-A antibodies in the type B blood serum react with the antigens on the type A blood cells, & vice versa, which produces agglutination or clumping of cells [Fig 6b].

Cross-matching blood types to determine compatibility is thus essential in blood transfusions.

In this process, blood donors and recipients are tested for the presence of antigens & antibodies that are incompatible.

Table (iii) summarizes the cell surface antigenic determinants of the serum antibodies present in the four major ABO blood types.

Individuals with blood type AB have both A & B antigens on their erythrocytes, but no anti-A & anti-B antibodies in their blood serum.

Type O individuals lack both ags, but carry both anti-A & anti-B abs in their blood serum.

Type O individuals are referred as UNIVERSAL DONORS, type O blood can be used in transfusion for individuals of any blood type if the blood is introduced slowly enough to permit sufficient dilution of the Anti-A & Anti-B abs present in the serum of the donor.

Type AB persons are consequently called UNIVERSAL RECIPIENTS.

**DEGREES OF ADVERSE REACTIONS IN TRANSFUSIONS:**

Transfusion of the wrong blood type causes various degrees of adverse reaction.
Incompatible blood. The red blood cells of the type A donor contain ag A, while the serum of the type B recipient contains anti-A abs that can agglutinate donor cells.

Agglutination particles can block the circulation in vital organs.

Activation of the complement by ab on the RBC's can cause haemolysis & anaemia. This sort of incorrect transfusion is very rare because of the great care taken by blood banks to ensure a correct match.
The severest reaction is massive hemolysis when the donated red blood cells react with recipient antibody & trigger the complement cascade (fig 3).

The resultant destruction of red cells leads to systemic shock & kidney failure brought on by the blockage of glomeruli (blood filtering apparatus) by cell debris.

Death is a common outcome.

Other reactions caused by RBC destruction are fever, anemia & jaundice.

A transfusion reaction is managed by immediately halting the transfusion, administering drugs to remove hemoglobin from the blood, and beginning another transfusion with RBC's of the correct type.

---

A CLOSER APPROACH: 
MICROFILE: THE ORIGIN OF ABO ANTIGENS.

The A and B genes each code for an enzyme that adds a terminal carbohydrate to RBC receptors during maturation.

RBC's of Type A contain an enzyme that adds N-acetylglucosamine to the receptor; RBC's of Type B have an enzyme that adds D-galactose; RBC's of Type AB contain both enzymes that add both carbohydrates, and RBC's of Type O lack the genes & enzymes to add a terminal molecule.

The genetics of ABO genes were once used to rule out paternity: for eg: if a man is type A, the mother type O, & the child type B, we know this man could not have fathered this child.

However this same logic cannot prove paternity. If the child is type A instead, it is this same logic for the man to believe...
father, but so could another man with blood type A.

Highly sensitive methods based on specific & variable MHC
and DNA fingerprinting have been developed to gather
more precise evidence of paternity, a maternity (in cases of
kidnapping, adoption, for instance).
NAME: Saswati Chakraborty
STD: B.Sc III yr
ROLL: 17

Adenosine Tri Phosphate (ATP)
<table>
<thead>
<tr>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. No.</td>
</tr>
<tr>
<td>----------</td>
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</tbody>
</table>
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METABOLISM

/ AN OVERVIEW

- Definitions of metabolism.
- Catabolism and Anabolism.
- Classification of micro-organisms on the basis of energy carbon sources.
- Bioenergetics.
- Coupling through ATP and through pyridine nucleotides.

/ INTRODUCTION: [Definition].

The term metabolism denotes all the organized chemical activities performed by a cell which comprise two general types:
- energy production
- energy utilization

The term intermediary metabolism is a rather incomplete definition which only highlights the sum total of all the enzymatic reactions occurring in the cell.

- Four specific functions of metabolism are:
  - (1) To extract chemical energy from the environment, either from organic nutrients or from sunlight.
  - (2) To convert exogenous nutrients into the building blocks or precursors of the macromolecular components of cells.
  - (3) To assemble the building blocks into proteins, nucleic acids, lipids
Fig: 4) The Metabolic Mill
and other characteristic cell components and
(4) to form and degrade those biomolecules required in special-
ized functions of cells.

/ TYPES OF METABOLIC REACTIONS. /

**Catabolism:**
- It is the enzymatic degradation, largely by oxidative processes, of relatively large nutrient molecules (e.g., polysaccharides, nucleic acids, fats, lipids, and proteins) from either the environment or the cell or from its own internal storage depot into a series of smaller, simpler molecules (e.g., lactate and acetyl-CoA, ammonia, urea).

**Anabolism:**
- Anabolism is the enzymatic synthesis of relatively large molecular components of cells, e.g., polysaccharides, nucleic acids, proteins, and lipids from simple precursors using energy, either from the environment or from within the cell.

(6) Catabolism is accompanied by a release of free energy inherent in the ordered size and structure of the complex molecules. A decrease in entropy, or increase in disorder, is required to convert the complex molecules into a series of simpler molecules, which is provided by the free energy released during the process of catabolism. The free energy is then converted into a form that can be used by the cell, usually in the form of phosphate bond energy of ATP.
Fig. 2: Catabolism, Anabolism, Amphibolism
(The 3 stages.)
AMPHIBOLIC PATHWAY:

Although the pathways of catabolism and anabolism are not identical, the stage III (Fig 12) constitutes a central meeting ground or pathway which is accessible to both. This central route is called an amphibolic pathway (amphi - dual).

CLASSIFICATION OF M.O.s ON THE BASIS OF ENERGY CARBON SOURCES:

- Division on the basis of utilization of carbon source:
  - Autotrophs
    - Photosynthetic autotrophs
    - Chemolithotrophs
  - Heterotrophs
    - Heterotrophs: feeders

- Division on the basis of energy source:
  - Phototrophs
  - Chemotrophs

- Division on the basis of oxidizing agent for nutrient breakdown:
  - (Aerobes) [those which grow in presence of O2]
  - (Anaerobes) [those which grow in absence of O2]
Fig 3 (a) The increase of entropy:

Copper blocks: Initial state

WARM COOL

Equilibrium state

Heat spontaneously flows from warm body to cool body.

Initial state

Equilibrium state

Gas molecules flow from zone of high pressure to zone of low pressure.

The increase of entropy or randomness in two physical systems such flows never reverse spontaneously.

THE SURROUNDINGS

The entropy of the surroundings may increase, stay constant or decrease.

THE SYSTEM

(constant P, V, T)

The entropy of the system alone may increase, stay constant or decrease, but its free energy always decreases to a minimum.

Fig 3 (b) Summary of Free Energy And Entropy
Bioenergetics:

(1) Entropy: Entropy is defined (for the moment) as the degree of disorder or randomness. \[ S \]

(2) Equilibrium: An equilibrium is defined as a state in which no further net chemical or physical change is taking place and in which temperature, pressure, and concentration are uniform throughout the system.

All "real" processes occurring in our physical world including the process of life are irreversible.

(3) Free energy: The change in entropy during a process is quantitatively related to changes in total energy of the system by a useful function called the free energy. \[ \Delta G \]

(4) Enthalpy: The change in function is known as enthalpy.

(5) Important Equations:

1. \[ \Delta G = \Delta H - T \Delta S \]
2. \[ \Delta H = \Delta E + \Delta PV \]
3. \[ \Delta G = \Delta E + T \Delta S \]
4. \[ \Delta E = \Delta G + T \Delta S \]
Coupling of an exergonic to an endergonic reaction. → Fig (6) (c)

Fig (7) → Transference of free energy from an exergonic to an endergonic reaction through the formation of a high energy intermediate compound. (c)

(Coupling of dehydrogenation and hydrogenation reactions by an intermediate carrier. → Fig (6b)

Exergonic Reactions

Syntheses
Muscular contraction
Nervous excitation
Active transport.

→ Fig (8) → Transduction of energy through a common high-energy compound to energy-requiring endergonic biologic processes.
**Bioenergetics of Coupled Reactions**

**Coupling - ATP & Pyridine Nucleotides**

First reaction in the glycolytic pathway.

The phosphorylation of glucose to glucose 6-P which is highly endergonic and would not proceed as such under physiological conditions.

(1) Glucose + P_i → Glucose 6-P + H_2O

(AG° = +12.8 kJ/mol)

(2) ATP → ADP + P_i (AG° = -36.8 kJ/mol)

Reaction couples with another reaction (hydrolysis of the terminal P of ATP) that is more exergonic.

(3) Glucose + ATP → Glucose 6-P + ADP (AG° = -23.0 kJ/mol)

---

**Interconversion of Adenine Nucleotides**

The enzyme adenosine kinase (myokinase) is present in most cells. It catalyzes the interconversion of ATP and AMP on one hand and ADP on the other.

(4) Adenosine - (P) \(\xrightarrow{\text{kinase}}\) Adenosine - (AMP)

\[ \text{adenosine} \rightarrow 2 \text{adenosine} \]

When ATP reacts to formAMP, inorganic pyrophosphate (PP_i) is formed, as occurs - [activation of long chain fatty acids].

(5) ATP + CoA \_SH + R \_COOH \(\xrightarrow{\text{kinase}}\) AMP + PP_i + R \_COA \_SH

(AG° = -4.6 kcal/mol)

(6) PP_i + H_2O \(\xrightarrow{\text{pyrophosphatase}}\) 2 P_i

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(5) Fig (5) - Phosphate cycles and interchange of adenine nucleotides.
A combination of the above reactions makes it possible for phosphate to be recycled and for adenine nucleotides to interconvert (fig. 6).

**Nucleoside Phosphates Related to ATP & ADP.**

By means of the enzyme nucleoside diphosphate kinase, nucleoside triphosphates similar to ATP but containing a different base from adenine, can be synthesized from their diphosphates e.g.

\[
\begin{align*}
\text{Nucleoside}_2 & \rightarrow \text{Nucleoside} \\
\{\text{diphosphate}\} & \rightarrow \text{ADP} + \text{UTP} \\
\text{ATP} + \text{UDP} & \leftrightarrow \text{ADP} + \text{UTP} \\
\text{ATP} + \text{GDP} & \leftrightarrow \text{ADP} + \text{GTP} \\
\text{ATP} + \text{CDP} & \leftrightarrow \text{ADP} + \text{CTP}
\end{align*}
\]

All of these triphosphates take part in phosphorylations in the cell. Similarly, nucleoside monophosphate kinases, specific for each purine or pyrimidine nucleoside, catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.

Thus, adenylate kinase is a specialized diphosphate kinase.

\[
\text{ATP} + \text{Nucleoside}\overset{(\text{P})}{\rightarrow} \text{ADP} + \text{Nucleoside}\overset{(\text{P})}{\rightarrow}\]

**Specific nucleoside diphosphate kinase.**
Fig. aside x above: Overview of Relationship of cellular components and energy yielding reactions of respiration.

"INTER METABOLIC RELATIONSHIPS"
The energy change of the ATP system.

Response of ATP generating ATP-utilizing reaction to

ATP-utilizing

ATP-generating

- ATP regulation

Energy change of ATP system
ATP - (Adenosine Triphosphate)

The Currency of Cell

Ball & Stick Model of Adenosine Triphosphate
AN OVERVIEW :-

- Introduction
- Structure
- Properties - Its high energy status
- Central Role of ATP-ADP system
- Modes of energy yielding metabolism
- Definition and nature of fermentation
- Respiration
- Photosynthesis
- Comparative account of these 3 modes
- Role of ATP in flagellar movement
**INTRODUCTION:**

ATP was first isolated from acid extracts of muscle in 1929 by Fiske and Subbarow. The structure was deduced some years later by degradation experiments and ultimately confirmed by total chemical synthesis by Todd and his colleagues in 1948. From its first discovery, ATP was suspected to play a role in cellular energy transfer, but it was not until 1939-1941 that Kipmann proposed it serves as a principal means of transfer of chemical energy in the cell.

