BIOTECHNOLOGY –PRINCIPLES AND PROCESSES-2

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Processes of Recombinant DNA Technology

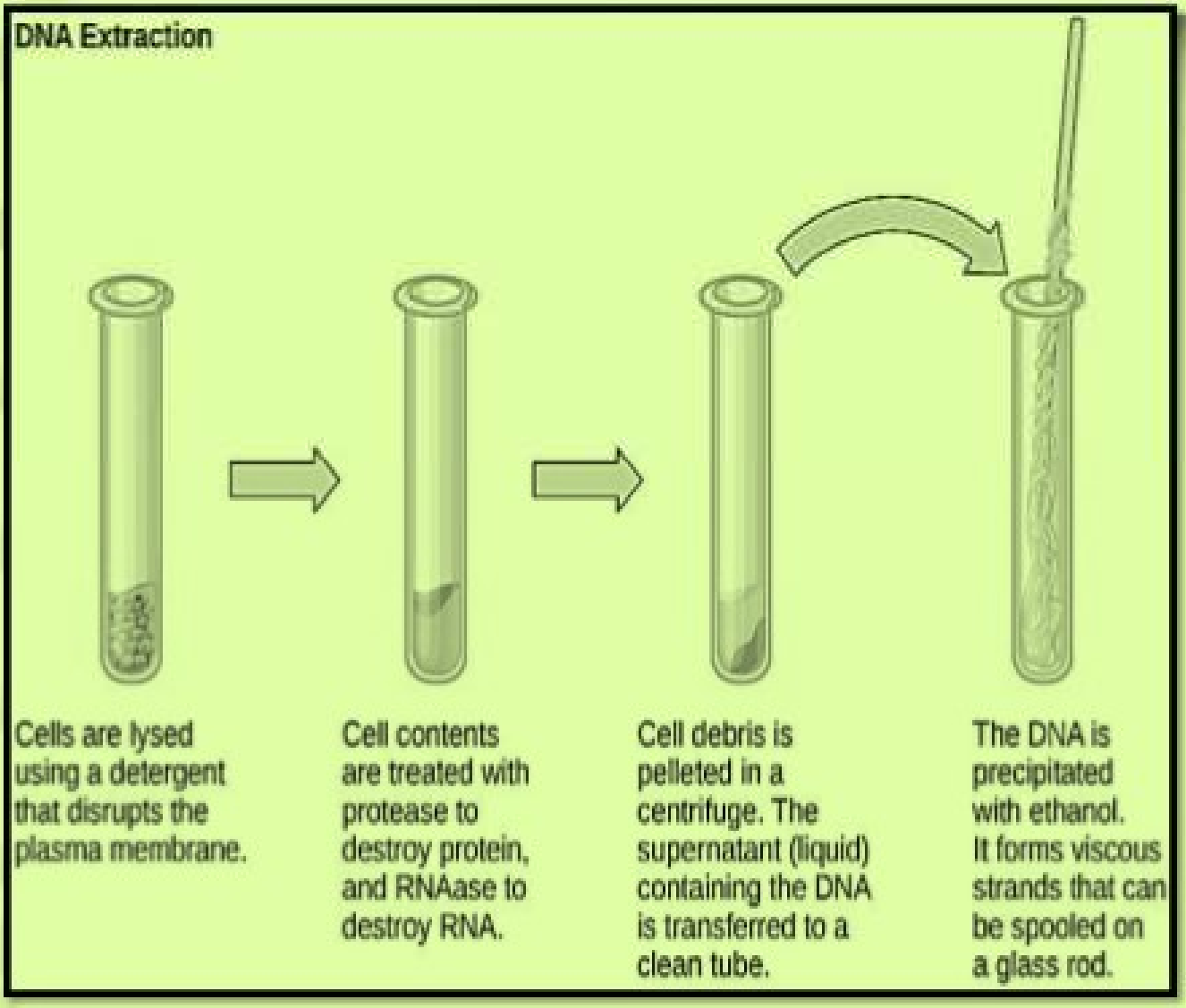
rDNA technology involves several steps in specific sequence such as.

