Any Questions Before We Begin?

Level 8 Cell Culture Processing (BIO08045)

Lecture 5 – "Energy & Mass Transfer Systems"

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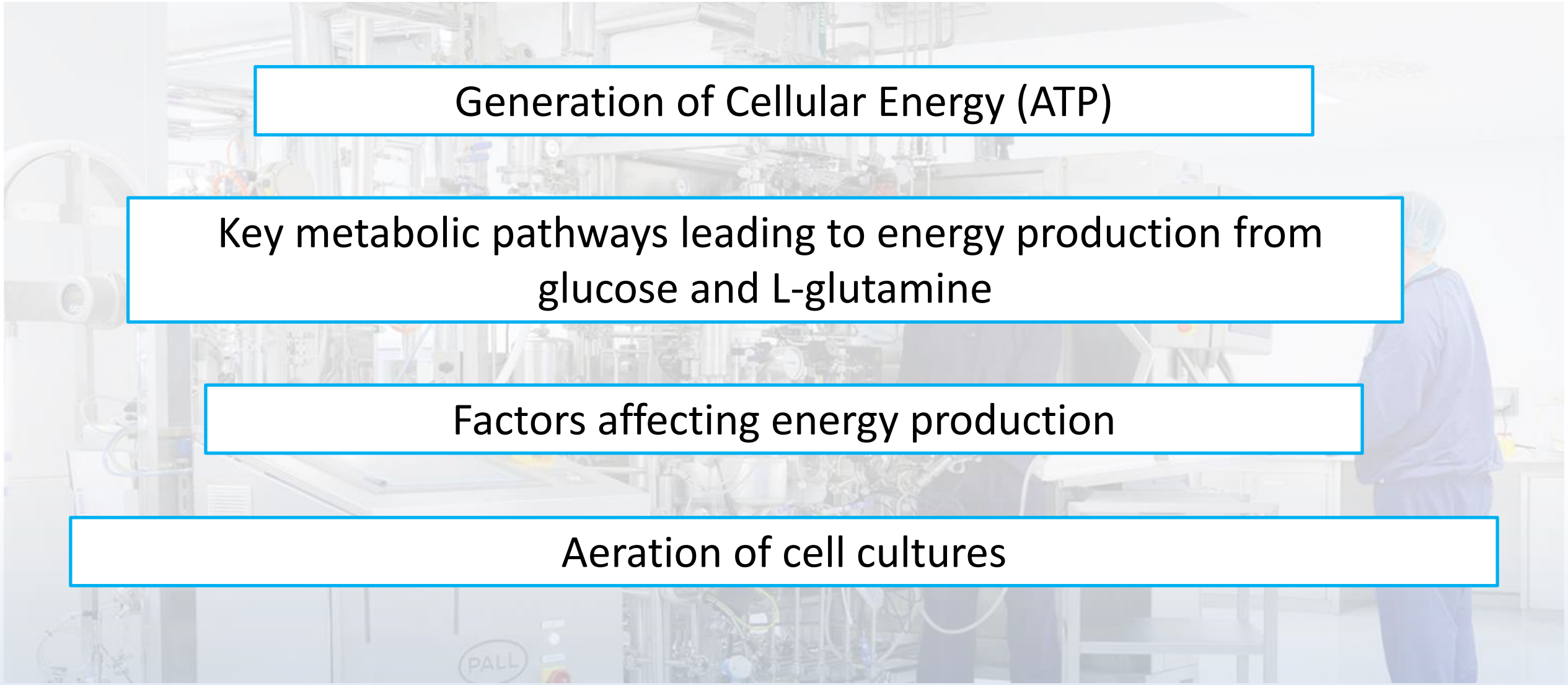
Learning Objectives



Generation of Cellular Energy (ATP)

Key metabolic pathways leading to energy production from glucose and L-glutamine

Factors affecting energy production

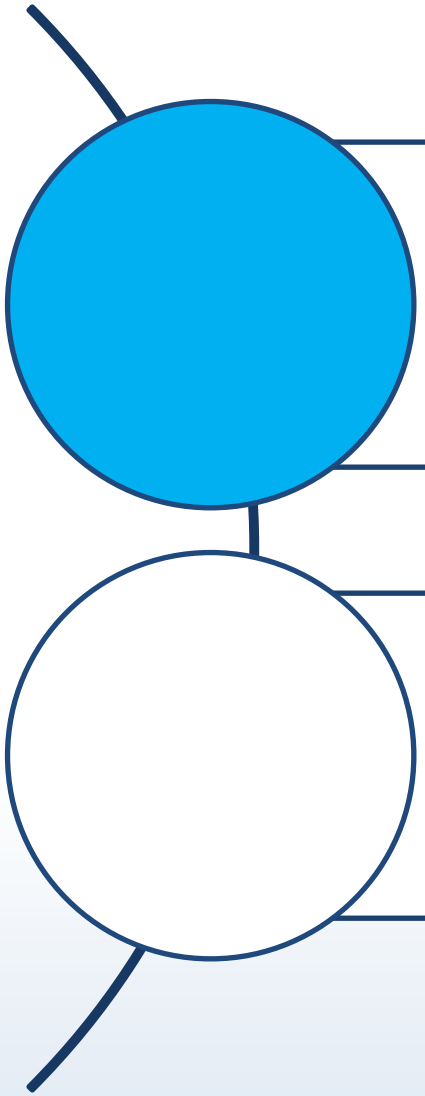
Aeration of cell cultures

Reading Material

- Links available in Moodle

1. Measuring kLa for Better Bioreactor Performance by James Kane - BioProcess International 10(3) March 2012
2. 2017_7 Factors affecting O_2 transfer in bioreactor (GE)
3. Meeting Increased Demands on Cell-Based Processes By Using Defined Media Supplements by Luke Dimasi - BioProcess International 9(8) September 2011 p.48-56
4. “What The Kla Tells You About The Oxygen Transfer In Your Bioreactor” by Lea Duppe – Infors-HT, 2020.