**STRUCTURE:**

ATP and ADP have the following structures:

![ATP and ADP structures]
(a) Fig. (i) STRUCTURE OF ADENYLIC ACID AND PHOSPHATE DERIVATIVES ADP AND ATP.

(b) Big (ii) Isotope Exchange Experiment showing that enzyme-bound ATP is formed from ADP and P_i in the absence of a proton motive force.
ATP is the "universal fuel" of the living cell.

It contains two high energy phosphate bonds (\(\gamma\)) and each stores about 12,000 calories and releases about 7,500 calories when broken.

ATP is produced by two series of reactions:

1. An aldhyde reacts with an inorganic phosphate to give hydrogen and an acid phosphate.

\[
\text{R-CHO + H}_2\text{PO}_4^- \rightarrow 2\text{H} + \text{R-C-O-P-OH} + \text{H}_2\text{O}
\]

2. The acid phosphate reacts with ADP to give an organic acid and ATP.

\[
\text{R-C-O-P-OH + ADP + H}_2\text{O \rightarrow R-C-OH} + \text{ATP}
\]

ATP due to its high energy bonds and \(\gamma\) groups is able to donate number of \(\gamma\) groups to a number of metabolic linkages, thereby converting them to activated forms.

Their increased free energy allows a phosphorylatic intermediate to participate in biosynthetic reactions.

The special reactivity of the high energy bonds of ATP is apparent when \(\Delta G^\circ\) (free energy) of their hydrolysis is compared with the \(\Delta G^\circ\) of hydrolysis of the phosphate of AMP attached to adenosine by an ester linkage. Therefore less reactive and formed as low energy bonds.

\[
\text{Adenosine} - (\text{P}) \rightarrow (\text{P}) + \text{H}_2\text{O} \rightarrow \text{Adenosine} - (\text{P}) + \text{P}
\]

\[
\text{ADP} \cdot \Delta G^\circ = 3.7 \text{ kcal}
\]
The chemiosmotic theory of proton electrochemical coupling.

One proton pump: ATP hydrolysis drives protons across the membrane. ATP buildup up on the inside side of the cell. ATP synthesis.

Equilibrium: between energy derived from ATP hydrolysis and energy needed to pump protons against the gradient.

Coupling

ATP as energy carrier

Fig (iii) - "ATP as energy carrier"

Second proton pump: this replenishes the proton gradient so that ATP synthesis can continue.

ATP utilization:

- ATP hydrolysis drives ion movement.
- Protons move down the proton gradient.
- ATP synthesis occurs as a result.

Coupling

ADP + Pi → ATP

High energy phosphates bonds

- O-P̄̄̄̄̄̄O-P̄̄̄̄̄̄O-P̄̄̄̄̄̄O-CH
- C-C-C-C-C-C
- OH ON

Ribose

Adenine
Adenosine ← $\text{C} \Rightarrow \text{C} + \text{H}_2\text{O} \rightarrow \text{Adenosine} ← \text{C} + \text{H}_2\text{O}

\begin{align*}
\Delta G^\circ &= -7.3 \text{ kcal}, \\
\text{AMP} &\quad \text{AMP}
\end{align*}

**Properties:**

(c) Its high energy status: ATP as energy carrier.

- Chemical reactions are coupled through common intermediates
  - Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is the substrate for the second.
  - **Example:**
    
    $\text{A} + \text{B} \rightarrow \text{C} + \text{D}$
    
    $\text{D} + \text{X} \rightarrow \text{Y} + \text{Z}$
    
    Here, **D** is the common intermediate.

- Because humans are isothermal, the only way in which energy can be transferred between chemical reactions for them to have a common intermediate that links them. In the example given above, **D** could be a carrier of chemical energy between the two reactions.

- ATP serves as a carrier of chemical energy between high energy phosphate donors and low-energy phosphate acceptors because it is a common intermediate in both energy delivering and energy requiring reactions of the cell (fig 1 to 3).
(Figure 1)

Some High Energy Compounds:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphocreatine</td>
<td><img src="image" alt="Phosphocreatine Structure" /></td>
</tr>
<tr>
<td>Phosphoethanolpyruvate</td>
<td><img src="image" alt="Phosphoethanolpyruvate Structure" /></td>
</tr>
<tr>
<td>Glucose - 6-phosphate</td>
<td><img src="image" alt="Glucose - 6-phosphate Structure" /></td>
</tr>
<tr>
<td>Glycerol - 3-phosphate</td>
<td><img src="image" alt="Glycerol - 3-phosphate Structure" /></td>
</tr>
</tbody>
</table>
**N-B Energy Change.**

The relative amount of high energy forms of ATP (ATP, ADP) can be calculated using the following formula:

\[ EC = \frac{1}{2} \left[ \frac{[ADP]}{[ATP]} + 2 \left[ \frac{[AMP]}{[ADP]} + \frac{[ADP]}{[ATP]} \right] \right] \]

- **Note:** If all adenine phosphates are ATP, \( EC = 1.0 \); if all AMP, \( EC = 0.5 \); if all ADP + ATP = AMP, \( EC = 0.5 \).
- **EC** vs % maximum reaction rate
- **The two roles for ATP**

**Free Energy and ATP.**

How does the energy in ATP specifically get utilized to power reactions in metabolism?

- **First Law: In any process, the total energy of the system and the surroundings remains constant.** Energy is not created nor destroyed, only transformed from one form to another.
- **Second Law: In any process, the entropy of the system and the surroundings increases.** Entropy is often thought of as disorder or randomness.

**The Ultimate Driving Machine:**

A new value for predicting the direction of chemical reactions

- **Free Energy:**
  - \( \Delta G = \Delta H - T \Delta S \)
  - \( \Delta G < 0 \): reaction is spontaneous
  - \( \Delta G > 0 \): reaction is not spontaneous
  - \( \Delta G = 0 \): reaction is at equilibrium

\[ \Delta G = \Delta G^\circ + RT \ln \left( \frac{[C]}{[A]} \right) \]
Liver mitochondria are incubated in the presence of glutamate. The rate of oxygen uptake from the medium measured by oxygen electrode, is initially low. As ADP is added, respiration speeds up until the ADP is phosphorylated to ATP. The latter can be measured as esterification of P:O. If all of the ADP is esterified, the P/O ratio is 0.00/2.0 = 2.7.
8. The energy carried by ATP is stored in its two terminal phosphate groups.

- ATP is composed of a molecule of adenosine to which three phosphate groups are attached. When one phosphate is removed, ADP (adenosine diphosphate) is produced; if two phosphates are removed, AMP (adenosine monophosphate) results.

- At physiological pH, ATP is highly negatively charged, having a total of three or four negative charges on its phosphate. ATP therefore forms a stable complex with Mg²⁺ and Mn⁺⁺

- The standard free energy of hydrolysis, ΔG°, is approximately -7000 cal/mole for each of the two terminal phosphate groups. Because of this large negative ΔG°, ATP is called a high energy phosphate compound.

- Compounds exist that contain phosphates with an energy higher than that of ATP. These very high compounds include phospho enol pyruvate, 1,3-bisphosphoglycerate, and phosphocreatine, all of which have a standard free energy of hydrolysis greater than -10,000 cal.

- Other phosphate containing compounds have low energy phosphates which have standard free energy of hydrolysis of less than -1000 cal. These include glucose-6-phosphate, glycerol-3-phosphate, and AMP.

- ATP thus occupies an intermediate position on the bioenergetic chain of phosphate containing compounds. ATP can serve as...
ATP carries energy between high and low energy compounds.

Very high energy phosphates donors:

- Phosphoenolpyruvate: -14.8 kcal
- 1,3-Bisphosphoglycerate: -11.4 kcal
- Phosphocreatine: -10.5 kcal

Energy transferred to ATP

ATP

Then to phosphate acceptors:

- Glucose-6-phosphate: -7.9 kcal
- Glycerol phosphate: -3.1 kcal

Low energy phosphates
an acceptor of phosphate groups from cellular phosphates containing higher energy phosphates. ATP can donate these phosphates to compounds in the cell forming phosphates of lower energy (TPP). There are no enzymes in cells that can transfer phosphate groups directly from very high-energy donors to low-energy acceptors without their first being transferred to ATP.

(c) **ATP IS THE UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS.**

- The central role of ATP in energy exchanges in biological systems was perceived by Fritz Lipman and by Herman Kalckar in 1941.

- ATP is a nucleotide consisting of an adenine ribose and a triphosphate unit. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety ATP is an rich molecule because its triphosphate unit contains two phosphoanhydride bonds.

\[
\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{P}_i + \text{H}^+ \quad (\Delta G^\circ = -7.3 \text{ kcal/mol})
\]

\[
\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{AMP} + \text{PP}_i + \text{H}^+ \quad (\Delta G^\circ = -7.3 \text{ kcal/mol})
\]

- ATP, AMP and ADP are interconvertible. The enzyme adenylate kinase (myokinase) catalyzes the reaction.

\[
\text{ATP} + \text{AMP} \rightleftharpoons \text{ADP} + \text{ADP}
\]

The free energy liberated in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy, such as muscle contraction. In vivo, ATP is formed from ADP and a glucose molecule are oxidized in chemotrophic or when light is trapped by phototrophs. This ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.
(Fig vi) Flow of phosphate groups from high-energy phosphate donors to low energy acceptors via ATP-ADP system.

- Phosphoenolpyruvate
- High energy P-donors
- ATP
- Phosphocreatine reservoir
- ~P
- ~P
- Low energy P-acceptors
- Glycerol phosphate

(Fig vii) The ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.

- Motion
- Active transport
- Biosynthesis
- Signal amplification
- Oxidation of fuel molecules
- Photosynthesis
CENTRAL ROLE OF ATP-ADP CYCLE

- ATP is continuously formed and consumed.

- ATP serves as the principle immediate donor of free energy in biological systems rather than as a long term storage form of free energy.

- In a typical cell, an ATP molecule is consumed within a minute following its formation. The turnover of ATP is very high.

- Phototrophs harvest the free energy in light to generate ATP, whereas chemotrophs obtain ATP by the oxidation of fuel molecules.

- In effect, an ATP/ADP cycle connects those processes which generate ~P to those processes that utilize ~P.

The processes that proceed ~P into this cycle involve:

(i) From reactions catalyzed by ATP synthetase which effectively reverses the hydrolysis of ATP.

(ii) Oxidative phosphorylation.

(iii) Embden-Meyerhof-Parnas pathway.

(iv) Incorporation of 1,3-diphosphoglycerate which after dehydrogenation forms 1,3-bisphosphoglycerate.
Transfer of high energy phosphate from intermediates of EMP to ADP.

1,3-Biphosphoglycerate

\[ \text{CO}_2 - \text{O} - \text{P} \]
\[ \text{H} - \text{C} - \text{OH} \]
\[ \text{CH}_2 - \text{O} - \text{P} \]

Phosphoglycerate kinase

ADP
ATP

\[ \text{COOH} \]
\[ \text{H} - \text{C} - \text{OH} \]
\[ \text{CH}_2 - \text{O} - \text{P} \]

3-Phosphoglycerate

\[ \text{COOH} \]
\[ \text{C} - \text{OH} \]
\[ \text{CH}_2 \]

Pyruvate kinase

ADP
ATP

\[ \text{COOH} \]
\[ \text{C} - \text{OH} \]
\[ \text{CH}_2 \]

(ENOL) Pyruvate

Spontaneous

\[ \text{COOH} \]
\[ \text{C} = \text{O} \]
\[ \text{CH}_3 \]

Keto (Pyruvate)
Fig IX: Role of ATP/ADP cycle in transfer of high energy phosphate. It is to be noted that ~P does not exist in a free state but is transferred in the reactions shown.
Yield of ATP from alcoholic fermentation.
Modes of Energy Yielding Metabolism.