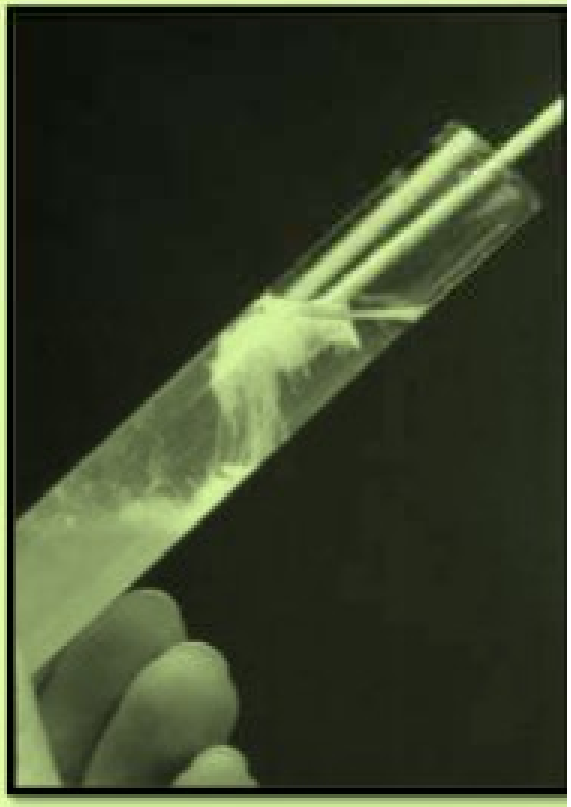
- Isolation of DNA
- Fragmentation of DNA by restriction endonucleases
- Isolation of desired DNA fragment
- Ligation of the DNA fragment into a vector
- Transferring the recombinant DNA into the host
- Culturing the host cells in a medium at large scale and extraction of the desired product

1. Isolation of Genetic material (DNA)

- Most of eukaryotic cells have DNA as genetic material cells
- To form rDNA, the DNA should be pure, i.e., free from all biomolecules
- DNA- located inside nucleus enclosed by plasma membrane, genes interwined with histone protein
- Cell have to break open to release DNA & other macro molecules (RNA, proteins, polysaccharides & lipids)
- Cells are lysed by lytic enzymes: **lysozyme** (bacteria), **cellulase** (plant cell), **chitinase** (fungus)
- Macromolecules- treated with specific enzymes to remove; RNA- **ribonuclease**, Proteins- **Protease**,
- Purified DNA precipitates with addition of **Ethanol**- appear as collection of fine threads in suspension

Steps for dna extraction





2. Cutting of DNA at specific location

- Purified DNA (source DNA/ desirable gene DNA & vector DNA) incubated with specified restriction enzymes for the process- **Digestion** at optimal condition
- Progression of restriction enzyme digestion is checked- agarose gel electrophoresis (source DNA & vector DNA)
- DNA- negatively charged so move towards positive electrode (anode)
- Once 'gene of interest' & cut vector with space for gene of interest is isolated, **DNA ligase** is added
- This step is a preparation of recombinant DNA

3. Amplification of Gene of Interest using PCR

- PCR (Polymerase Chain Reaction)- technology used to amplify a single or a few copies of a piece of DNA to generate thousands to millions of copies of a particular DNA sequence *in vitro*.
- Components required- Primers (small chemically synthesized oligonucleotides that are complementary to the regions of DNA), Nucleotides & DNA polymerase- incubated together
- Steps in PCR

1. **Denaturation** of double stranded DNA by heating- single stranded DNA
2. Primer is added- binds to complementary region- **Annealing**
3. DNA polymerase- polymerization with nucleotides in medium; genomic DNA act as template
4. **Replication** repeated many times- 1 billion copies of desirable DNA- **Amplification** & involve 'thermostable DNA polymerase'- *Thermus aquaticans*- to facilitate replication even at high temperature (denaturation)