Lecture Topics

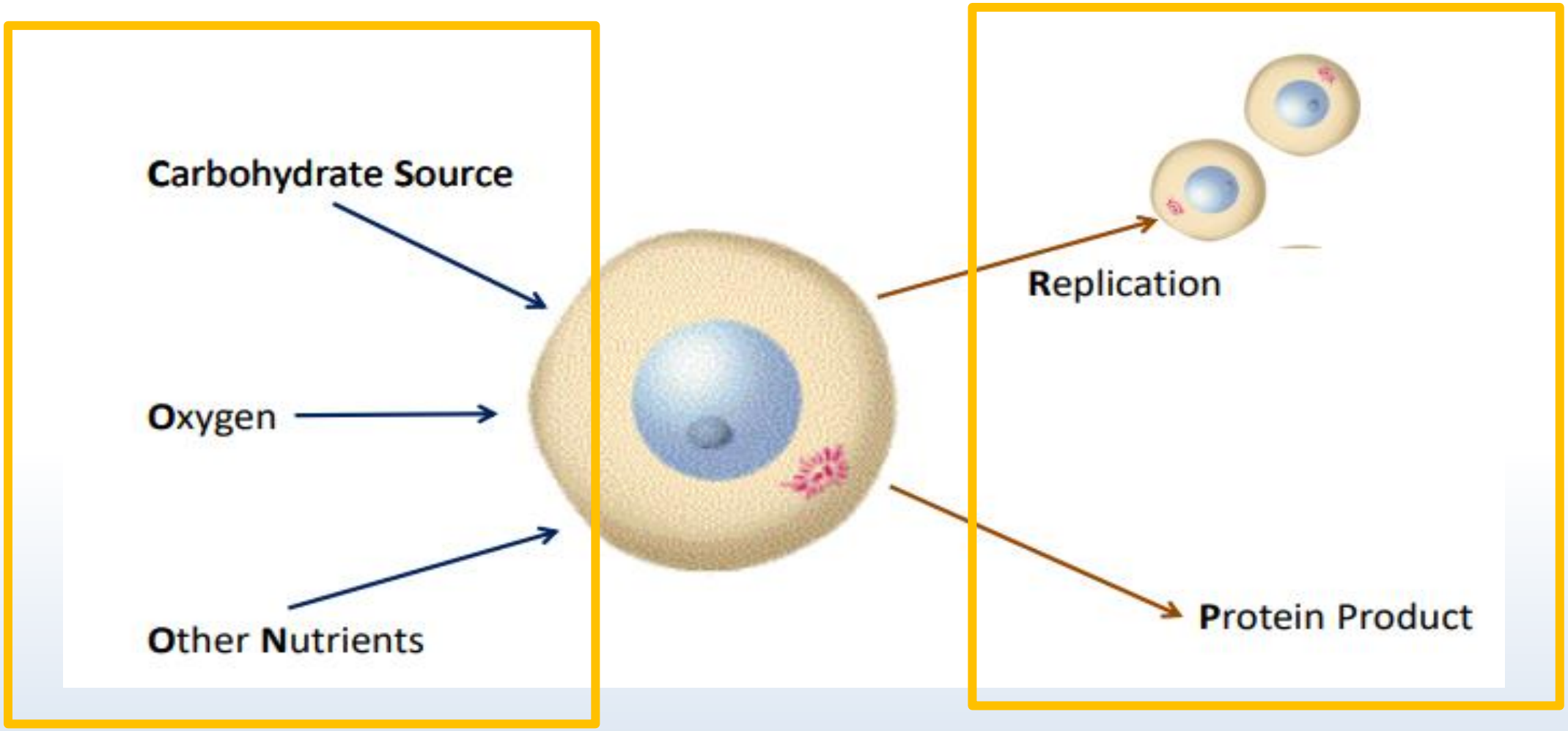


Energy Systems in Cells

Mass Transfer Systems in Cells

Cell Inputs and Outputs

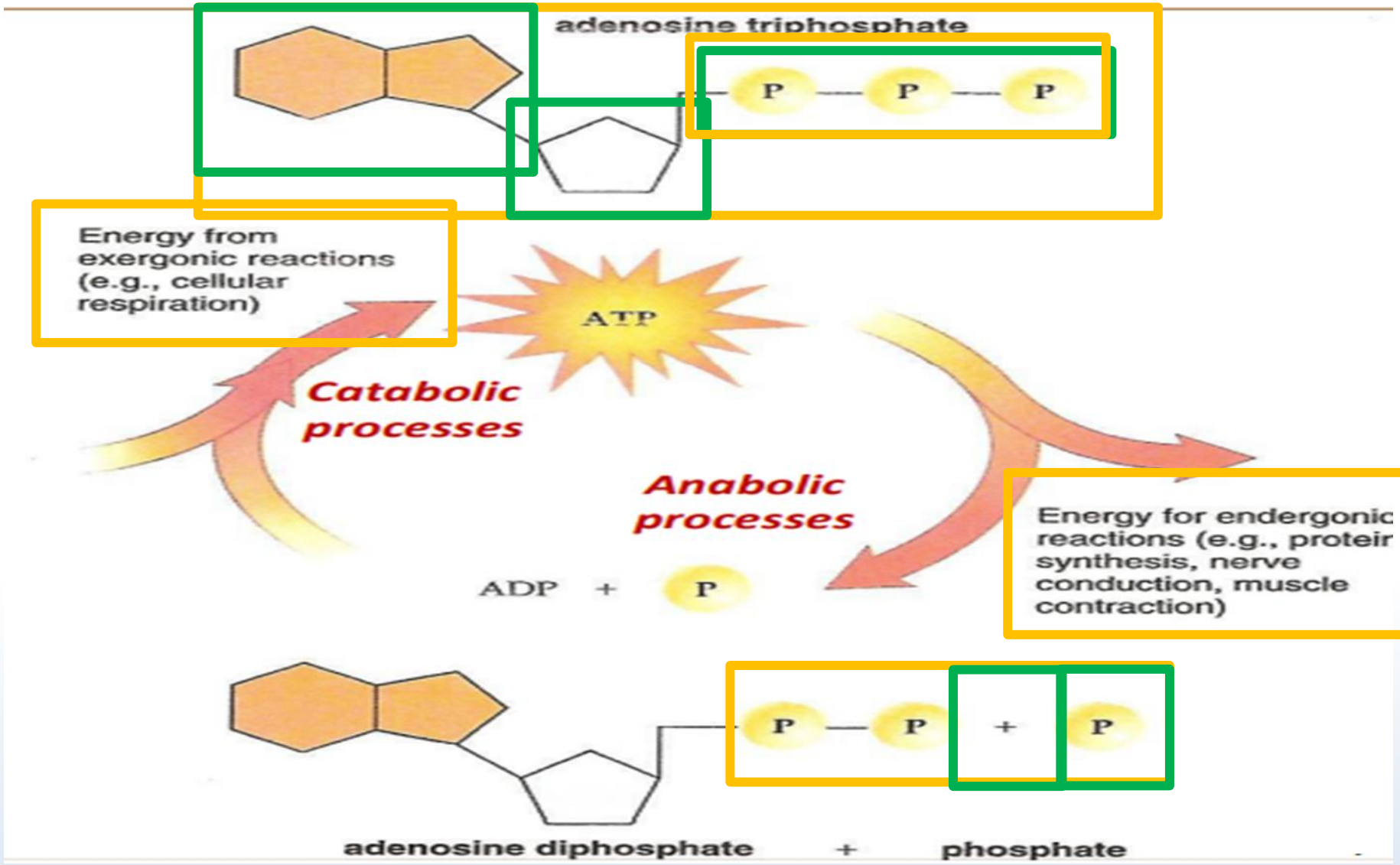
- Environmental conditions: pH, moisture, temperature, salinity



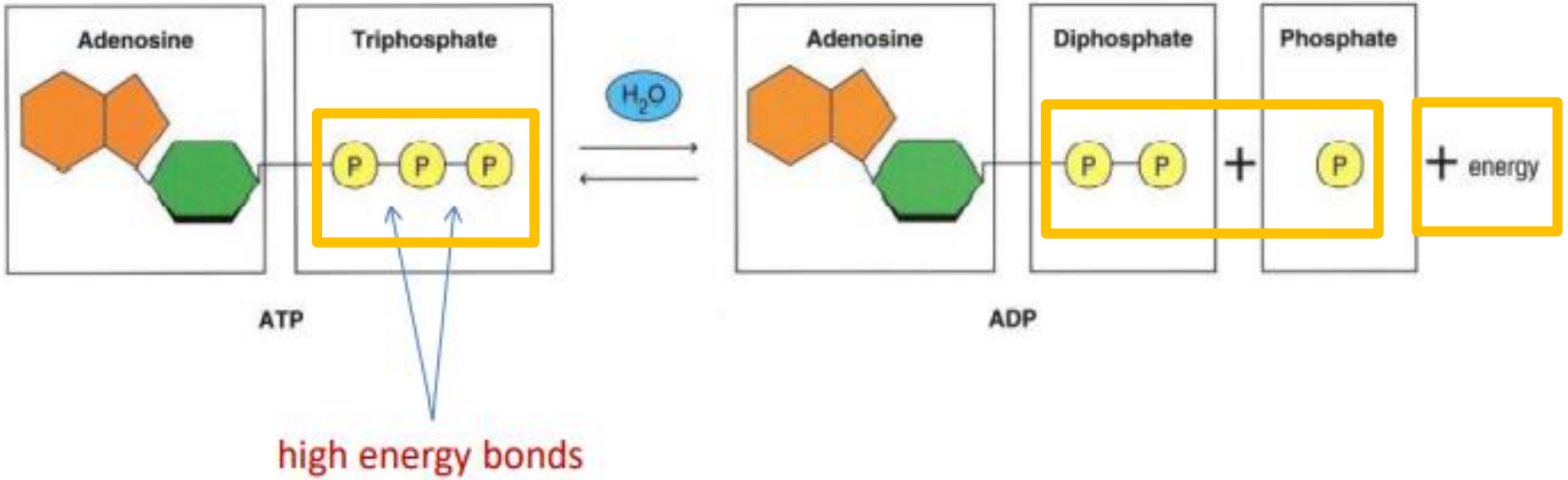
Energy and Cellular Activity

- **Energy** is one of the key constituents of all cellular activity.
  - Metabolism is the sum of all of the chemical reactions that take place in a cell.
  - Exergonic reactions are those where energy is released while Endergonic reactions require an input of energy to take place.
- **Glucose substrate** breakdown during cellular respiration provides a release of energy for the build-up of ATP in the mitochondria of cells.
- **ATP (Adenosine Triphosphate)** is a carrier of energy between exergonic and endergonic reactions.
  - ATP is a nucleotide composed of the nitrogen-containing base adenine and the 5-carbon sugar ribose and three phosphate groups

Energy Balance in Cell Activity



ATP Energy Reaction



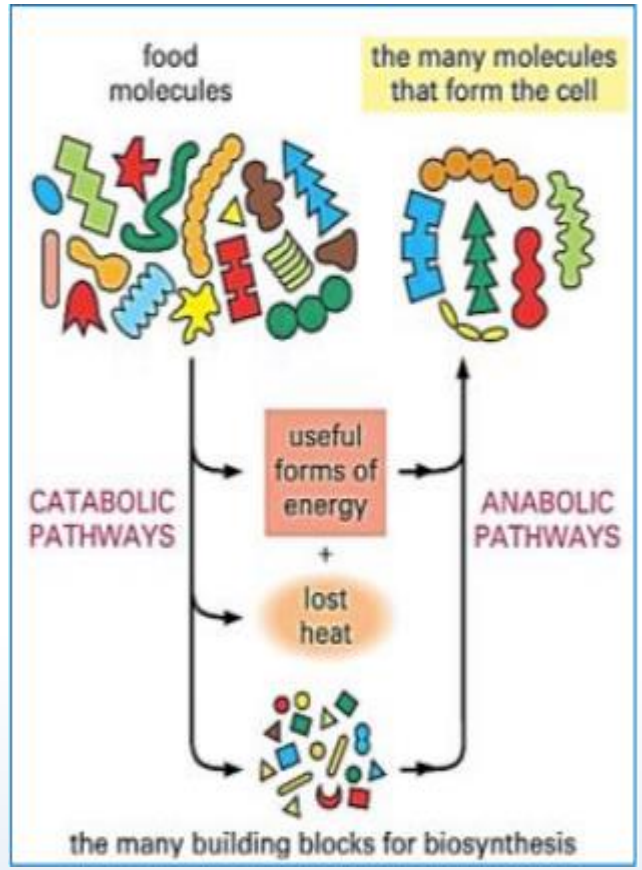
- ATP = **A**denosine **T**ri**P**hosphate
  - Phosphate – phosphate bonds – high energy bonds
  - Release ca. 7.3kcal of energy