Generation of ATP is the fundamental mechanics by which some free energy can be trapped.

In fact, most is dissipated in the form of heat. The role of ATP in coupling energy to biosynthesis is summarised in the figure (X).

COMPARISON OF FERMENTATION/RESPIRATION

- Fermentation: Dissimilation and Definition.
  
  In the strict sense, the term fermentation refers to those energy-yielding pathways in which organic compounds act as both electron donors and electron acceptors.

  During fermentation, microorganisms obtain energy from organic compounds without utilizing oxygen.

  The process of fermentation takes place in two stages:

  1. Glucose is broken down to pyruvate with the release of two pairs of hydrogen atoms.

  2. Pyruvate or compounds derived from pyruvate are reduced by the hydrogen atoms released in the first stage.

   **Fermentation**

   - Lactic acid fermentation
     - Homolactic fermentation
     - Heterolactic fermentation
   - Alcoholic fermentation

Diagram:

- Fermentation
  - Lactic acid
    - Homolactic fermentation
    - Heterolactic fermentation
  - Alcoholic fermentation
The respiratory chain and the sites of entry of electrons from various substrates. Also shown the probable sites of energy conservation leading to ATP formation.

The decline in free energy as electron pairs flow down the respiratory chain to oxygen. Each of the three segments denoted in color yields sufficient energy to generate a molecule of ATP from ADP and phosphate.
**Respiration**

- Respiration is another major energy yielding react.
- Oxidative phosphorylation
- Electron transport chain

### Partial reactions of oxidative phosphorylation:

1. **ATPase activity**
   
   \[
   \text{ATP} + \text{H}^+ + \text{H}^2 \text{O} \rightarrow \text{ADP} + \text{Pi} + \text{P} \text{E} \text{P} \text{A} \text{c} \text{i} \text{d} \text{e}
   \]
   
2. **ATP Phosphorylation**

   \[
   \text{AMP} + \text{P} + \text{P} \rightarrow \text{AMP} + \text{P} \text{O} \text{4} + \text{Pi}
   \]

3. **Phosphorylation by nicotine**

4. **ADP - ATP exchange**

   \[
   ^{14} \text{C} \text{AMP} + \text{P} + \text{P} \rightarrow ^{14} \text{C} \text{AMP} + \text{P} \text{O} \text{4} + \text{Pi}
   \]

### Synthesis by ETC.

- ATP is synthesized by transporting electrons through a series of molecules, with fixed orientation, in the cell membrane.

#### Microbial metabolic process including respiration—Photosynthesis:

<table>
<thead>
<tr>
<th>Primary donor</th>
<th>Carrier 1 (red)</th>
<th>Carrier 2 (red)</th>
<th>Carrier 3 (red)</th>
<th>Red acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized donor</td>
<td>Carrier 1 (red)</td>
<td>Carrier 2 (red)</td>
<td>Carrier 3 (red)</td>
<td>Terminal electron acceptor</td>
</tr>
</tbody>
</table>

- Each number of the chain is capable of being reduced by reacting with the carrier molecule that proceeds it and oxidized by the carrier that follows it.
Schematic illustration of the coupled processes of electron transport and oxidative phosphorylation. Using the proton motive force of the electrochemical proton gradient generated by the pumping of protons across the mitochondrial inner membrane, ATP synthase catalyzes the synthesis of one ATP molecule for each pair of protons pumped out. In this way, 3 molecules of ATP are made for the 5 pairs of electrons pumped out as one pair of electrons is transported through the respiratory electron chain to oxygen.

(Fig XIX)

Exchange of ADP and ATP across the inner mitochondrial membrane by the ATP carrier. The carrier is inhibited by heavy ions of oxaloacetic acid, which bears some resemblance to the ATP molecule.

(Fig XIX)

Oxidative phosphorylation of ADP.

(* Phosphorylation of ADP and accumulation of cations are alternative processes during electron transport."

---

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Photosynthesis

Schematic Representation for electron transport in photosynthetic bacteria under aerobic anaerobic conditions:

(Cis-Vi) light

NAD$^-\rightarrow$ NADH + H$^+$

Aniosphate $\rightarrow$ ATP

Sulfate $\rightarrow$ ATP

$\rightarrow$ ATP

ATP $\rightarrow$ ADP

NADH + H$^+$

PUMARATE $\rightarrow$ SUCCINATE

PAD

NAD$^+$ + H$^+$

The time of electrons in cyclic phosphorylation in the photosynthetic light reactions, only ATP is produced.
(a) Effect of pH on ΔG of hydrolysis of ATP (25°C)

(b) Proton motive force drives flagella

(c) Channeling of high energy phosphate groups into different biosynthetic pathways via the ribonucleoside diphosphates and ribonucleoside monophosphates.
Role of ATP in Flagellar Movement:

- Bacterial flagella filaments appear to have no machinery for converting chemical and mechanical energy. For example, flagellin, the flagellar protein molecule, has no enzymatic activity and no detectable ATPase activity, such as is present in eukaryotic flagella of unicellular microorganisms (Fig. xvi).

  (Fig. xvii) aside.

- It is therefore a misconception that in prokaryotic flagellar movement ATP plays a role. Rather, here proton motive force comes into play.

- Generally in eukaryotic ciliary and flagellar movement ATP may play a significant role.

Summary:

- Energy changes of chemical reactions can be analyzed quantitatively in terms of the first and second laws of thermodynamics, which are combined into the equation $\Delta G = \Delta H - T \Delta S$. Under conditions in which biological reactions occur, i.e., at constant temperature and pressure, chemical reactions proceed in such a direction that at equilibrium, the entropy of the system plus surroundings is at a maximum and the free energy of the system alone is at a minimum. Every chemical reaction has a characteristic standard free energy $\Delta G$ of the system alone, at a minimum standard temperature and pressure, with all reactants and products at 1 M conc and $\rho^{0} = 1$.

- ATP is the energy currency of cell.
- ATP is generated by Respiration, photosynthetic and fermentation.
- ATP is vital for all biological life processes.
(o) Fig. (xxi) Schematic Representation of the Functions of ATP.
Numbers in parentheses refer to reactions in Table given aside.
(Fig xxii) Bonds designated by  are characterized by large negative $\Delta G^\circ$ of hydrolysis. $N$ refers to a nucleoside.
<table>
<thead>
<tr>
<th>Reaction or reaction type</th>
<th>Example of stoichiometry</th>
<th>Nature of reactant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative phosphorylation</td>
<td>$A_{red} + B_{ox} + P_i + ADP \rightarrow A_{ox} + B_{red} + ATP$</td>
<td>Unknown</td>
</tr>
<tr>
<td>Photosynthetic phosphorylation</td>
<td>$A_{red} + B_{ox} + ADP + P_i \overset{h^+}{\rightarrow} A_{ox} + B_{red} + ATP$</td>
<td>Unknown</td>
</tr>
<tr>
<td>Glycolysis - Phosphorylation</td>
<td>$\begin{align*} &amp; \text{D- Glycerol dehydrogenase} - \text{D- phosphate} + P_i + \text{NAD}^+ \ &amp; \text{I, D- diphaspho- D- glycerate} + \text{NADH} \ &amp; \text{I, D- diphaspho- D- glycerate} + \text{ADP} \ &amp; \text{D- Phospho- D- glycerate} + \text{ATP} \end{align*}$</td>
<td>$- \text{C} - \text{O} -$</td>
</tr>
<tr>
<td>Enolase (Phosphoenolpyruvate hydratase)</td>
<td>$2\text{-phospho-D- glycerate} \rightarrow \text{phosphoenolpyruvate} + \text{P}_i$</td>
<td>$\text{H} - \text{C} - \text{O} -$</td>
</tr>
<tr>
<td>$\alpha$- Oxoglutarate dehydrogenase</td>
<td>$\text{K- oxoglutarate} + \text{NAD}^+ + \text{CoASH} \rightarrow \text{Succinyl-SCoA} + \text{NADH}$</td>
<td>$\text{O} - \text{P} - \text{OH}$</td>
</tr>
<tr>
<td>Plus succinyl-CoA synthetase</td>
<td>$\begin{align*} &amp; \text{Succinyl-SCoA} + \text{ADP} + \text{P}_i \rightarrow \text{Succinyl-ATP} + \text{CoASH} \ &amp; \text{GTP} + \text{ADP} \rightarrow \text{GDP} + \text{ATP} \end{align*}$</td>
<td>$\text{0} - \text{C} - \text{O} -$</td>
</tr>
<tr>
<td>Various kinases (ATP : donor phosphotransferases)</td>
<td>$\text{ATP} + \text{Acetyl-CoA} \rightarrow \text{Acetylphosphate} + \text{ADP} + \text{H}_2\text{O}$</td>
<td>$\text{C} - \text{O}$</td>
</tr>
<tr>
<td>&amp; $\text{ATP} + \text{Creatine} \rightarrow \text{Creatine phosphate} + \text{ADP(U)}$</td>
<td>$\text{C} - \text{N}$</td>
<td></td>
</tr>
<tr>
<td>Acyl transfersases (CoA-phosphate - transferase)</td>
<td>$\text{Acetyl phosphate} + \text{HSCoA} \rightarrow \text{acetyl-SCoA} + \text{P}_i$</td>
<td>$\text{C} - \text{O}$</td>
</tr>
<tr>
<td>Various synthetase (X:R)</td>
<td>$\text{ATP} + \text{L-glutamate} + \text{L-cysteine} \rightarrow \text{L-glutamyl-L-cysteine} + \text{ADP} + \text{P}_i$</td>
<td>$\text{C} - \text{N}$</td>
</tr>
<tr>
<td>Nucleoside diphosphate kinases (ATP : nucleoside diphosphate)</td>
<td>$\text{ATP} + \text{NDP} \rightarrow \text{ADP} + \text{NTP}$</td>
<td>$\text{P} -$</td>
</tr>
<tr>
<td>Various nucleosidyl transferases</td>
<td>$\text{ATP} + \text{FMN} \rightarrow \text{FAD} + \text{P}_i$</td>
<td>$\text{C} - \text{P} - \text{O}$</td>
</tr>
</tbody>
</table>
(a) Fig (axlii) Donor Functions of ATP

**Class II A:**

- Adenosine-5'-P
- Class III
- N9-adenine

**Class II A2:**

- Adenosine-5'-P = 0

(b) Fig (axlii)

<table>
<thead>
<tr>
<th>Class ATP acts as donor of</th>
<th>Formation of</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>II. Phosphodiesterase (phospho-phosphatases, 2.7.2.1)</td>
<td>A. Hydride or equivalent (Enzyme + ATP = Enzyme + ADP)</td>
<td>Acetate + ATP $\rightarrow$ Acetate + ADP</td>
</tr>
<tr>
<td>III. Pyrophosphatase (pyrophosphatases, 2.7.1.6)</td>
<td>A. Acceptor pyrophosphate</td>
<td>D-glyceraldehyde + ATP $\rightarrow$ D-glyceraldehyde + ADP</td>
</tr>
<tr>
<td>IV. Adenosine-3'-phosphate</td>
<td>A. Enzyme-bound acyl adenylate and transfer to acceptor (6.11.4.2)</td>
<td>ATP $\rightarrow$ ADP + P;</td>
</tr>
<tr>
<td></td>
<td>B. Dimnucleotide coenzymes (2.7.2)</td>
<td>$\text{ATP} + \text{R} \rightarrow \text{AMP} + \text{P;}$</td>
</tr>
<tr>
<td></td>
<td>C. Polyadenylate (2.7.2.4)</td>
<td>$[\text{ATP} + \text{R} \rightarrow \text{AMP} + \text{P};$</td>
</tr>
<tr>
<td></td>
<td>S-adenosyl methionine (2.4.2.12)</td>
<td>ATP + methionine $\rightarrow$ S-adenosyl methionine + trimetaphosphate</td>
</tr>
</tbody>
</table>

**Driving force for reaction**

- Synthetases and ligases (class 6)
<table>
<thead>
<tr>
<th>Reaction or reaction type</th>
<th>Example of deamination</th>
<th>Example of restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C_2R = C-O-AMP] + Hz</td>
<td>[C_2R = C-O-R]</td>
<td>[C_2R = C-O-AMP] + Hz</td>
</tr>
</tbody>
</table>
Diseases -
Their Mini Definitions

Blood Grouping

Sampath Chakraborty
B.Sc II Yr
Roll 17
REFERENCES

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I. **Blood Staining:**

**Diseases & Their Mini Definitions.**

- **Neutrophils:**
  - Increase: (1) **Septic Endocarditis**
  - Increase in number → (2) **Pus Formation**

**Septic Endocarditis:** *(Kahr′-di′-tis)*

- Exudative and proliferative inflammation alteration of the endocardium,
- Usually characterized by the presence of vegetations on the surface of the endocardium (within the heart) or in the endocardium itself, and most commonly involving heart valve, but also affecting the inner lining of the cardiac chambers or the endocardium elsewhere.
- **Causal organisms:** *Streptococci, Staphylococci, Enterococci*
- Gonococci & Gram negative bacilli.