Polymerase chain reaction

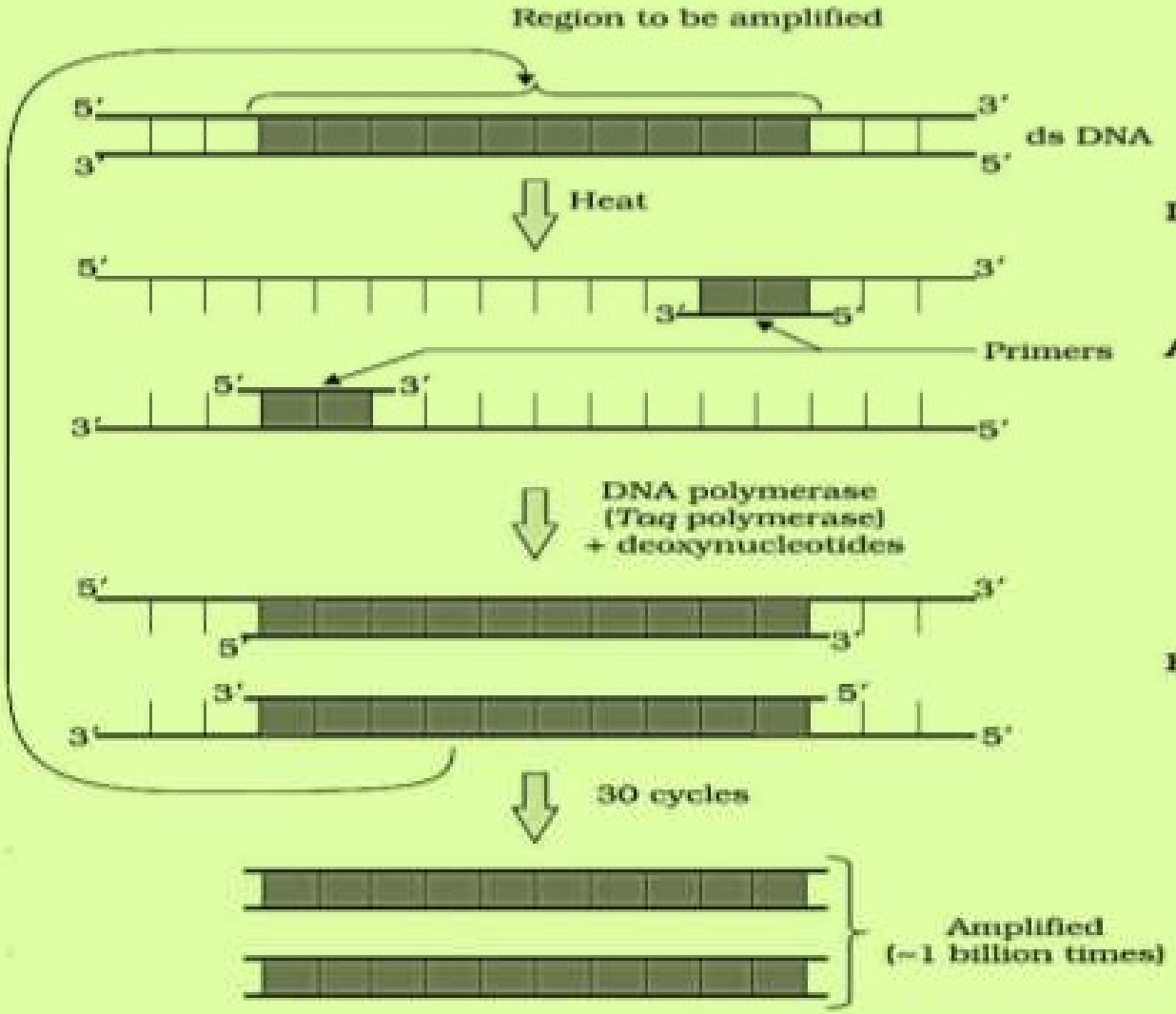