Principal Functions of ATP

- **ATP is a carrier of energy within cells**. It is the common energy currency because it supplies energy for many different types of reactions. It can be thought of as a small rechargeable battery unit
- In living organisms the three primary applications of ATP are as follows:
  1. ATP supplies the energy needed to synthesise macromolecules that form the cell and cellular byproducts e.g. MAbs synthesis.
  2. ATP supplies the energy needed to transport substances both into and out of cells via the cell plasma membrane
  3. ATP supplies the general energy needed to enable the cilia and flagella organelles in the cell to beat and move the cell about; the chromosomes to move within the cell nucleus etc

Energy – Its Applications in Cells

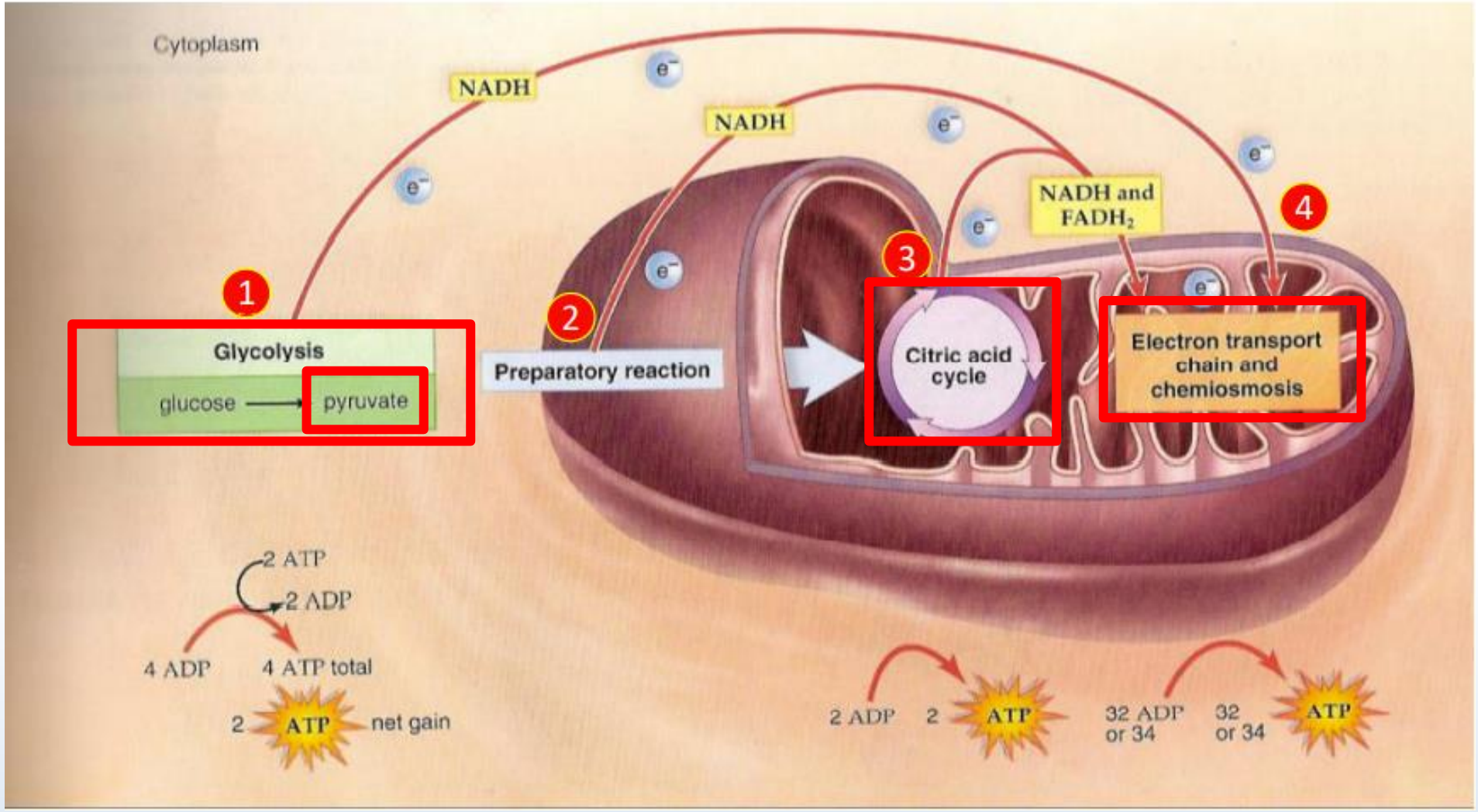
- **Energy Uses in Cells:**
  - 1. for transport purposes e.g. across cell membranes
  - 2. for biosynthesis (anabolic process) e.g. macromolecules
  - 3. for polymerization e.g. linking of peptides and polypeptides
  - 4. for maintenance e.g. regulation of pH, osmotic pressure, motility etc.
- All biochemical reactions = metabolism
  - Anabolic are biosynthetic
  - Catabolic are energy generating