**Pus Formation:**

- A protein rich liquid inflammation product made up of cells (leukocytes) a thin fluid (liquefied pus) & cellular debris.
- **Causal organism:** *Strep pyogenes, Staph aureus, etc.*
- **Scarlet Fever**
  - An acute disease caused by Group A β-hemolytic streptococci, marked by pharyngitis and a skin rash caused by an erythrogenic toxin produced by the organism.
  - The rash is a diffuse, bright red erythema and desquamation, the skin begins as fine scaling with eventual peeling of the palms and soles.

- **Leukopenia**
  - Reduction in the number of leukocytes in the blood below about 5000 per cubic mm. Basophilic Leukopenia pertains to Basophilia.

- **Ricketsial Disease**
  - Caused by Rickettsia.

- **Increase in number of Eosinophils**
  - SCARLET FEVER

- **Increase in Basophils**
  - Basophilia
  - Viral Infection
  - Leukopenia

- **Increase in number of Monocytes**
  - RICKETSIAL DISEASE
  - Rocky Mountain Spotted Fever.
Rocky Mountain spotted fever

- Infection with Rickettsia rickettsii
- Transmitted by ticks; marked by fever, muscle pain, & weakness followed by a macular petechial (red spot due to escape of small amount of blood) eruption that begins on the hands & feet & spreads to the trunk and face, with other symptoms in the CNS & elsewhere.
BLOOD GROUPING

The basis of human ABO isoantigens and blood types.

→ The existence of human blood types was first demonstrated by an Austrian pathologist, Karl Landsteiner in 1904.
→ While studying incompatibilities in blood transfusions, he found that the serum of one person could clump the red blood cells of another.
→ Landsteiner identified four distinct types, subsequently called the ABO blood groups.
→ Like the MHC antigens on white blood cells, the ABO isoantigen markers on red blood cells are genetically determined and composed of glycoproteins.
→ These ABO antigens are inherited as two (one from each parent) of three alternative alleles: A, B, and O.
→ A & B alleles are dominant over O and codominant with one another.
→ This mode of inheritance gives rise to four blood types (phenotypes), depending on the particular combination of genes.
→ Thus, a person with an AA or AO genotype has Type A blood; genotype BB or BO gives Type B; genotype AB produces Type AB, and genotype OO produces Type O.

Important points about blood types.
They are named for the dominant antigens.
The RBCs of type O persons have antigens, but not A & B antigens.
TABLE 1

**CHARACTERISTICS OF ABO BLOOD GROUPS.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype A antigen</th>
<th>Prevalence in Population**</th>
<th>Serum Content of Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>Neither</td>
<td>Most common</td>
<td>Both anti A &amp; anti-B</td>
</tr>
<tr>
<td>AA, AO</td>
<td>A</td>
<td>Second most common</td>
<td>Anti-B</td>
</tr>
<tr>
<td>BB, BO</td>
<td>B</td>
<td>Third most common</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>Least common</td>
<td>Neither antibody</td>
</tr>
</tbody>
</table>

**Legend:**
- Capital letters generally denote antigen; lowercase denotes antibody.
- True of most large population of mixed racial & ethnic groups.

**FIG 1. AGGLUTINATION REACTIONS CONTROLLED BY THE ABO BLOOD TYPE LOCUS IN HUMANS:**

- Legend: Red blood cells (erythrocytes) of the type indicated at the top of each slide are mixed with blood serum of the type indicated below each reaction mixture (circle).
- A clumped pattern of cells within a circle indicates that agglutination occurs.

- Group O: Anti B Serum + Anti A Serum
- Group A: Anti B Serum + Anti A Serum
- Group B: Anti B Serum + Anti A Serum
- Group AB: Anti B Serum + Anti A Serum
Tissues other than RBC's carry A & B antigens.

GENETIC BASIS: ABO BLOOD TYPE ALLELES IN HUMANS

Table 2: Genotypes & their corresponding Phenotypes (Blood group types) for the ABO locus (human)

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>PHENOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I^A I^B</td>
<td>A</td>
</tr>
<tr>
<td>I^B I^B</td>
<td>B</td>
</tr>
<tr>
<td>I^A I^O</td>
<td>AB</td>
</tr>
<tr>
<td>I^O I^O</td>
<td>O</td>
</tr>
</tbody>
</table>

One of the most firmly established series of multiple alleles in humans involves the genetic locus controlling the blood types A, B, AB & O.

The ABO locus has three common alleles: I^A, I^B, & I^O. I^A & I^B are codominant; I^O is recessive (i.e., I^O homozygotes have no ABO antigens on their RBC's; I^A I^O & I^B I^O heterozygotes have A & B antigens, respectively, on their RBC's).

The ABO locus controls the type of glycolipids found on the surface of erythrocytes, apparently by specifying the type of glycosyl transferases (enzymes catalyzing the synthesis of oligosaccharides) synthesized in the RBC's.

The specific types of glycolipids on the red cell surface provide the antigenic determinants that react with specific antibodies present in the blood serum.

Humans, like all other mammals, produce antibodies & circulate them in the blood serum as a defense mechanism against foreign substances.

Fortunately, no antibodies are synthesized in normal individuals.
### TABLE 3.
**Blood Transfusion Compatibilities for the ABO Blood Groups.**

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Terminal Sugars of Antigens Present</th>
<th>Antibodies Present</th>
<th>Red Cell Types Agglutinated</th>
<th>Transfusions Accepted From</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A (galactosamin)</td>
<td>Anti-B</td>
<td>B, AB</td>
<td>A or O</td>
</tr>
<tr>
<td>B</td>
<td>B (galactose)</td>
<td>Anti-A</td>
<td>A, AB</td>
<td>B or O</td>
</tr>
<tr>
<td>AB</td>
<td>A (galactosamin)</td>
<td>None</td>
<td>None</td>
<td>A, B, AB, AB + O</td>
</tr>
<tr>
<td>O</td>
<td>None</td>
<td>Anti-A &amp; Anti-B</td>
<td>A, B and AB</td>
<td>O</td>
</tr>
</tbody>
</table>

**Fig. 2.**

**Legend:** In this test, a drop of blood is mixed with a specially prepared antiserum known to contain antibodies against the A, B, and Rh antigens.

**Fig. 2:** If that particular ag is not present, the R.B.C.'s in that droplet do not agglutinate and form an even suspension.
|-----------|----------|

- that react with antigens present on the individual's own cells. However, when type A blood & type B blood are mixed, the anti-A antibodies in the type B blood serum react with the antigens on the type A blood cells, & vice versa, which produces clumping of cells (fig 60).

- Cross-matching blood types to determine compatibility is thus essential in blood transfusions.

- In this process, blood donors and recipients are tested for the presence of antigens & antibodies that are incompatible.

- Table (iii) summarizes the cell surface antigens determine. & the serum antibodies present in the four major ABO blood types.

- Individuals with blood type AB have both A & B antigens on their erythrocytes, but no anti-A & anti-B antibodies in their blood serum.

- Type O individuals lack both ags, but carry both anti-A & anti-B abs in their blood serum.

- **Type O individuals are referred as UNIVERSAL DONORS**, type O blood can be used in transfusions for individuals of any blood type if the blood is introduced slowly enough to permit sufficient dilution of the Anti-A & Anti-B abs present in the serum of the donor.

- Type AB persons are consequently called **UNIVERSAL RECEPTORS**.

**DEGREES OF ADVERSE REACTIONS IN TRANSFUSIONS:**

Transfusion of the wrong blood type causes various degrees of adverse reaction.
Legend:

(a) Incompatible blood. The red blood cells of the type A donor contain ag A, while the serum of the type B recipient contains anti-A ags that can agglutinate donor cells.

(b) Agglutination particles can block the circulation in vital organs.

(c) Activation of the complement by ab on the RBC's can cause haemolysis & anaemia. This sort of incorrect transfusion is very rare because of the great care taken by blood banks to ensure a correct match.
The severest reaction is massive hemolysis when the donated red blood cells react with recipient antibody & trigger the complement cascade (fig 3).

The resultant destruction of red cells leads to systemic shock & kidney failure brought on by the blockage of glomeruli (blood filtering apparatus) by cell debris.

Death is a common outcome.

Other reactions caused by RBC destruction are fever, anemia & jaundice.

A transfusion reaction is managed by immediately halting the transfusion, administering drugs to remove hemoglobin from the blood, and beginning another transfusion with RBC's of the correct type.

A CLOSER APPROACH
MICROFILE: THE ORIGIN OF AB0 ANTIGENS

The A and B genes each code for an enzyme that adds a terminal carbohydrate to RBC receptors during maturation. RBC's of Type A contain an enzyme that adds N-acetyl glucosamine to the receptor; RBC's of Type B have an enzyme that adds D-galactose; RBC's of Type AB contain both enzymes that add both carbohydrates, and RBC's of Type 0 lack the genes & enzymes to add a terminal molecule.

The genetics of AB0 ags were once used to rule out paternity. For eg., if a man is Type A, the mother type O, & the child type B, we know this man could not have fathered this child.

However, this same logic cannot prove paternity. If the child is type A instead, it is this same logic for the man to believe...
Fig 4.
GENETIC BASIS FOR AB AGS ON RBC.

Type A

Type B

Type AB

Type O

Code Guide:

Terminal sugar

common receptor

RBC of Type A and B do not have the antigens A and B on their surfaces. RBC of Type AB have both A and B antigens on their surface. RBC of Type O do not have A or B antigens on their surface but can have other antigens.
Highly sensitive methods based on specific & variable NH

other, but so could some other man with blood like a

ringer, evidence of paternity or maternity. The

DNA fingerprinting have been developed to gather

at a much earlier age, but no indication.
METABOLISM

ATP

Name: Saswati Chakraborty
Std: B.Sc III Yr
Roll: 17

Adenosine Triphosphate
<table>
<thead>
<tr>
<th>References</th>
</tr>
</thead>
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AN OVERVIEW

- Definitions of metabolism.
- Catabolism and Anabolism.
- Classification of micro-organisms on the basis of energy carbon sources.
- Bioenergetics.
- Coupling through ATP and through pyridine nucleotides.