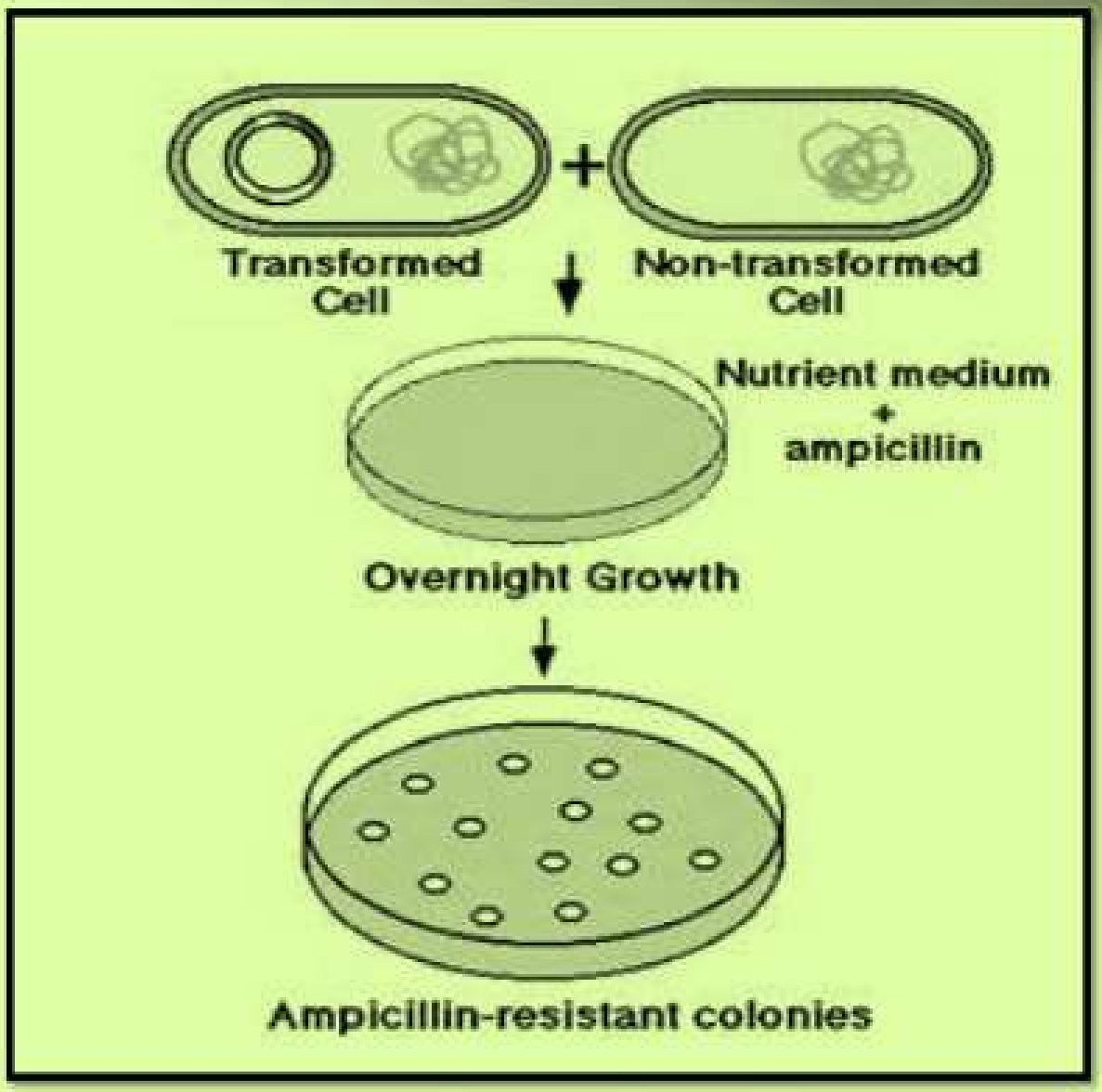