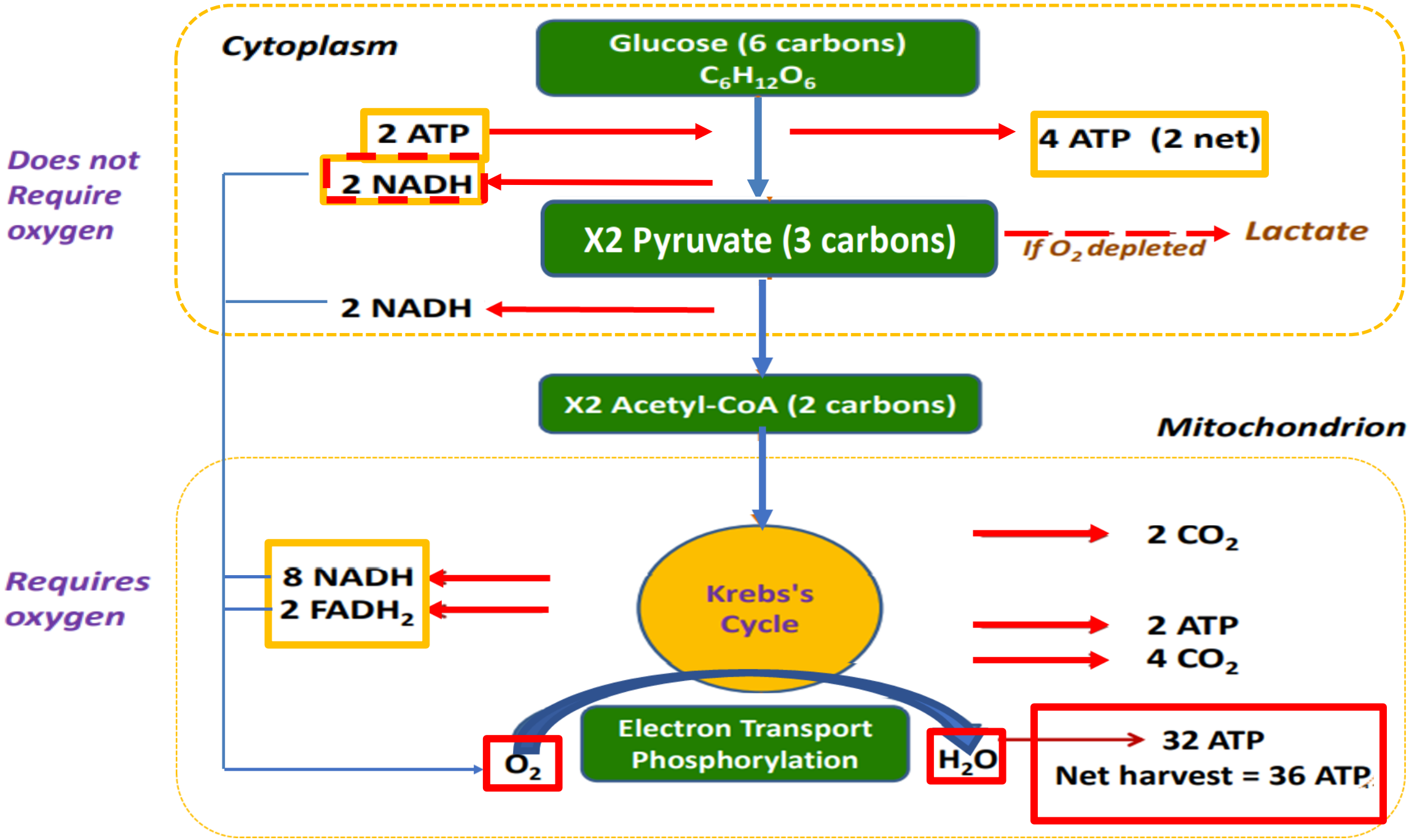


Cells can produce energy in two ways

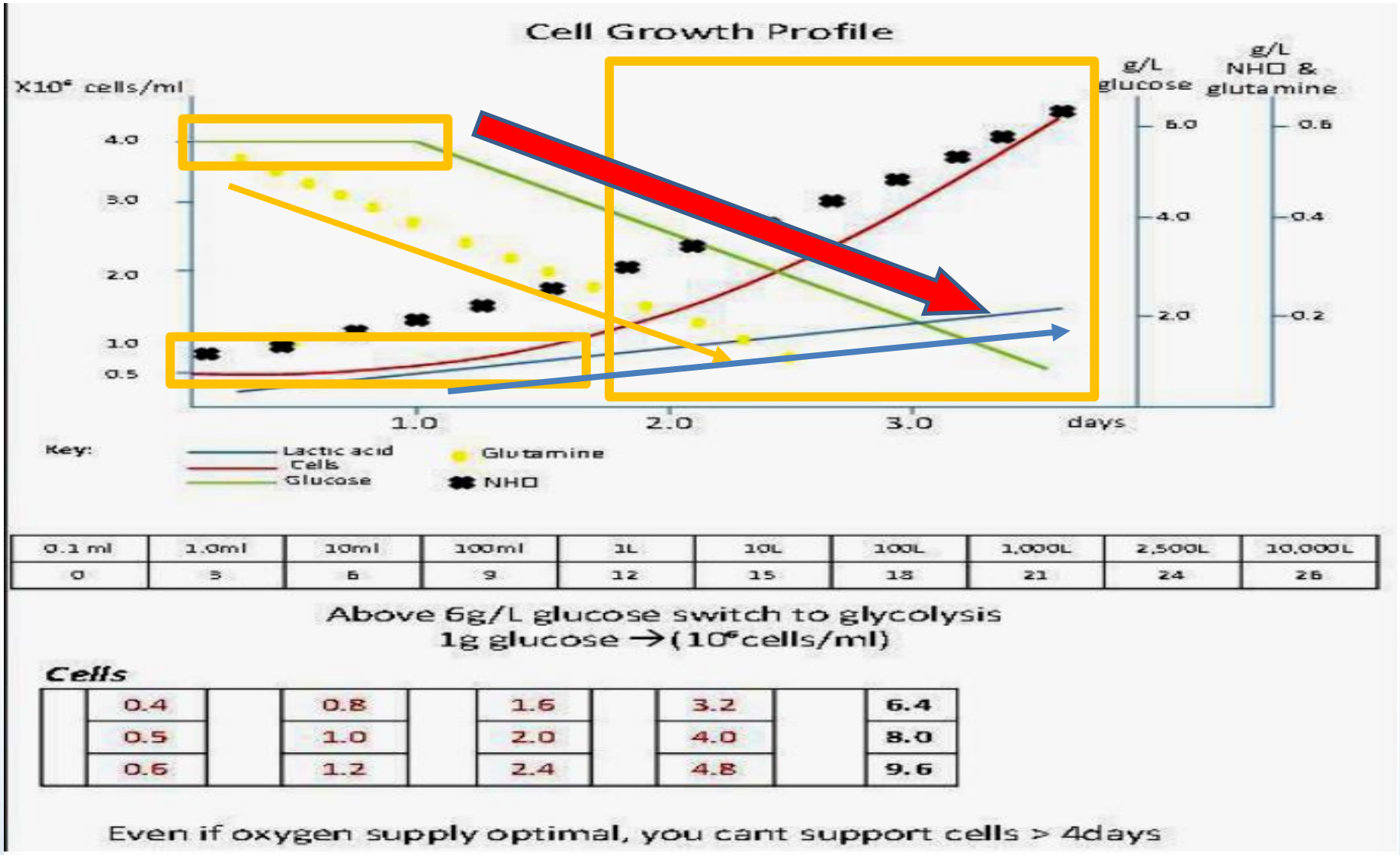
1. From glycolysis.
   - Occurs in cell cytoplasm.
   - Very fast but inefficient.
   - Produces 2 ATP quickly (energy units) but glucose is not fully oxidised. Instead produces byproduct (lactate) which is potentially toxic.
   - Cells switch to this pathway when energy demand is high and oxygen supply into TCA cycle is limiting.
2. From Krebs cycle.
   - Very efficient. Requires a plentiful supply of O_2.
   - Results in complete oxidation of glucose to CO_2 and water and produces 38 ATP (energy equivalents) but takes time because Krebs cycle is very complex.
   - Occurs in mitochondria in cell.

4 Phases of Glucose Breakdown




Cytoplasm
Glucose (6 carbons)
$C_6H_{12}O_6$
Does not Require oxygen
2 ATP
2 NADH
4 ATP (2 net)
X2 Pyruvate (3 carbons)
If O_2 depleted
Lactate
2 NADH
X2 Acetyl-CoA (2 carbons)
Mitochondrion
Requires oxygen
8 NADH
2 $FADH_2$
Krebs's Cycle
2 CO_2
2 ATP
4 CO_2
O_2
Electron Transport Phosphorylation
H_2O
32 ATP
Net harvest = 36 ATP

Cell Growth Profile



0.1 ml	1.0ml	10ml	100ml	1L	10L	100L	1,000L	2,500L	10,000L
0	3	6	9	12	15	18	21	24	26

Above 6g/L glucose switch to glycolysis
1g glucose → (10^6 cells/ml)

Cells

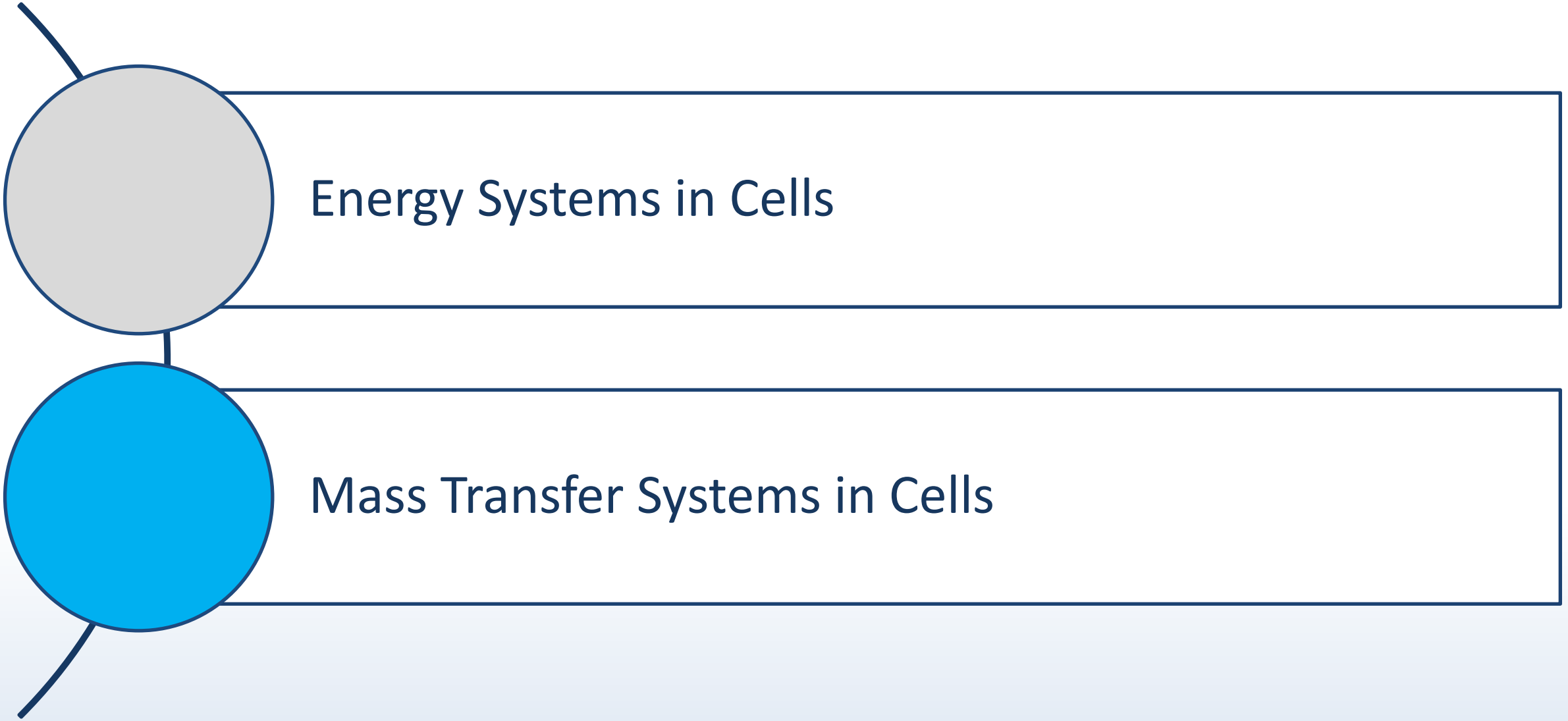
	0.4		0.8		1.6		3.2		**6.4**
	0.5		1.0		2.0		4.0		**8.0**
	0.6		1.2		2.4		4.8		**9.6**

Even if oxygen supply optimal, you cant support cells > 4days

Catabolic Reactions

- **Catabolic Respiration (Aerobic):**
  - **In presence of oxygen**, electrons are transferred to oxygen to yield H_2O through the respiratory chain in the cell mitochondria.
  - ATP yield is 38 mole / mole glucose as compared to only 2 mole ATP/glucose for fermentation process – respiration yields more ATP.
  - Instead of lactate being formed, pyruvate is oxidized through the TCA cycle to CO_2:

Lecture Topics



Mass Transfer

- A study of how critical nutrients and gases (O_2) are Mass Transferred from point of entry in the bioreactor to point of use in the cell structure.
- *kLa - the volumetric mass-transfer coefficient that describes the efficiency with which oxygen can be delivered to a bioreactor for a given set of operating conditions*
  - Advisable to run kLa measurements routinely in many bioprocesses

Dissolved Oxygen

- Dissolved oxygen (DO) is often the limiting substrate in fermentation and cell-culture systems.
  - For bacteria and yeast cultures, the critical oxygen concentration is usually 10–50% of air saturation. Above that critical level, the oxygen concentration no longer limits growth.
  - For optimum growth - maintain DO levels above the critical value by sparging (bubbling gas through) the bioreactor with air or pure oxygen.
- To be effective, the mass transfer rate of oxygen (kLa) to the liquid broth must equal or exceed the rate at which growing cells take up that oxygen i.e. OTR > OUR

kLa and Cell Growth Curve

- During batch cell culture, OUR (or OTR) is initially low during the lag phase (Fig 2), where cells are self-synthesizing and there is little gain of cell density.
- As cell density increases during the exponential phase, OUR increases until OTR becomes a limiting rate, as determined by the mass transfer of oxygen into the bulk liquid.