INTRODUCTION: [Definition.]

The term metabolism denotes all the organized chemical activities performed by a cell which comprise two general types:

- energy production
- energy utilization

The term intermediary metabolism is a rather incomplete definition which only highlights a mere sum total of all the enzymatic reactions occurring in the cell.

Four specific functions of metabolism are:

1. To extract chemical energy from the environment, either from:
   - organic nutrients or from sunlight.
2. To convert exogenous nutrients into the building blocks or precursors of the macromolecular components of cells.
3. To assemble the building blocks into proteins, nucleic acids, lipids,
(4) To form and degrade those biomolecules required in specialized functions of cells.

**TYPES OF METABOLIC REACTIONS**

- **Catabolism**: It is the enzymatic degradation, largely by oxidative reactions, of relatively large nutrient molecules (carbohydrates, nucleic acid, fats, lipids, proteins) coming either from the environment or from the cell or from its own reserves, finally reducing them to a series of smaller, simpler molecules and releasing energy, e.g., lactic acid, acetate, ammonia, and urea.

- **Anabolism**: Synthesis of relatively large molecular components of cells, e.g., polysaccharides, nucleic acid, proteins, and lipids from simple precursor molecules.

(5) Catabolism is accompanied by the release of free energy inherent in the complex structure of large organic molecules and the decrease in entropy. It requires input of free energy which is furnished by the phosphate bond energy of ATP.
Fig 2: (c) CATABOLISM, ANABOLISM, AMPHIBOLISM
(The 3 stages.)
AMPHIBOLIC PATHWAY:

Although the pathways of catabolism and anabolism are not identical, the stage III (Fig. 2) constitutes a central meeting ground or pathway which is accessible to both. This central route, is called an amphibolic pathway (amphi- dual).

CLASSIFICATION OF M.O.S ON THE BASIS OF ENERGY CARBON SOURCES:

1> Division on the basis of utilization of carbon source.

- **Autochroms**
  - Autotrophs: self-trophos, photosynthesis
  - Chemolithotrophs

- **Heterotrophs**
  - Hetero + other trophos: heterotrophs

2> Division on the basis of energy source

- **Phototrophs**
  - Phototrophs: photosynthesis

- **Chemotrophs**
  - Chemoorganotrophs
  - Chemo lithotrophs

3> Division on the basis of oxidizing agent for nutrient breakdown

- **(Aerobic)**
  - Those which grow in presence of O₂

- **(Anaerobic)**
  - Those which grow in absence of O₂
Fig 3 (c) The increase of entropy:

- Copper blocks: Initial state
  - WARM
  - COOL

  Equilibrium state
  - Heat spontaneously flows from warm body to cool body.

- Initial state
  - Fine particles

  Equilibrium state
  - Gas molecules flow from zone of high pressure to zone of low pressure.

The increase of entropy or randomness in two physical systems such flows never reverse spontaneously.

---

Fig (d) (i) Summary of Free Energy and Entropy:

**THE SURROUNDINGS**

The entropy of the surroundings may increase, stay constant or decrease.

**THE SYSTEM**

(Conservation of P, V, T)

- The entropy of the system alone may increase, stay constant or decrease, but its free energy always decreases to a minimum.
Bioenergetics:
(Terminologies Involved).

1. **Entropy**: Entropy is defined (for the moment) as the degree of disorder or randomness. \[ S \]

2. **Equilibrium**: An equilibrium is defined as a state in which no further net chemical or physical change is taking place and in which temperature, pressure and concentration are uniform throughout the system.

All "real" processes occurring in our physical world including the processes of life are irreversible.

3. **Free Energy**: Entropy changes during chemical reactions are not always easily measured or calculated. However, the change in entropy during a process is quantitatively related to changes in total energy of the system by a function called the free energy. \[ \Delta G \]

4. **Enthalpy**: The change in function is known as enthalpy.

5. **Important Equations**:
   - \( \Delta G = \Delta H - T \Delta S \)
   - \( \Delta H = \Delta E + \Delta PV \)
   - \( \Delta G = \Delta E + T \Delta S \)
   - \( \Delta E = \Delta G + T \Delta S \)
"Coupling - Reaction"

Coupling of an exergonic to an endergonic reaction → Fig (6) (o)

Coupling of dehydrogenation and hydrogenation reactions by an intermediate carrier → Fig (6b)

Fig (7) → Transference of free energy from an exergonic to an endergonic reaction through the formation of a high energy intermediate compound. (e)

(Exergonic Reactions)

Syntheses
Muscular contraction
Nervous excitation
Active transport

Fig (8) → Transduction of energy through a common high-energy compound to energy-requiring (endergonic) biologic processes.
**Bioenergetics of Coupled Reactions**

- **Coupling - ATP & Pyridine Nucleotides**
  - First reaction in the glycolytic pathway.
  - The phosphorylation of glucose to glucose 6-P, which is highly endergonic and would not proceed as such under physiological conditions.

1. \( \text{Glucose} + P_i \rightarrow \text{Glucose 6-P} + H_2O \)  
   \( \Delta G^0' = +12.8 \text{ KJ/mol} \)

2. \( \text{ATP} \rightarrow \text{ADP} + P_i \)  
   \( \Delta G^0 = -36.8 \text{ KJ/mol} \)
   - Reaction couple with another reaction (hydrolysis of the terminal phosphate of ATP) that is more exergonic.

3. \( \text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose 6-P} + \text{ADP} \)  
   \( \Delta G^0' = -23.8 \text{ KJ/mol} \)

**Interconversion of Adenine Nucleotides**

- The enzyme adenylic kinase (myokinase) is present in most cells. It catalyzes the interconversion of ATP & AMP on the one hand and ADP on the other.

4. \( \text{Adenosine} \rightarrow \text{AMP} \rightarrow \text{AMP} \rightarrow \text{Adenosine} \)  
   \( \text{Adenylic Kinase} \rightarrow \text{Adenosine} \rightarrow \text{AMP} \rightarrow \text{AMP} \)
   \( \text{AMP} \rightarrow \text{AMP} \rightarrow \text{Adenosine} \)

5. When ATP reacts to form AMP, inorganic pyrophosphate (PPi) is formed, as occurs - [activation of long chain fatty acids].

6. \( \text{ATP} + \text{COA} \cdot \text{SH} + \text{R} \cdot \text{COOH} \xrightarrow{\text{Phosphorlyase}} \text{AMP} + \text{PPi} \cdot \text{R} \cdot \text{COA} \cdot \text{COA} \)  
   \( \Delta G^0' = -4.6 \text{ Kcal/mol} \)

7. \( \text{PPi} + H_2O \xrightarrow{\text{inorganic pyrophosphatase}} 2P_i \)
(5) Fig (5) - Phosphate cycles and interchange of adenine nucleotides.
A combination of the above reactions makes it possible for phosphate to be recycled and the adenine nucleotides to interconvert (fig. 1).

**Nucleoside Phosphates Related to ATP & ADP.**

By means of the enzyme nucleoside diphosphate kinase, nucleosides triphosphates similar to ATP but containing a different base from adenine, can be synthesized from their diphosphates e.g.

\[
\text{Nucleoside} \rightarrow \text{Nucleoside diphosphate} \rightarrow \text{Nucleoside triphosphate}
\]

- \[ \text{ATP} + \text{UDP} \rightarrow \text{ADP} + \text{UTP} \]
- \[ \text{ATP} + \text{GDP} \rightarrow \text{ADP} + \text{GTP} \]
- \[ \text{ATP} + \text{CDP} \rightarrow \text{ADP} + \text{CTP} \]

All of these triphosphates take part in phosphorylations in the cell. Similarly nucleoside monophosphate kinases, specific for each purine or pyrimidine nucleoside, catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.

Thus adenylate kinase is a specialized diphosphate kinase.
Hexose monophosphate or pentose phosphate pathway:

- Glucose
- Starch
- NADPH
- NADP
- ATP
- ADP
- Triose phosphate
- ADP
- ATP
- NAD
- NADPH
- NADH
- Phenolic compounds
  - Caffeine, tyrosine, tryptophan
- Anthocyanins
- Indole phytohormones
  - Indole-3-acetic acid (IAA)
- Ethanol
- Lactic acid
- Alanine (amino acid)
- Acetyl CoA
- Pyruvate

Phosphoenolpyruvate:

- NAD
- NADH
- ADP
- ATP

Protein:

Cell wall components:
- Cellulose, hemicellulose, xylo-
- Avellane, galactans, mannans, pectic substances.

Glycerol (of triglycerides for oils, fats and other lipids)

Amino acids (serine, cysteine)

Isoprenoids (chlorophyll, phytol, tach, carotenoids)

Phytohormones (side chain of cytokinins, gibberellins, abscisic acid)

Steroids

Cuticular compounds

Fatty acids

Protein
Fig. aside above: – Overview of Relationship of cellular components and energy yielding reactions of respiration –

"INTER METABOLIC RELATIONSHIPS"
ATP - (Adenosine Triphosphate)

The Currency Of Cell

Ball & Stick Model of Adenosine Triphosphate
ADENOSINE TRIPLE PHOSPHATE 

AN OVERVIEW:-

- Introduction
- Structure
- Properties - its high energy status
- Central role of ATP-ADP system
- Modes of energy yielding metabolism
  - Definition and nature of fermentation
  - Respiration
  - Photosynthesis
- Comparative account of these 3 modes
- Role of ATP in flagellar movement
INTRODUCTION:

ATP was first isolated from acid extracts of muscle in 1929 by Fiske and Subbarow. The structure was deduced some years later by degradation experiments and ultimately confirmed by total chemical synthesis by Todd and his colleagues in 1948. From its first discovery, ATP was suspected to play a role in cellular energy transfer, but it was not until 1939-1941 that Lipmann proposed it serves as a principal means of transfer of chemical energy in the cell.

STRUCTURE:

ATP are phosphate-transferring coenzymes having the following structure:

\[
\begin{align*}
N &= \text{C} - \text{NH}_2 \\
\text{H} &\quad \text{H} &\quad \text{H} &\quad \text{H} &\quad \text{H} &\quad \text{OH} &\quad \text{OH} &\quad \text{OH} \\
\text{N} &= \text{C} - \text{N} &\quad \text{C} - \text{C} &\quad \text{C} - \text{C} &\quad \text{C} - \text{C} &\quad \text{O} &\quad \text{P} &\quad \text{O} - \text{P} &\quad \text{O} - \text{P} &\quad \text{O} \\
\text{H} &\quad \text{H} &\quad \text{H} &\quad \text{H} &\quad \text{H} &\quad \text{O} &\quad \text{O} &\quad \text{O} \\
\end{align*}
\]

(ADP)
(a) Fig (i)
STRUCTURE OF
ADENYLC ACID
AND PHOSPHATE
DERIVATIVES ADP
AND ATP.

(b) Fig (ii)
Isotope Exchange Experiment showing that enzyme-bound
ATP is formed from ADP and Pi in the absence of a proton
motive force.
ATP is the "universal fuel" of the living cell.

It contains two high energy phosphate bonds (\( \sim \)) and each stores about 12,000 calories and releases about 7,800 calories when broken.

ATP is produced by two series of reactions:

1. An aldehyde reacts with an inorganic phosphate to give hydrogen and an acid phosphate.

\[
\begin{align*}
R-CHO + H_2PO_4^- &\rightarrow 2H + R-C=O - P-OH + H_2O \\
\end{align*}
\]

2. The acid phosphate reacts with ADP to give an organic acid and ATP.

\[
\begin{align*}
R-C=O - P-OH + ADP + H_2O &\rightarrow R-C=O - OH + ATP \\
\end{align*}
\]

ATP due to its high energy bonds and PO\(_4\) groups is able to donate number of PO\(_4\) groups to a number of metabolic linkages, thereby converting them to activated forms.