Figure 11.6 Polymerase chain reaction (PCR) : Each cycle has three steps: (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers

4. Insertion of Recombinant DNA into Host cell/ organism

- Insertion of rDNA possible by making host '**competent**'
- Achieved by- Chemical treatment, microinjection, biolistic & gene gun method or disarming pathogen
- Phenotype of organism alters when rDNA is transferred to host
- Eg.- rDNA with gene resistance against antibiotic Ampicillin transferred to *E. coli* (host)- phenotype alters; resistant against ampicillin- transformants
- When plated on agar plate with ampicillin- transformed will grow (change in phenotype) & non- transformed- die
- Presence of ampicillin resistance gene- select transformed cells & considered as **Selectable marker**


Transformed Cell
Non-transformed Cell
Nutrient medium
+
ampicillin
Overnight Growth
Ampicillin-resistant colonies

5. Obtaining the Foreign gene product

- Alien DNA binds with cloning vector- rDNA, transferred to suitable host (bacteria, plant or animal cell)
- rDNA- expresses itself (target protein) under appropriate optimal conditions & after cloning of rDNA to particular number of copies
- Target protein- produce in large scale
- In **heterologous host** if protein encoding gene is expressed- **recombinant protein**
- Heterologous host- gene or gene fragment that does not naturally present in host & the gene expresses itself (recombinant protein)
- Cells which harbor cloned genes of interest- grown in laboratory in small scale
- Culture of dividing host- used to extract desired protein & then purified by different separation techniques

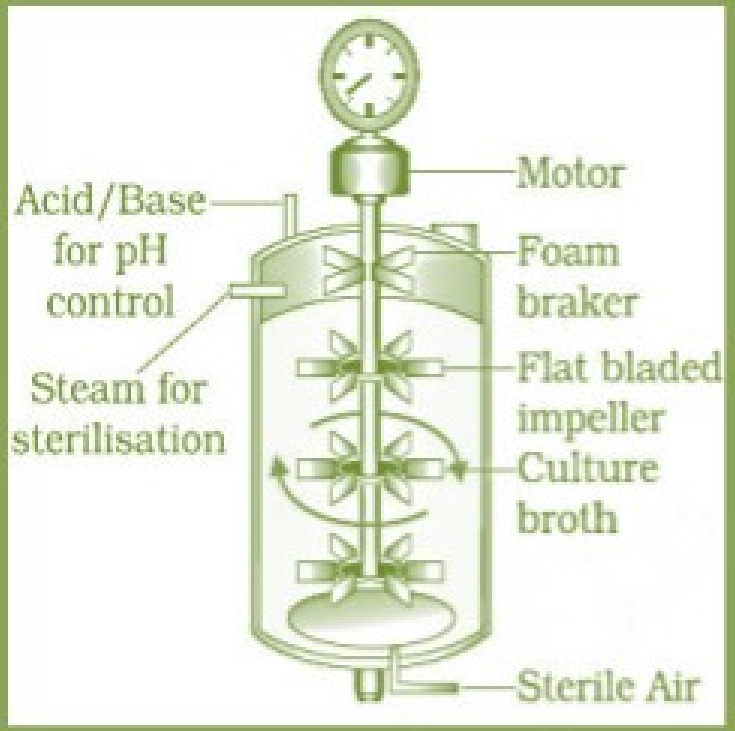
- In large scale cells can be cultured in continuous culture system
- Here the used medium is drained out form one side & fresh medium added from other to maintain the cells- physiologically most active log/ exponential phase
- This culture method produce a larger biomass leading to higher yields of desired protein
- Biomass- biological material derived from living, or recently living organisms
- To produce large quantities of products- **Bioreactors** are employed (100- 1000 liters)- large volumes of culture is processed
- Bioreactors- vessels in which raw materials (recombinant organism like microbial plant, animal or human cell & culture medium with ambient condition)- biologically converted into specific products (protein/ enzymes)
- Optimal growth is achieved with growth conditions- temperature, pH, substrate, salts, vitamins & oxygen