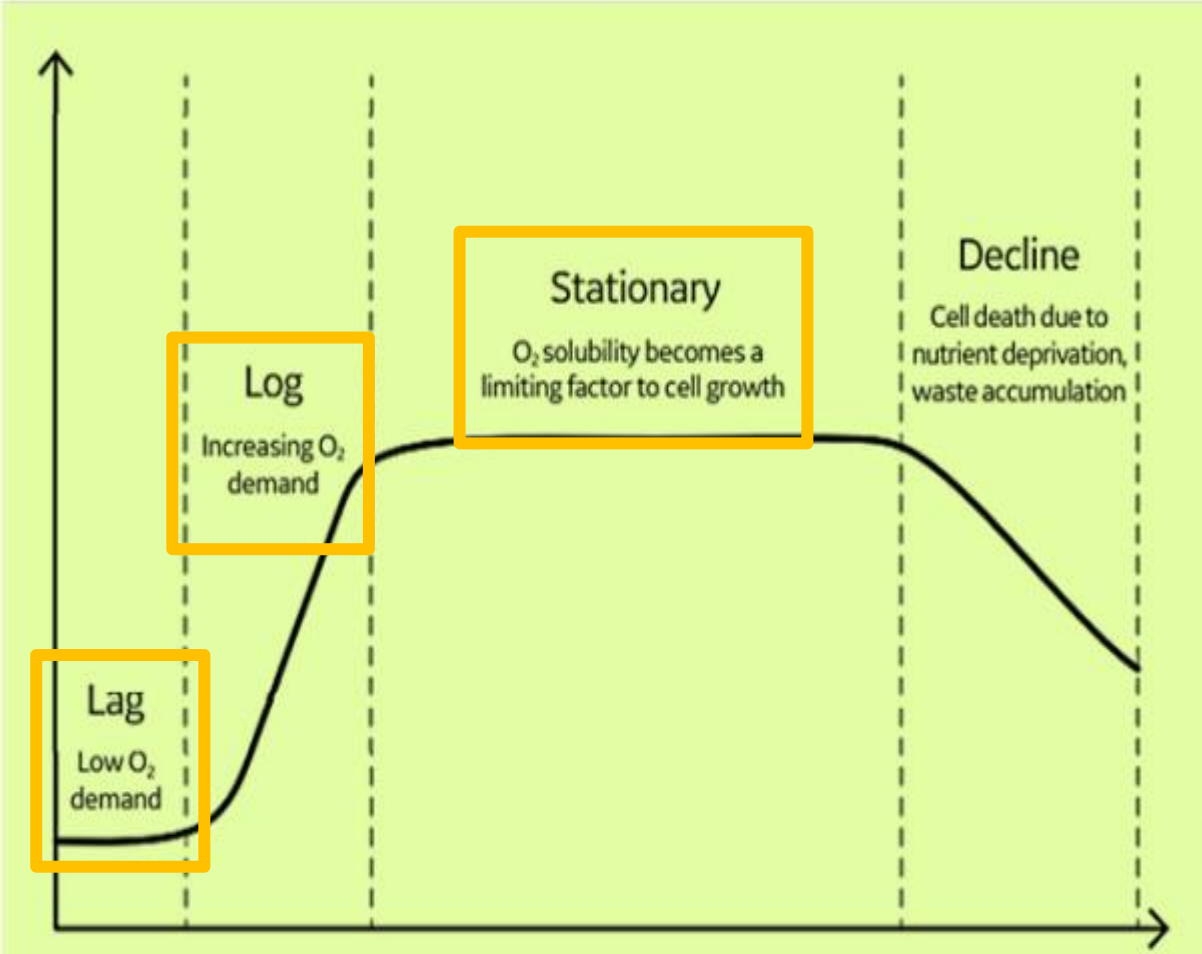


Fig 2. Phases of cell growth.

Bioprocess Mass Transfer

3 phases of mass transfer are involved as follows:

Gas

Liquid

Solid

3 phase boundaries are also involved as follows:

Gas - liquid

Solid - liquid

Liquid - liquid

Mass transfer across phase boundaries is very important and requires efficient performance in the following areas:

Mixing / agitation

Aeration / hold- up

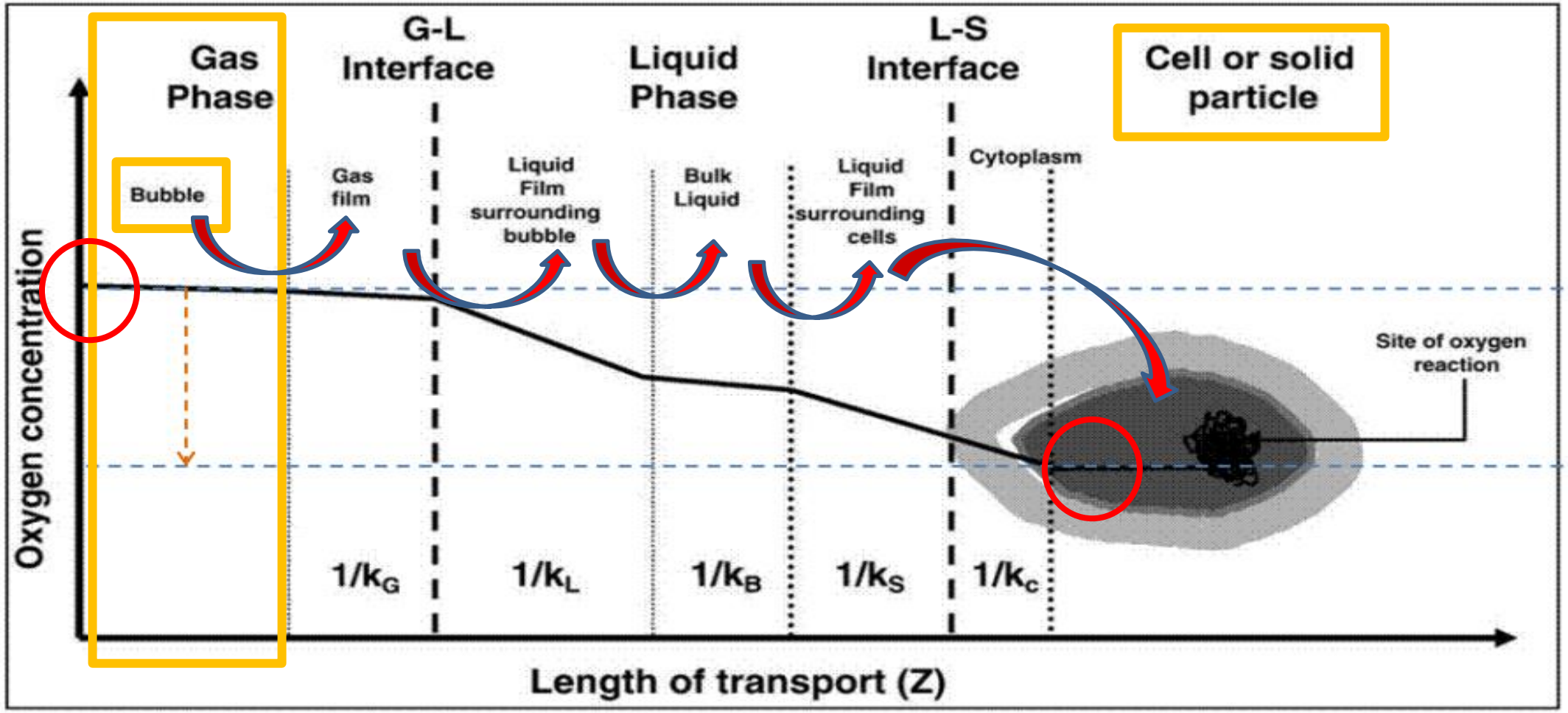
Shear control

Heat transfer

Delivering Oxygen to Cells

- In cell culture, oxygen is a key substrate for growth, production, and maintenance activities.
- Cells obtain their oxygen in free and non-compound forms, called dissolved oxygen (DO).
- One of the most important functions of bioreactors is providing DO to cells continuously through a process called aeration.
- Aeration in the bioreactor typically occurs when:
  1. Oxygen diffuses through overlay to the cell culture medium interface (lab-scale and small scale systems)
  2. Oxygen from the spargers dissolves in the cell culture through convection with the help of agitation (pilot and production scale bioreactors)

Resistance to Oxygen Transfer from Air Bubble to Cell



From: Gomez, E., and Garcia-Ochoa, F., "Bioreactor Scale-Up and Oxygen Transfer Rate in Microbial Processes: An Overview," Biotechnology Advances, vol. 27, 2009, pp. 153-176.