Their increased free energy allows a phosphatidic intermediate to participate in biosynthetic reactions.

The special reactivity of the high energy bonds of ATP is apparent when \( \Delta G^0 \) (free energy) of their hydrolysis is compared with the \( \Delta G^0 \) of hydrolysis of the phosphate of AMP attached to adenosine by an ester linkage. Therefore less reactive and formed as low energy bonds.

Adenosine - \( \sim P \sim \sim + H_2O \rightarrow \text{Adenosine} - \sim P + P \)

\( \text{ADP} \quad \Delta G^0 = -7.3 \text{ kcal} \)
The chemiosmotic theory of proton electrochemical coupling.

- One proton pump: ATP hydrolysis drives protons across the membrane, building up a potential gradient that drives ATP synthesis.

- Equilibrium: a proton pump is needed to pump protons against their gradient.

- Figure (iii) - "ATP AS ENERGY CARRIER"
Adenosine $\rightarrow$ \( \text{AM}^{+} \text{P} \rightarrow \text{AMP} \rightarrow \text{ADP} \rightarrow \text{ATP} \) 

\( \Delta G_0^\circ = -7.3 \text{kcal} \)

---

**Properties:**

- Its high energy status: ATP as energy carrier.

**A. Chemical reactions are coupled through common intermediates**

- Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is the substrate for the second.
  
  e.g. given the reactions 
  
  \[ A + B \rightarrow C + D \]
  
  \[ D + X \rightarrow Y + Z \]

  Here \( D \) is the common intermediate.

- Because humans are isothermal, the only way in which energy can be transferred between chemical reactions for them to have a common intermediate that links them. In the example given above, \( D \) should be a carrier of chemical energy between the two reactions.

- ATP serves as a carrier of chemical energy between high energy phosphate donors and low energy phosphate acceptors because it is a common intermediate in both energy delivering and energy requiring reactions of the cell (fig 7-3).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphocreatine</td>
<td><img src="image" alt="Phosphocreatine" /></td>
</tr>
<tr>
<td>Phosphoanhydride</td>
<td><img src="image" alt="Phosphoanhydride" /></td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td><img src="image" alt="Glucose-6-phosphate" /></td>
</tr>
<tr>
<td>Glycerol-3-phosphate</td>
<td><img src="image" alt="Glycerol-3-phosphate" /></td>
</tr>
</tbody>
</table>
**N-B ENERGY CHANGE.**

The relative amount of high energy forms of ATP (ATP, ADP) can be calculated using the following formula:

\[ EC = \frac{1}{2} \frac{[ADP]}{[ATP]} + 2 \frac{[AMP]}{[AMP] + [ADP] + [ATP]} \]

Note that if all adenine phosphates are ATP, \( EC = 1 \); if all AMP
\( EC = 0 \); if all ADP + ATP = AMP, \( EC = 0.5 \)

- **EC vs % maximum reaction rate**
- **The two roles for ATP**

---

**FREE ENERGY AND ATP.**

How does the energy in ATP specifically get utilized to power reactions in metabolism?

- **The laws of Thermodynamics - First law:** In any process, the total energy of the systems and the surroundings remains constant. Energy is not created nor destroyed; however, it can be transformed from one form to another.

- **Second law:** In any process, the entropy of the system and the surroundings increases. Entropy is often thought of as disorder or randomness.

---

**THE ULTIMATE DRIVING MACHINE.**

- A new value for predicting the direction of chemical reactions

Free energy:

\[ 
D\eta = \Delta H - T \Delta S 
\]

- \( \Delta H \): enthalpy change
- \( \Delta S \): entropy change
- \( T \): absolute temperature

\[ D\eta = \Delta H - T \ln \left( \frac{[C][D]}{[A][B]} \right) \]
Liver mitochondria are incubated in the presence of glutamate. The rate of O₂ uptake from the medium measured by oxygen electrode, is initially low. By ADP is added, respiration speeds up until the ADP is phosphorylated to ATP. The latter can be measured as esterification of P; if all of the ADP is esterified, the P/O ratio is 3.0/2.0 = 1.5.
The energy carried by ATP is stored in its two terminal phosphate groups.

- ATP is composed of a molecule of adenine to which three phosphate groups are attached. If one phosphate is removed, ADP (adenosine diphosphate) is produced; if two phosphates are removed, AMP (adenosine monophosphate) results.

- At physiological pH, ATP is highly negatively charged, having a total of three or four negative charges on its phosphates. ATP therefore forms stable complexes with Mg²⁺ and Mn²⁺.

- The standard free energy of hydrolysis, ΔG°, is approximately -7300 cal/mole for each of the two terminal phosphate groups. Because of this large negative ΔG°, ATP is called a high energy phosphate compound.

- Compounds exist that contain phosphates with an energy higher than that of ATP. These very high energy compounds include phosphocreatine, 1,3-bisphosphoglycerate, and phosphodiesters, all of which have a standard free energy of hydrolysis greater than -10,000 cal.

- Other phosphate-containing compounds have low energy phosphates, which have a standard free energy of hydrolysis of less than -1000 cal. These include glucose-6-phosphate, fructose-6-phosphate, and AMP.

- ATP thus occupies an intermediate position on the bioenergetic scale of phosphate-containing compounds. ATP can serve as
ATP carries energy between high and low energy compounds.

Very high energy phosphates donors:
- Phosphoenolpyruvate: -14.8 kcal
- 1,3-Bisphosphoglycerate: -11.9 kcal
- Phosphocreatine: -10.6 kcal

Energy transferred to ATP:

Low energy phosphates:
- Glucose-6-phosphate
- Glycerol phosphate

$\Delta G^\circ$ (standard free energy of hydrolysis of phosphates) in kcal/mol
an acceptor of phosphate groups from cellular phosphates containing higher energy phosphates. ATP can donate these phosphates to compounds in the cell forming phosphates of lower energy ($Q^p$). There are no enzymes in cells that can transfer phosphate groups directly from very high-energy donors to low-energy acceptors without their first being transferred to ATP.

(c) ATP IS THE UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS.

- The central role of ATP in energy exchanges in biological systems was perceived by Fritz Lipman and by Hermann Kalckar in 1941.
- ATP is a nucleotide consisting of an adenine ribose and a triphosphate unit. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety. ATP is an rich molecule because its triphosphate unit contains two phosphoanhydride bonds.

\[
\begin{align*}
\text{ATP} + \text{H}_2\text{O} & \rightarrow \text{ADP} + \text{Pi} + \text{H}^+ \quad (\Delta G^\circ = -7.3 \text{ kcal/mol}) \\
\text{ATP} + \text{H}_2\text{O} & \rightarrow \text{AMP} + \text{PPi} + \text{H}^+ \quad (\Delta G^\circ = -7.3 \text{ kcal/mol})
\end{align*}
\]

- ATP, AMP and ADP are interconvertible. The enzyme adenylate kinase (amp kinase) catalyzes the reaction:

\[
\text{ATP} + \text{AMP} \rightarrow \text{ADP} + \text{ADP}
\]

The free energy liberated in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy such as muscle contractility. In vivo ATP is formed from ADP plus a carbon fuel molecule that are oxidized in chemotrophs or when light is trapped by phototrophs. This ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.
(Fig vi) → (4) Flow of phosphate groups from high-energy phosphate donors to low-energy acceptors via ATP-ADP system.

- Phosphoenolpyruvate
- High energy P-donors
- ATP
- Low energy P-acceptors
- Glycerol 3-phosphate
- Phosphocreatine reservoir

(Fig vii) →

- Motion
- Active transport
- Biosynthesis
- Signal amplification

ATP → ADP

Photosynthetic or oxidation of fuel molecules

(5) The ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.
**CENTRAL ROLE OF ATP-ADP CYCLE**

- ATP is continuously formed and consumed.
  - ATP serves as the principle immediate donor of free energy in biological systems rather than as a long term storage form of free energy.
  - In a typical cell, an ATP molecule is consumed within a minute following its formation. The turnover of ATP is very high.
  - Motion, active transport, signal amplification, and biosynthesis can occur only if ATP is continuously regenerated from ADP.

- Phototrophs harvest the free energy in light to generate ATP, whereas chemotrophs form ATP by the oxidation of fuel molecules.

- In effect, an ATP/ADP cycle connects those processes which generate \( \sim P \) to those processes that utilize \( \sim P \).

- The processes that feed \( \sim P \) into this cycle involves:
  1. From reactions catalyzed by ATP synthase which actively reverses the hydrolysis of ATP.
  2. Oxidative Phosphorylation.
  3. Embden-Meyerhof Pathway.
  4. Incorporation of \( \sim P \) into 3-phosphoglyceraldehyde which after dehydrogenation forms 1,3-biphosphoglycerate.
Transfer of high energy phosphate from intermediates of EMP to ADP.

1,3-Biphosphoglycerate

Phosphoglycerate kinase

ADP → ATP

S-phosphoglycerate

Phosphoenolpyruvate

Pyruvate kinase

ADP → ATP

Pyruvate

Spontaneous

α-Ketoglutarate
(Fig iv) - Role of ATP/ADP cycle in transfer of high energy phosphate. It is to be noted that ⊳ P does not exist in a free state but is transferred in the reactions shown.
**Catabolism**

**Energy Source**
- ATP
- ADP
- Heat

**Metabolic Products**

**Biosynthesis**
- Biopolymers (e.g., proteins)
- Intracellular pools of precursors

**Photosynthesis**
- Light
- Photosynthetic machinery

**Yield of ATP from Alcoholic Fermentation**

Glucose

\[ 2(ADP + P_i) + 2ATP \rightarrow 2NADH + 2H^+ \]

Pyruvate

\[ \text{Pyruvate} \rightarrow 2\text{CO}_2 + 2\text{NADH} + 2\text{H}^+ \]

Acetaldehyde

\[ \text{H} - \text{C} = \text{O} + \text{CH}_3 \]

Ethanol

\[ \text{CH}_2 - \text{OH} + \text{CH}_3 \]

Alcohol dehydrogenase
Modes of Energy Yielding Metabolism.

- Generation of ATP is the fundamental mechanisms by which some free energy can be trapped.
- In fact, most is dissipated in the form of heat. The role of ATP in coupling energy to biosynthesis is summarised in the figure below.

Comparison of Fermentation/Respiration

**Fermentation vs. Respiration**
- **Fermentation** is the process by which microorganisms obtain energy from organic compounds without utilizing oxygen.
- **Respiration** is the process of using oxygen to break down organic compounds in the presence of oxygen.

During fermentation, microorganisms obtain energy from organic compounds without utilizing oxygen.

The process of fermentation takes place in two stages:

1. Glucose is broken down to pyruvate with the release of two pairs of hydrogen atoms.
2. Pyruvate or compounds derived from pyruvate are reduced by the hydrogen's released in the first stage.

### Fermentation

- **Alcoholic Fermentation**
- **Lactic Acid Fermentation**
- **Homolactic Fermentation**
- **Heterolactic Fermentation**
The respiratory chain and the points of entry of electrons from various substrates. Also shown is the probable site of energy conservation leading to ATP formation.

(Fig xil)

Pyruvate → Succinate → F0 → O2

Malate
Succinate
Oxaloacetate
α-Hydroxy acyl CoA
Fatty acyl CoA
Glyceraldehyde

(Fig xli)

The decline in free energy as electron pairs flow down the respiratory chain to oxygen. Each of the three segments denoted in color yields sufficient energy to generate a molecule of ATP from ADP and phosphate.

\[ E_0^{\text{NAD}} \rightarrow E_0^{\text{F1}} \rightarrow E_0^{\text{F2}} \rightarrow E_0^{\text{O2}} \]

Energy yield:

- NAD: 0.37 V or 13.7 kJ
- F1: 0.30 V or 12.6 kJ
- F2: 0.22 V or 9.0 kJ
- O2

Energy released:

- 0.63 V or 23.6 kJ
**Respiration**

- Respiration is another major energy yielding react.
- Oxidative phosphorylation
- Electron transport chain.