Bioreactors

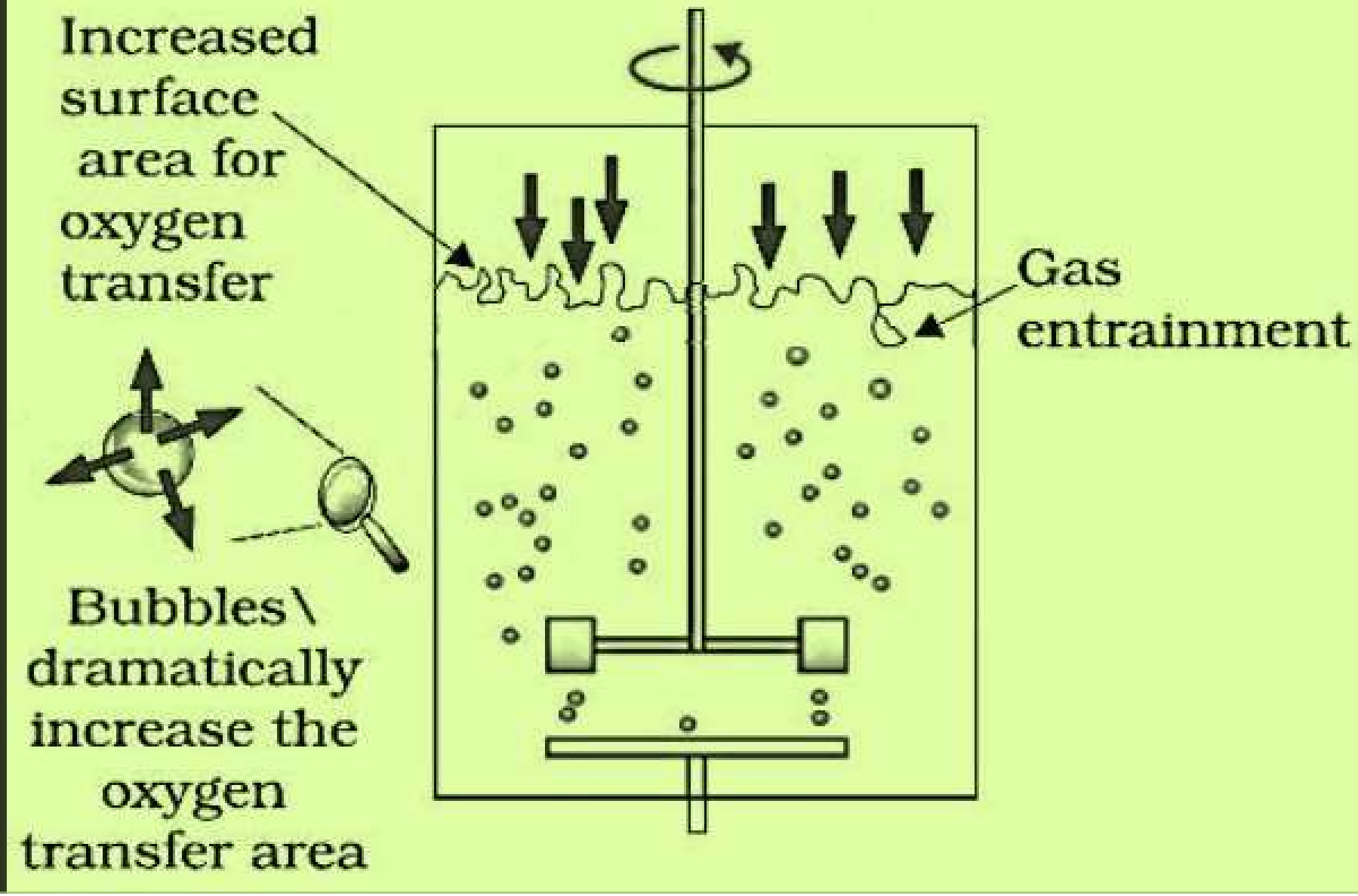
- **Bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells in large scale**.
- A bioreactor provides the **optimal conditions** for achieving the desired product by providing **optimum growthconditions** (**temperature, pH, substrate, salts, vitamins, oxygen**).

Simple Stirred-tank bioreactor

- It is usually cylindrical or with **a curved base** to **facilitate the mixing of the reactor contents.** The stirrer facilitates even mixing and oxygen availability throughout the bioreactor.
- Alternatively air can be bubbled through the reactor.
- The bioreactor has an agitator system, an oxygen delivery system and a foam control system,
- a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.



Spranged stirred tank Bioreactor



Downstream Processing

- The processes include separation and purification, which are collectively referred to as downstream processing.
- Strict quality control testing for each product is also required.

THANK YOU