Mass Transfer of O_2

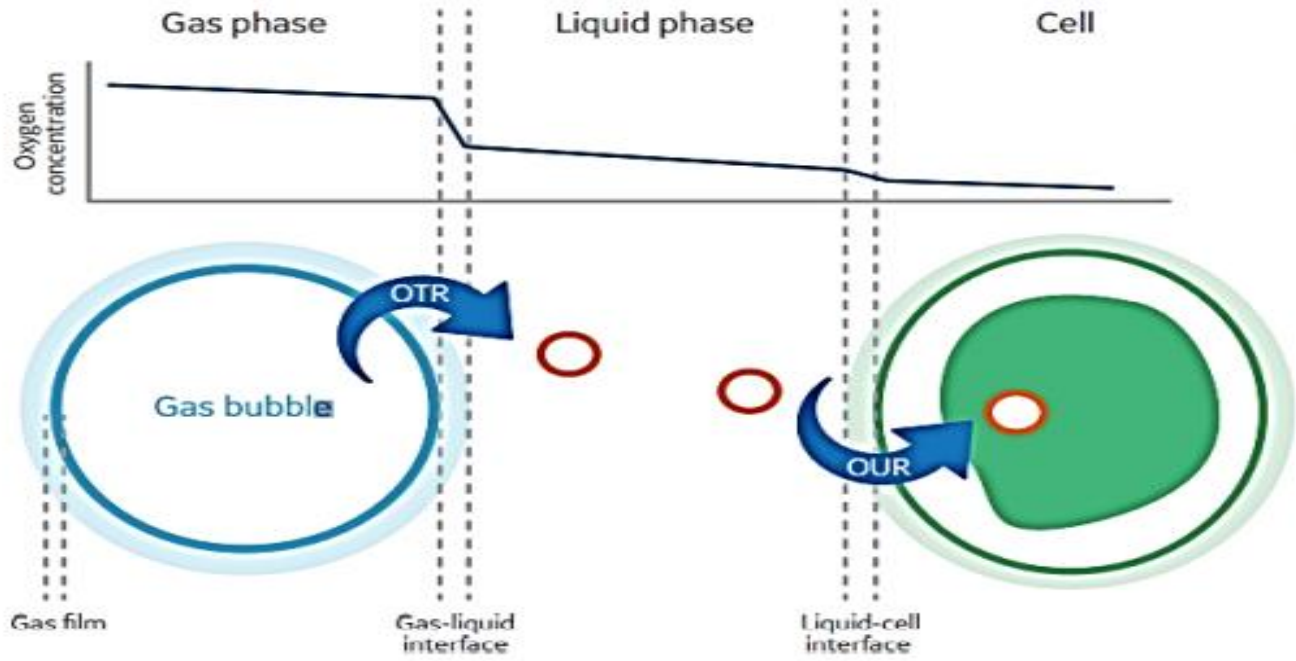


Fig 1. Diagram of a gas bubble in liquid, showing how the bubble is released, solubilized, and transferred to a cell.

Cell line	OUR [10^{-13} mol/cell/h]
DG44 (CHO)	2
CHO	5.0–8.04
NSO (myeloma)	2.19–4.06
MAK (hybridoma)	4.6
FS-4 (human diploid cells)	0.5
HFN7.1 (hybridoma)	2

Agitation disperses the oxygen bubbles and promotes mass transfer of the gas bubbles through the gas-liquid (cell culture medium) interface (Fig 1). The rate of oxygen transfer (OTR) from gas to liquid interface is a function of physicochemical properties of the cell culture medium, the geometrical parameters of the bioreactor, and presence of cells. Oxygen supply is carefully controlled for optimal cell growth by manipulating bioreactor parameters.

Gas - Liquid Mass Transfer Boundaries

- It is a combination of the following resistances:
  1. Diffusion from bulk gas to gas - liquid interface.
  2. Passage through the gas- liquid interface.
  3. Diffusion through the poorly mixed liquid film to well mixed bulk liquid.
  4. Transport through the bulk liquid to the liquid film surrounding the cell.
  5. Transport through the liquid film at the cell interface.
  6. Transport across the cell membrane into the cell and internal diffusion to reactive sites e.g. mitochondria.

  – **Note that antifoam and protective agents such as pluronic acid, increase O_2 solubility but also increase mass transfer resistance.**

Oxygen Transfer

- **Oxygen Transfer is important because it is:**
  1. A non-reacting gas in aqueous solutions.
  2. A major substrate for aerobic processes.
  3. Poorly soluble in aqueous culture media.
  4. Frequently growth limiting.
  5. Often dictates the bioreactor configuration.

- Solubility of O_2 in 1 litre H_2O at 20°C is: 0.3mM = 9 ppm = 9mg l^{-1}
  - Solubility decreases with increase in temperature and salt concentration.

Oxygen Uptake Rate

Like any substrate, oxygen follows the Monod equation:

$$\mu = \mu_m \frac{S}{K_s + S}$$

μ = specific growth rate

μm = max specific growth rate

S = substrate conc.

Ks = conversion factor

For oxygen, this is given by:

$$OUR = q_{O2} \cdot X = q_{O2m} \frac{C_L}{K_{O2} + C_L} X$$

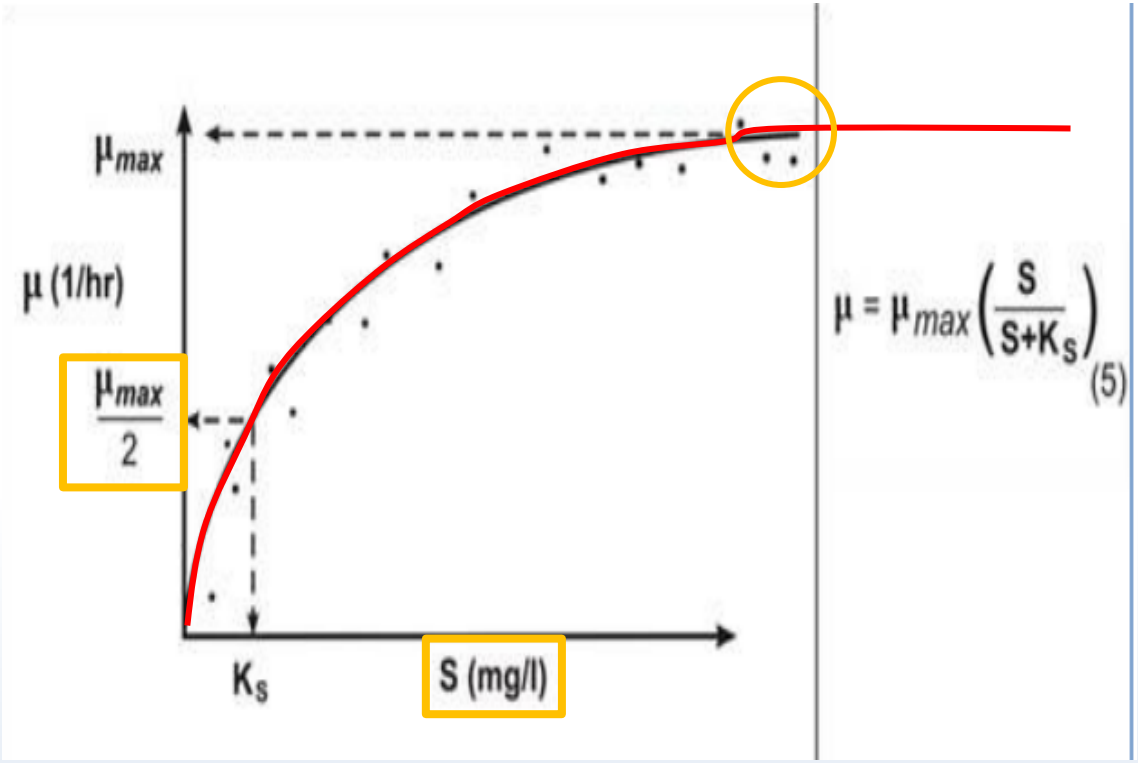
q_{O2} = specific oxygen uptake rate (mmol O_2 g^{-1} h^{-1})

q_{O2m} = max specific OUR (mmol O_2 g^{-1} h^{-1})

K_{O2} = saturation constant for O_2 (mM)

C_L = dissolved O_2 conc.

X = cell conc. (g/l)



Oxygen Transfer Rate (OTR)

Air Liquid

$$N_A = k_L a (C^* - C_L) = OTR$$

N_A = volumetric mass transfer rate (mM O_2 l^{-1} h^{-1}).

k_L = mass transfer coefficient at phase boundary (ms^{-1}).

a = volumetric mass transfer area ($m^2m^{-3} = m^{-1}$).

k_La = volumetric mass transfer coefficient (s^{-1}) i.e. per unit time.