**Partial reactions of oxidative phosphorylation:**

1. **ATPase activation.**
   \[ ATP + H^+ + H_2O \rightarrow ADP + Pi \]

2. **ATP Phosphorylation.**
   \[ AMP + P + \text{isotope} \rightarrow AMP + P + \text{isotope} \]

3. **Phosphate water exchange.**
   \[ \text{HPO}_4^{2-} + H_2^{18}O \rightarrow \text{H}^{18}O^+ + \text{H}_2O \]

4. **ADP - ATP exchange.**
   \[ {^{14}}C \text{AMP} + P + \text{isotope} \rightarrow \text{ADP} + {^{14}}C \text{AMP} + P + \text{isotope} \]

**Synthesis from substrate level phosphorylation.**

- ATP is formed from ADP by transfer of an \( \Delta G^0 \) of \( \Delta G^0 \) ADP + P in substrate level phosphorylation.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{H}_2\text{O} \quad \text{CH}_2 \\
\text{COOH} & \quad \text{COOH} \\
2 \text{PGA} & \quad \text{P.E.P. Acid}
\end{align*}
\]

**Synthesis by ETC.**

- ATP is synthesized by transferring electrons through a series of molecules with fixed orientation in the cell membrane.

<table>
<thead>
<tr>
<th>Redox</th>
<th>Carrier</th>
<th>Carrier</th>
<th>Carrier</th>
<th>Red acceptor</th>
</tr>
</thead>
</table>
| I ox  | I red  | I ox   | III red | Terminal |}

- Each number of the chain is capable of being reduced by reacting with the carrier molecule that proceeds it and oxidized by the carrier that follows it.
Schematic illustration of the coupled processes of electron transport and oxidative phosphorylation. Using the proton motive force of the electrochemical proton gradient generated by the pumping of protons across the mitochondrial inner membrane, ATP synthase catalyzes the synthesis of one ATP molecule for each pair of protons pumped out. In this way, 3 molecules of ATP are made for the 5 pairs of electrons pumped out as one pair of electrons is transported through the respiratory carrier chain to oxygen.

Exchange of ADP and ATP across the inner mitochondrial membrane by the ATP carrier. The carrier is inhibited by a variety of agents that block the respiratory electron transport chain, which bears some resemblance to the ATP molecule.

Oxidative phosphorylation of ADP.
* Photosynthesis

Schematic Representation for electron transport in photosynthetic bacteria under aerobic and anaerobic conditions:

(Etq V1)

NADP + ADP + P → NADPH + ATP + H₂

(P800)

Light

NADPH + H⁺ → NAD⁺ + H⁺

(ETP) A TP + ADP + P → ADP + H⁺ + P → ATP

(Pummerate → Succinate)

PAD

NADH + H⁺ → (PMN)

NAD⁺ + H⁺

(0.8) + (0.8) Standard Redox Potentials (NAD/NADH)

The time of electrons in cyclic phosphorylation in the photosynthetic light reactions, only ATP is produced.
(c) Effect of pH on AG° of hydrolysis of ATP

Proton motive force drives flagella

Cell wall

Cell membrane

Rotary motor:

H⁺, H⁺, H⁺

~P ➔ ATP ➔ UTP ➔ ATP

Polysaccharides

~P ➔ GTP ➔ ATP ➔ ATP

Proteins

~P ➔ GTP ➔ ATP ➔ ATP

Lipids

~P ➔ GTP ➔ ATP ➔ ATP

RNA

DNA

Channeling of high energy phosphate groups into different biosynthetic pathways via the ribonucleotidase and deoxyribonucleotidase sides (2'-5' phosphodiesterase).
ROLE OF ATP IN FLAGELLAR MOVEMENT:

- Bacterial flagella filaments appear to have no machinery for interconverting chemical and mechanical energy. For example, flagellin, the flagellar protein molecule, has no enzymatic activity i.e. no detectable ATPase activity (such as in plant and flagella of unicellular microorganisms) (Fig xvi) - aside.

- It is therefore a misconception that in prokaryotic flagellar movement, ATP plays a role. Rather, here, proton motive force comes into play.

- Generally in eukaryotic cells, flagellar movement ATP may play a significant role.

SUMMARY:

- Energy changes of chemical reactions can be analyzed quantitatively in terms of the first and second laws of thermodynamics which are combined into the equation $\Delta G = \Delta H - T \Delta S$. Under conditions in which biological reactions occur i.e. at constant temperature and pressure, chemical reactions proceed in such a direction that at equilibrium the entropy of the system plus surroundings is at a maximum and the free energy of the system alone is at a minimum. Every chemical reaction has a characteristic standard free energy $\Delta G$ of the system alone at a maximum.

- Standard temperature and pressure with all reactants and products at 1 M concentration and $T = 298$ K.

- ATP is the energy currency of cells.

- ATP is generated by respiration, photosynthesis, and fermentation.

- ATP is vital for all biological life processes.
(b) Fig xxii Schematic Representation of ATP functions.
Numbers in parentheses refer to reaction(s) in Table given aside.
Bonds designated by ~ are characterized by large negative $\Delta G^{\circ}$ of hydrolysis. R refers to a nucleoside.
<table>
<thead>
<tr>
<th>Reaction or reaction type</th>
<th>Example of stoichiometry</th>
<th>Nature or function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative phosphorylation</td>
<td>$\text{A}<em>\text{red} + 2\text{B}</em>\text{ox} + \text{P}<em>\text{i} + \text{ADP} \rightarrow \text{A}</em>\text{ox} + 2\text{B}_\text{red} + \text{ATP}$</td>
<td>Unknown</td>
</tr>
<tr>
<td>Photosynthetic phosphorylation</td>
<td>$\text{A}<em>\text{red} + 2\text{B}</em>\text{ox} + \text{ADP} + \text{P}<em>\text{i} \xrightarrow{\text{hv}} \text{A}</em>\text{ox} + 2\text{B}_\text{red} + \text{ATP}$</td>
<td>Unknown</td>
</tr>
<tr>
<td>Triose-Phosphate dehydrogenase plus glyceraldehyde kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{D}-\text{glycerald} \rightarrow \text{B}-\text{phosphate} + \text{P}_\text{i} + \text{NAD}^+ \rightarrow \text{B}-\text{dipospho}-\text{D-} \rightarrow \text{Glyceraldehyde} + \text{ADP} \rightarrow \text{B}-\text{phospho}-\text{D-} \rightarrow \text{Glyceraldehyde} + \text{ATP}$</td>
<td></td>
</tr>
<tr>
<td>Enolase (Phosphoenolpyruvate hydratase)</td>
<td>$2\text{-Phospho}-\text{D-} \rightarrow \text{Glyceraldehyde} \rightarrow \text{phosphoenolpyruvate} + \text{H}_\text{2} \rightarrow \text{O}$</td>
<td>$\text{H} - \text{C} - \text{O}$-</td>
</tr>
<tr>
<td></td>
<td>$\text{Phosphoenol pyruvate} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP}$</td>
<td>$\text{O} - \text{C} - \text{O}$-</td>
</tr>
<tr>
<td>$\alpha$- Oxoglutarate dehydrogenase plus succinyl-CoA synthetase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$(\text{Succinyl}-5\text{-}\text{COA} + \text{ADP} + \text{P}<em>\text{i} \rightarrow \text{succinate} + \text{GTP} + \text{C} + \text{H}</em>\text{2} \rightarrow \text{O}$</td>
<td>$\text{O} - \text{P} - \text{OH}$</td>
</tr>
<tr>
<td></td>
<td>$\text{GTP} + \text{ADP} \rightarrow \text{GDP} + \text{ATP}$</td>
<td>$\text{O} - \text{C} - \text{O}$-</td>
</tr>
<tr>
<td>Acyl transferases (e.g. phospho- and transacylases)</td>
<td>$\text{Acetyl phosphate} + \text{H}<em>\text{2} \text{COA} \rightarrow \text{acetyl-SCOA} + \text{P}</em>\text{i}$</td>
<td>$\text{O} - \text{C} - \text{O}$-</td>
</tr>
<tr>
<td>Various synthetases (X:R)</td>
<td>$\text{ATP} + \text{L-glutamate} + \text{L-cysteine} \rightarrow \text{L-glutamyl-} \L_\text{cysteine} + \text{ADP} + \text{P}_\text{i}$</td>
<td>$\text{O} - \text{C} - \text{O}$-</td>
</tr>
<tr>
<td>Nucleoside diphosphate kinases (ATP: nucleoside, diphosphate phosphotransferase)</td>
<td>$\text{ATP} + \text{NDP} \rightarrow \text{ADP} + \text{NTP}$</td>
<td>$\text{O}$-</td>
</tr>
<tr>
<td>Various nucleotidyl transferases</td>
<td>$\text{ATP} + \text{FMN} \rightarrow \text{FAD} + \text{PP}_i$</td>
<td>$\text{O} - \text{P} - \text{O}$-</td>
</tr>
</tbody>
</table>
(c) Fig (xxii) Donor Functions of ATP

Class IV

Adenosine-5'-O
-0-P=O

Class II A2:

Class II A1:

Formations of

A. Acceptor pyrophosphate
   Transfer to acceptor (6,436.24)

B. Dimedone coenzymes (2.7.7)

C. Polyadenylate (2.7.7)

S. Adenosyl methionine (2.4.2.18)

Driving force for reaction

Example:

Acetate + ATP \xrightarrow{\text{acceptor pyrophosphate}} \text{AMP} + ADP

D-glucose + ATP \xrightarrow{\text{D-glucose-6-P}} \text{ADP} + \text{AMP}

ATP + D-ribose-5-P \xrightarrow{\text{AMP} + PP} \text{AMP}

ATP + NADPH \xrightarrow{\text{AMP} + PP} \text{AMP}

ATP + FAD \xrightarrow{\text{AMP} + PP} \text{AMP}

ATP + methionine \xrightarrow{\text{AMP} + PP} \text{AMP} + \text{methionine}
<table>
<thead>
<tr>
<th>Reaction or reaction type</th>
<th>Example of stoichiometry</th>
<th>Nature of ( \text{E} ) (Eq. ( \text{E} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various ligases (synthesis of groups 6-1 and 5-2, first step)</td>
<td>( \text{ATP} + \text{CH}_2 + \text{RO}_2^- \rightarrow \left[ \text{CH}_2.\text{R} - \text{C} - \text{O} - \text{AMP} \right] )</td>
<td>( \left[ \text{CH}_2.\text{R} - \text{C} - \text{O}^- \right] )</td>
</tr>
<tr>
<td>Various ligases (second step)</td>
<td>( \left[ \text{CH}_2.\text{R} - \text{C} - \text{O} - \text{AMP} \right] + \text{HZ} \rightarrow \text{AMP} + \text{R} - \text{C} - \text{O} - \text{X} )</td>
<td>( \left[ \text{CH}_2.\text{R} - \text{C} - \text{O}^- \right] )</td>
</tr>
<tr>
<td>ATP: D-ribose-5-phosphate kinase</td>
<td>( \text{ATP} + \text{D}-\text{ribose-5-phosphate} \rightarrow \text{AMP} + \text{P}_{\text{RPP}} )</td>
<td>( \text{CH}_2 - \text{O}^- )</td>
</tr>
<tr>
<td>Phosphoribosylpyrophosphate</td>
<td>( \text{PRP} + \text{O}_6\text{P} \rightarrow \text{orotidine-5-phosphate} )</td>
<td>( \text{CH}_2 - \text{O}^- )</td>
</tr>
<tr>
<td>Various NMP pyrophosphohydrolases, pentosylamin-P</td>
<td>( \text{PRP} + \text{O}_6\text{P} \rightarrow \text{orotidine-5-phosphate} )</td>
<td>( \text{CH}_2 - \text{O}^- )</td>
</tr>
<tr>
<td>Various ligases (glycolysis)</td>
<td>( \text{ATP} + \text{L}-\text{pantoate} + \beta-\text{alanine} \rightarrow \text{L}-\text{pantoate} + \text{AMP} + \text{PP} )</td>
<td>( \text{C} - \text{O}^- )</td>
</tr>
</tbody>
</table>
**Introduction:**

- Ricker et al (1948) isolated Vit B12 from liver concentrate.
- Halo bacterium salinar in animal protein assay for the treatment of pernicious anemia.
- Recovery of Vit B12 from Streptomyces griseus.