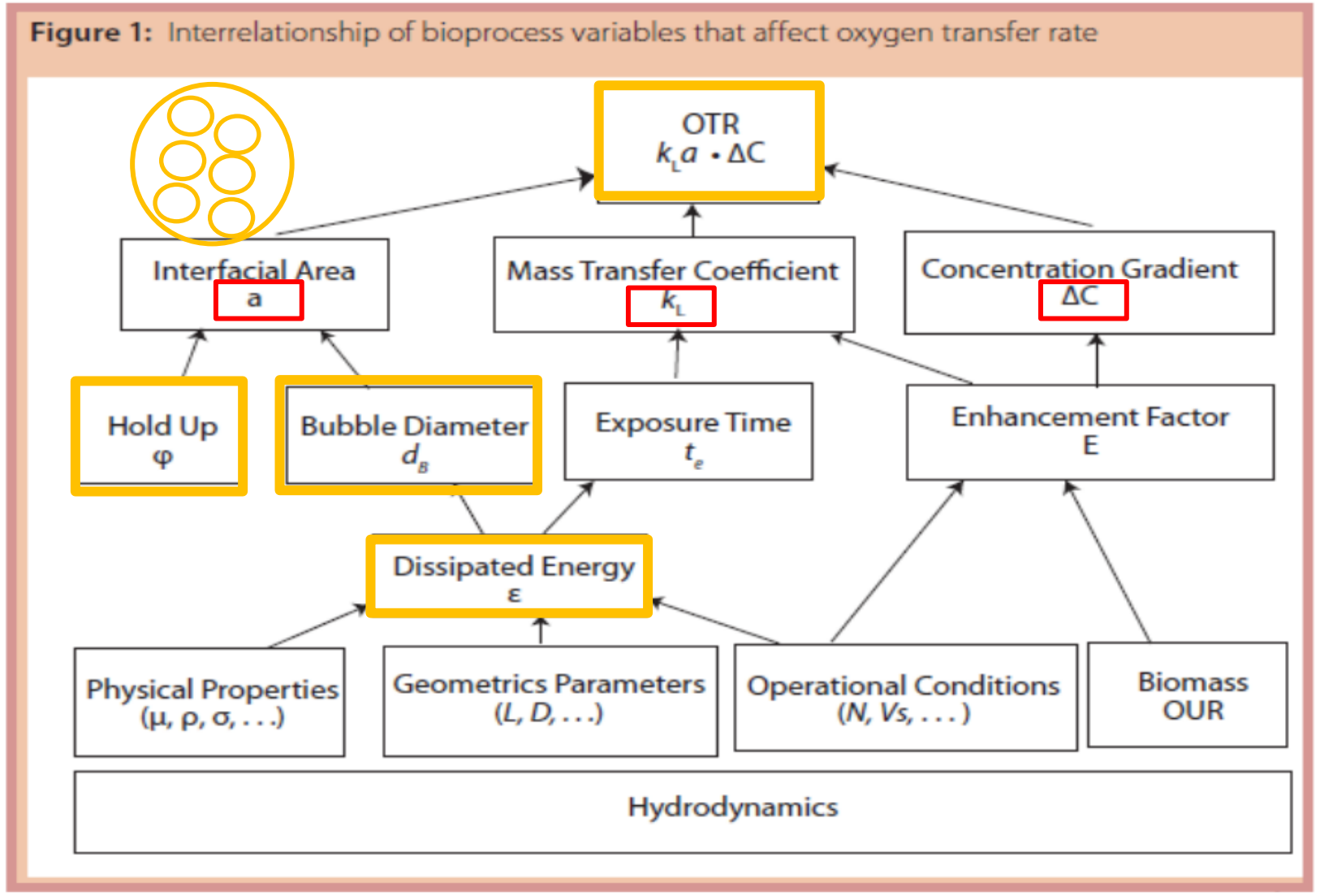
C^* = dissolved gas concentration in phase boundary (mM l^{-1}).

C_L = dissolved oxygen concentration (mM l^{-1}).

OTR = oxygen transfer rate (mM l^{-1} h^{-1}).

Factors affecting OTR

The oxygen transfer rate (OTR) of a bioreactor is strongly influenced by the hydrodynamic conditions being used in the bioprocess.



Factors affecting kLa

- Multiple variables - kLa is influenced by many variables including

Bioreactor size and design	Temperature
Sparging of gas	pH
Agitation and mixing	Salt content
Media type	Antifoaming agents

- Every time one of those factors changes, the dynamics of the bioprocess, including kLa, change as well.
- Scientists have relied to a large extent on the built-in functionality of the bioprocesser to maintain proper oxygen flow rate

Adapted from: 'Measuring kLa for Better Bioreactor Performance' by J Kane BioProcess International 10(3) March 2012

kLa – Why is it important ?

- Mass transfer is a very important element of bioreactor operation.
- The design/operation of bioreactors for aerobic fermentation must take the oxygen requirements into account and **should be able to replace oxygen in the medium faster than it is consumed i.e. OTR > OUR**

- The kLa value allows us to directly compare the conditions between different reactors and also allows us to check that the reactor is set-up in the same way every time.
  - Since we can use the k_La to compare conditions between reactors it can also be used as a scale-up criterion i.e. constant k_La at 2L, 500L and 2000L bioreactors.

kLa in System Scalability

- kLa values are particularly useful in the following scenarios:
- **Scenario 1: Evaluating scalability within the same bioreactor platform**
  - The conventional scale-up of bioprocesses is based on physicochemical and geometric similarity i.e. maintain the same media and conditions (temp., pH, conc., cell density etc.) and the geometric ratio (H:D) of the bioreactor
  - kLa is kept constant for this scenario
  - The OTR should remain constant for a bioreactor platform with geometric similarity
  - Bioreactor physical characteristics at the different scales are altered to provide the necessary OTR at controlled temperature, pH, and DO to achieve the target cell density

kLa in System Scalability

- **Scenario 2: Technical transfer across different bioreactor designs**
  - During the comparison, kLa is utilized as a target performance metric when a process is transferred from one bioreactor platform to another design.
  - Bioreactor hardware design (e.g., stirrer geometry and aeration-sparger option) and running parameters (e.g., gas flow rate or power input) are altered to achieve a similar kLa, providing a similar cell density

Key Variables that Impact kLa Values

- Any change to process and engineering parameters or to physical characteristics will have an impact on kLa and should be considered when evaluating bioreactor platforms and performing scaling calculations.
- Here are four key variables that can affect kLa values (Fig 3):

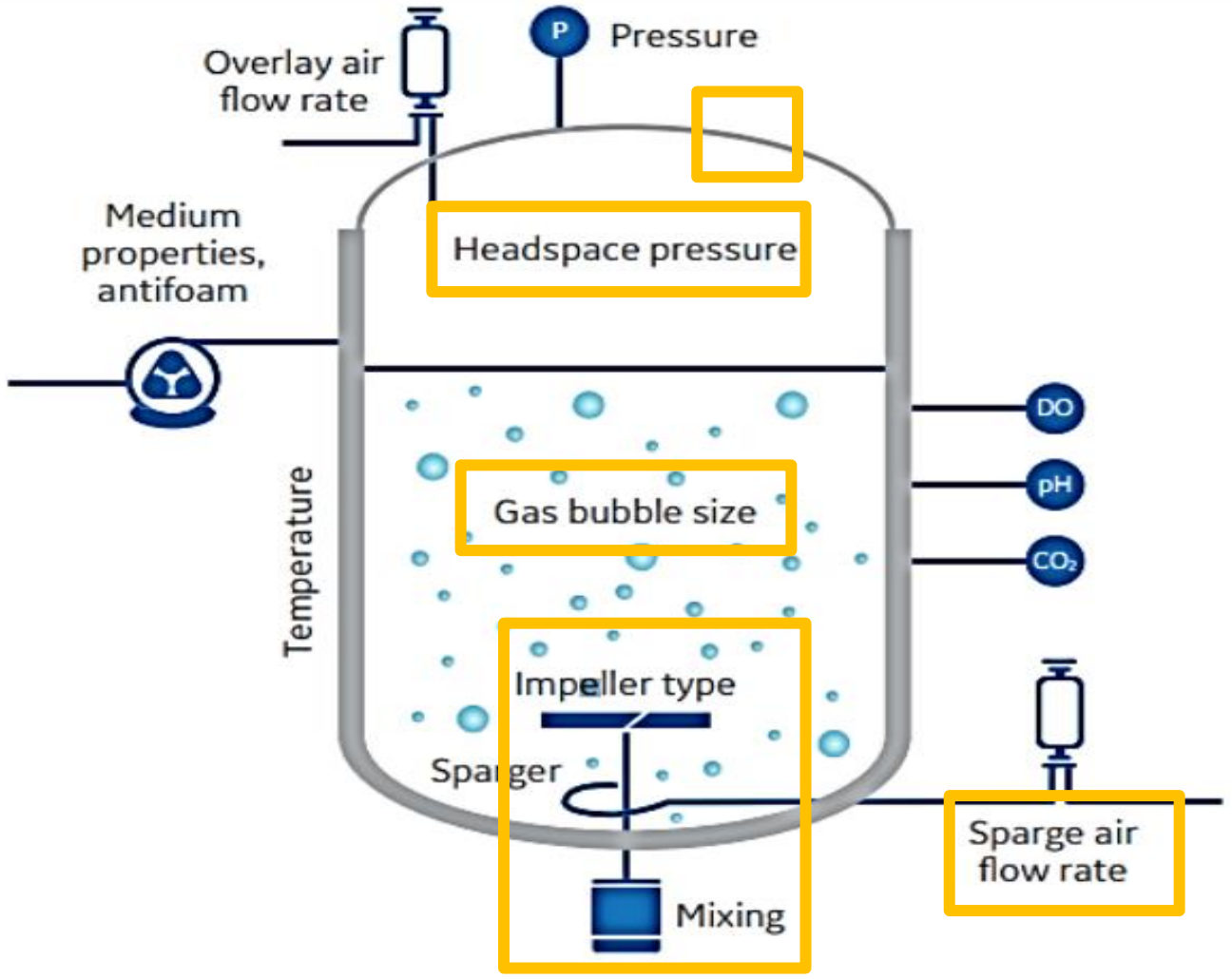


Fig 3. Diagram of a bioreactor process showing key factors that can influence k_La values.