**Microorganism used:**

- **Actinomyetes:** Nocardia spp, Streptomyces albidosflavus, 
  - **Bacteria:** Aerobacter aerogenes, Bacillus megaterium, Halo bacterium salinar, Pseudomonas spp, etc.

**Uses:**

- Human and animal nutrition (medicine and microbiology).
- When introduced in stock feed at 10 - 15 mg/ton it increases the reversible protein utilization of poultry, swine etc.
- It increases body weight considerably.
- Stimulates appetite & growth rate in children.
- Effective in treatment of pernicious anemia.

**Other method:**

1. Mycelium separated from whole beer suspended in water.
2. Reducing the pH suspension to 5 with H2SO4.
3. Heating it to boiling.
4. Centrifuging / Filtering.
5. Evaporating the liquor to stir up under vacuum.
6. Drying.
7. Vit B12 / 100 mg / final product.
Ready Reference Pattern: Trump Card

Title: Vitamin B12 (Pribolamide) production

No. of Books/Journals/Websites Referenced: 6

**SCREENING:** Bacillus megaterium; Streptomyces spp. etc.

**POSSIBLE GENETIC ENGINEERING:**
B. megaterium NRRL-933; S. olivaceus NRRL-B-1125

**LABORATORY SCALE**

**PILOT PLANT**

FERMENTER

**AERATION AND AGITATION**
Vigorous aeration with 
steam air 0.25-0.5 vol/vol of medium/min

**PROCESS CONTROL**
- pH = 7.3
- Temp = 28°C
- Duration = 4-6 days
- Stirring rate = 250 rpm for 1.5-2 hrs

**SEPARATION:** Centrifugation & filtration whole beans for 70-90 mins

**ISOLATION & PURIFICATION OF PRODUCT**

**FORMULATION, PACKAGING, STORAGE & MARKETING**

**DISTRIBUTION**

**SCALING UP**

**MEDIA COMPONENTS**
- Dextrose
- Peptide
- Yeast extract
- Calcium chloride
- Soyabean oil
- pH

**Fermentation Waste Heat**

**DOWNSTREAM PROCESSING**
Cell (Biomass) - Solid is separated by drying in an atmosphere at 120°C

**Fermentation Procedure:**
1. INTRODUCTION:— Penicillin is the first antibiotic produced on large scale. It is active against *G*+ve bacteria but rarely against *G*-ve ones. It hampers cell wall synthesis and is almost non-toxic to mammals except for the allergic reactions.

2. HISTORY:— It was first observed by Sir Alexander Fleming (in 1929) accidentally while studying air microflora. He observed Pen-P produced by *Penicillium notatum*. Later Chain et al. (in 1940) and Abraham et al. (in 1941) published their observations.

3. PENICILLIN MOLECULE & PRECURSORS (STRUCTURES):—

<table>
<thead>
<tr>
<th>Precursor</th>
<th>R-Side Chain</th>
<th>Pen-Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₆H₅CH₂COOH</td>
<td>Pen-G</td>
<td>CH₃C=NH-CHCH₃</td>
</tr>
<tr>
<td>Phenyl acetic acid</td>
<td>Benzyl pen</td>
<td></td>
</tr>
<tr>
<td>HOOC-C₆H₄CH₂COOH</td>
<td>Pen-V</td>
<td></td>
</tr>
<tr>
<td>Hydroxy phenylacetic acid</td>
<td>Hydroxy benzyl penicillin</td>
<td></td>
</tr>
<tr>
<td>C₆H₅CH₂COOH</td>
<td>Pen-F</td>
<td></td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>Dihydro penicillin</td>
<td></td>
</tr>
<tr>
<td>CH₃CH₂CH=CHCH₂</td>
<td>Pen-K</td>
<td></td>
</tr>
</tbody>
</table>

4. STRAINS USED:— *P. notatum*; *P. crysogenum* (from mouldy fruits) mutagenic agents (X-rays, UV-rays, MBA etc) → 8 – 176. Recently, still high yielding strains have been discovered.
5. FERMENTATION PROCEDURE: 5-1 MEDIA INOCULATION MEDIA:

<table>
<thead>
<tr>
<th>KOFFLER'S SPORLAN MED.</th>
<th>FOXSTERS et al.'S MED.</th>
<th>JACKSONS MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone:</td>
<td>Dextrose</td>
<td>Corn steep liquor:</td>
</tr>
<tr>
<td>- 5 g</td>
<td>- 30 g</td>
<td>- 3.5 g</td>
</tr>
<tr>
<td>Sugar beet molasses</td>
<td>NaNO₃</td>
<td>lactose</td>
</tr>
<tr>
<td>- 5 g</td>
<td>- 0.6 g</td>
<td>- 3.5 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>K₂HPO₄</td>
<td>Glucose</td>
</tr>
<tr>
<td>- 4 g</td>
<td>- 1.5 g</td>
<td>- 1.8 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>MgSO₄·7H₂O</td>
<td>CaCO₃</td>
</tr>
<tr>
<td>-0.1 g</td>
<td>- 0.5 g</td>
<td>- 1.0 g</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>CaCl₂</td>
<td>K₂HPO₄</td>
</tr>
<tr>
<td>- 0.05 g</td>
<td>- 0.25 g</td>
<td>- 0.4 g</td>
</tr>
<tr>
<td>Agar - agar</td>
<td>D/D</td>
<td>Edible oil</td>
</tr>
<tr>
<td>- 15 g</td>
<td>- 1.5 g</td>
<td>- 0.025 g</td>
</tr>
<tr>
<td>D/D</td>
<td></td>
<td>D/W</td>
</tr>
<tr>
<td>- 11 g</td>
<td></td>
<td>- 0.005 g</td>
</tr>
</tbody>
</table>

(1946) (1946) (1958)

5.2 PREPN & INOCUN OF INOCULUM

1. Suspension of spores (from heavily sporulated cultures) in water.
2. Add spores in nutrient solution.
3. Incubate for 5-7 days at 84°C for spores to germinate.
4. Inoculate directly in tank.
5. Incubate for 24 hrs - 48 hrs in tank and maintain agitation for spore growth.

Note: avoid contamination.
Do not add foresores.

CHARACTERISTIC CHANGES IN 3 PHASE OF PEN. PROD:

<table>
<thead>
<tr>
<th>CONDITIONS</th>
<th>PHASE I (Growth)</th>
<th>PHASE II (Mature)</th>
<th>PHASE III (Decline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pen production pH</td>
<td>slight shift high</td>
<td>maximal</td>
<td>slight decrease</td>
</tr>
<tr>
<td>Mycelium length</td>
<td>short</td>
<td>rapid growth high N content</td>
<td>slow growth low N content</td>
</tr>
<tr>
<td>Liquid and Ammonia</td>
<td>high</td>
<td>released into medium slow release</td>
<td>utilized non stable</td>
</tr>
<tr>
<td>Nitrates</td>
<td>slow</td>
<td>used slowly</td>
<td>non used</td>
</tr>
<tr>
<td>NaN₃</td>
<td>slow</td>
<td>used slowly</td>
<td>non used</td>
</tr>
<tr>
<td>Sodium P</td>
<td>slow</td>
<td>used slowly</td>
<td>non used</td>
</tr>
</tbody>
</table>

Screening: Penicillin

GENETIC ENG: NRRL 1981; G-176 etc.

Scaling up

Lab / Scale

Pilot Plant

Aerobic Digestion

Media

Sterilization 121°C for 20 min

Waste Heat

DISTRI BUTTON PROGRESS

Formulaion; Packaging & Marketing

CELL BIOMASS

Downstream Process

Isolation & Purification

Waste Heat

Liquid

LIPID

4
**Introduction & History:**

- **Bulany** (1957) - by shaken flask technique obtained 400 mg/ml Mo's: Gluconobacter sp. & Usitago mayeis.
- **First amino acid on a commercial scale by form**
- **Casida (1956)**: Escherichia coli auxotrophic mutant (now: ATCC 12408). Indirect method: DAP → L-lysine.
- **Other D.A.P producing strains**
  - Corynebacterium diphtheria; Mycobacterium tuberculosis (Dr. Wark 1951).
  - E. coli auxotrophic mutant (Barri 1952).

**Method: Submerged culture technique**

**Indirect or Dual fermentation**

**Escherichia coli**

- **L-lysine**
- **E. coli auxotrophic mutant (now: ATCC 12408)** lacks: lysine deaminase & x-DAP deaminase

**Inoculation (1-5%)**

- **Inoculation media**: Glycerol 6%, CSL 4%, (NH₄)₂HPO₄ 0.5%, pH 7-7.5 slightly alkaline with KOH

**Fermentation media**: Glycerol 6%; CSL 4%; (NH₄)₂HPO₄ 0.5%; CaO 0.5%

**Accumulation of DAP**

- **Optimal yield**: 9 mg/ml
- **Conditions**: DAP accumulation → Nα-DAP → L-lysine
- **Separation**: Centrifugation

**Process control**

- **Temp**: 28°C; pH 7-7.5
- **20 lb/sq in² pressure duration - 3 days.

**Conditions**: - pH 7-7.5
- Temp: 28°C, 14 hrs
- Aeration: 15 l/s
The Direct Method (Kimoshita et al. 1958)

**Micrococcus glutaminus**

- **Base medium:**
  - Glucose: 1.5% (NH₄)₂SO₄: 1.5%
  - KH₂PO₄: 0.05%
  - NaCl: 0.05%
  - MgSO₄: 7H₂O: 0.025%
  - CaCO₃

- **Cultural med.:**
  - Glucose: 10%, (NH₄)₂SO₄: 3%
  - CaCO₃: 2%
  - L-histidine: 400 mg/l
  - Proteins: 75 mg
  - KH₂PO₄: 0.1%
  - MgSO₄: 7H₂O: 0.008%
  - Lmethionine: 300 mg

**Duration:** 4 days

**Fermentation:**
- Black strap molasses + Hydrolysed soybean
- pH 5.5
- temp: 28°C
- Duration: 60 days

**Condition:**
- kg Cl₂
- (NH₄)₂SO₄
- temp: 28°C
- Duration: 60 days

**Acidity:**
- The clarified broth with hydrochloric acid

**Absorption of l-lysine in an ion exchange column in ammonium form
**

**Reaction with HCl**

**Crystallization of l-lysine hydrochloride**

**Marketing:**
- yield: 1.5-2.0 g/l
- 40-45 ml/l
- (starting with 90 ml/l: increased to 160 ml/l of sugar)

Uses of L-lysine:
- Food supplement
- Growth factor in number of strains which prevent protein deficiency diseases (Keshavan-ker)

Important Features:
- Enzyme mix encourages the free strains
- Bacterial culture must be in excess
- Penicillin addition inhibits glutamine production in the homoserine-less strain