Key Variables that Impact kLa Values cont'd

- **MIXING** is used to eliminate gradients of concentration (cell, gas, medium, and nutrient), temperature, and other properties.
  - Mixing time is widely used to characterize mixing efficiency in a bioreactor; efficiency is one of the most significant factors affecting both performance and scale-up in a bioreactor.
- **Gas bubble size and residency time are highly dependent upon three mixing conditions impeller type, speed, and location(s).**
- kLa values generally increase as tip speed increases.
  - However, tip speed is proportional to shear forces that can lead to cell death. Bioreactors, therefore, are designed with different impeller types, combinations, and locations to achieve target kLa values without creating these shear forces.
- Generally, kLa values are closely associated with impeller design, with Rushton typically higher than paddle, which is typically higher than marine and pitched impeller

Key Variables that Impact kLa Values cont'd

- **GAS BUBBLE SIZE**
  - When gas bubble size decreases, surface area and gas residency time increases, causing bubbles to stay in the culture longer.
  - Thus, there is a greater opportunity for oxygen to release mass transfer into the cell culture medium. An increase in this oxygen residence time improves kLa
- **SPARGER CHARACTERISTICS**
  - kLa values will vary widely with sparger characteristics, including number, pore size, and surface area, because these factors affect bubble size, gas velocity, and flow rates

Key Variables that Impact kLa Values cont'd

- **AIR FLOW RATE**
- Higher oxygen availability drives kLa increases.
  - Increasing oxygen supply to a bioreactor drives this availability and can be controlled by modifying concentration (air vs O_2 enrichment) and volumetric flow.
  - Although high kLa values are desirable, it is important to consider the actual operating conditions and implications to cell viability and associated process costs.
  - For example, **high air flow rates can cause cell damage due to shear forces. Excessive foam might also be generated**, requiring a high concentration of antifoam that could hinder downstream processing. Additionally, higher air flow rates require a larger exhaust filter area, driving consumable cost increases.

Key Variables that Impact kLa Values cont'd

- **PROPERTIES OF THE LIQUID OR MEDIUM**
- During cell culture, small bubbles collide and coalesce to form larger bubbles, decreasing surface area (a) and subsequently kLa
- Be aware of reported kLa values in which high salt concentrations are used, because this can prevent bubble coalescing
- Antifoaming agents are used to influence surface tension, resulting in reduced bubble coalescence and foaming.
  - However, this principle does not always lead to increases in OTR wherein antifoam also reduces bubble mobility, which subsequently reduces the kLa

Key Variables that Impact kLa Values cont'd

- **TEMPERATURE**
- Increasing temperatures inversely affects both the volumetric mass transfer coefficient and oxygen solubility in culture medium.
- Oxygen solubility in pure water falls with increasing temperature (i.e., -0.5×10^{-3} kg/m^{-3} between 35°C and 30°C).
- Therefore, it is important to note the temperature conditions from vendor-supplied characterization data.

Oxygen Transfer Rate (OTR)

- How to increase the OTR rate for a bioreactor:

  a) Increase the kLfactor by increasing the agitator rotation speed or by increasing the oxygen inflow velocity.

  b) Increase the volumetric mass transfer area (a) by reducing the oxygen bubble size. Bubble size can range from micro-bubbles of 0.1mm up to 5mm diameter – avg. around 2 - 3mm.

  c) Increase the oxygen concentration of the incoming gas by using oxygen/air mixtures or in some cases pure oxygen.

  d) Minimise the dissolved oxygen concentration of the culture medium - this depends on phase of growth of cells e.g. exponential vs. steady-state phases. The faster the cells are growing the more uptake of oxygen.

Steady State Conditions

For steady state conditions in a bioreactor: OTR = OUR

$$OUR = qO_2m.\ X = k_La\ (C^* - C_L) = OTR$$

If OUR > OTR then limit on cell growth and product synthesis because of oxygen depletion.

If OTR > OUR then excessive metabolic reaction leading to excess metabolite generation e.g. ammonia, CO_2 etc.

Methods for Measuring Bioreactor kLa

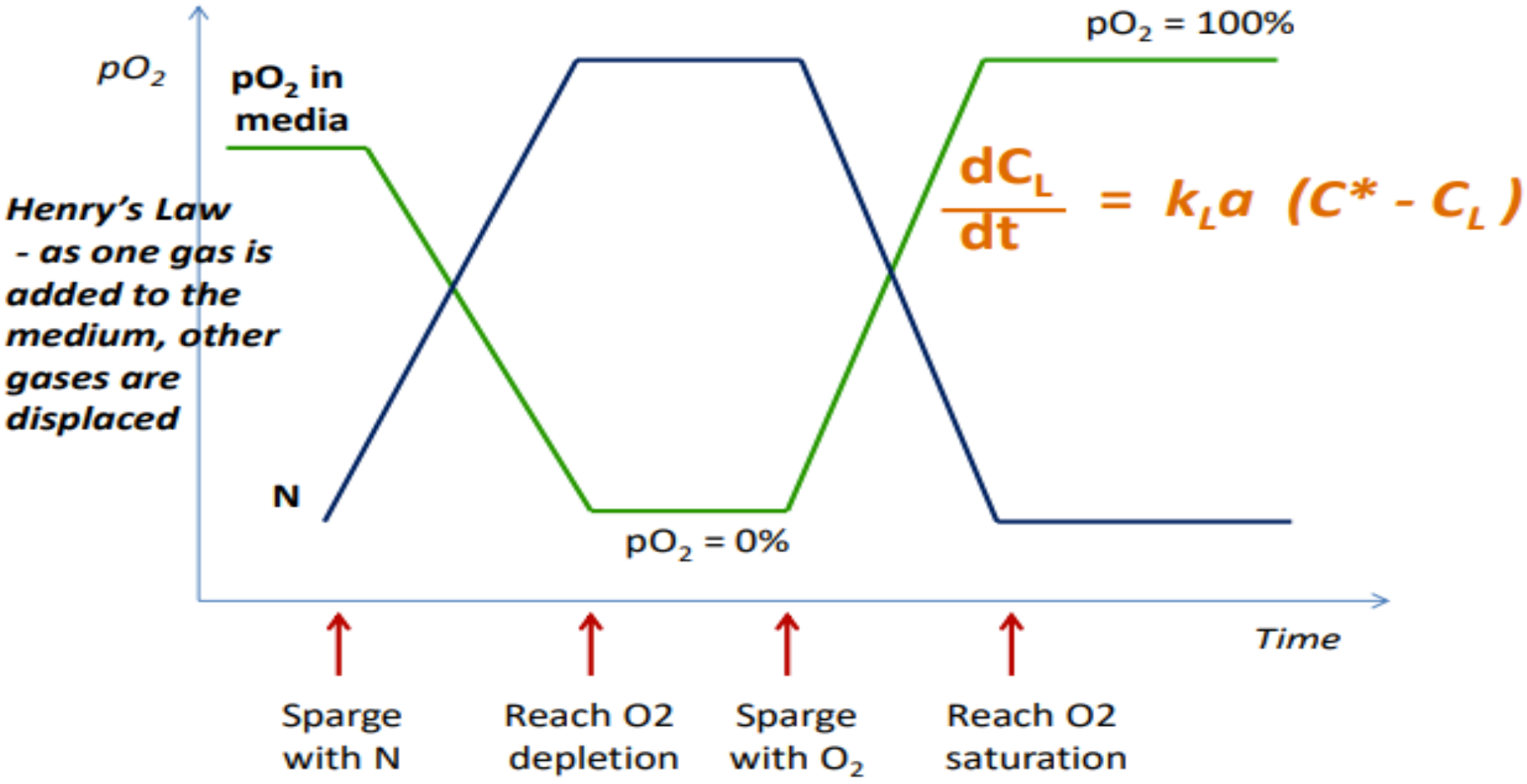
1. Non-steady state (Dynamic Response) method.
2. Steady state method (used mainly for microbial).
3. Chemical reaction method e.g. sulphite (rarely used).

Methods for Measuring Bioreactor kLa

- **Dynamic Response Method.**
  - Fill reactor with water or media solution.
  - Determine C* - solubility of gas under normal operating conditions (temp., pH, pressure gas composition, media composition etc.)
  - Sparge with N_2 to calibrate pO_2 electrode to 0%.
  - Sparge with air / O_2 to calibrate pO_2 electrode for 100% saturation.
  - Sparge with N2 to provide pO_2 of 0%.
  - Sparge with air or O_2 and measure slope of increase in pO_2 with time:

$$\frac{dC_L}{dt} = k_L a\,(C^* - C_L) \quad \text{or} \quad \frac{-d(C^* - C_L)}{dt\,/\,(C^* - C_L)} = k_L a$$

kLa - Dynamic Response Method

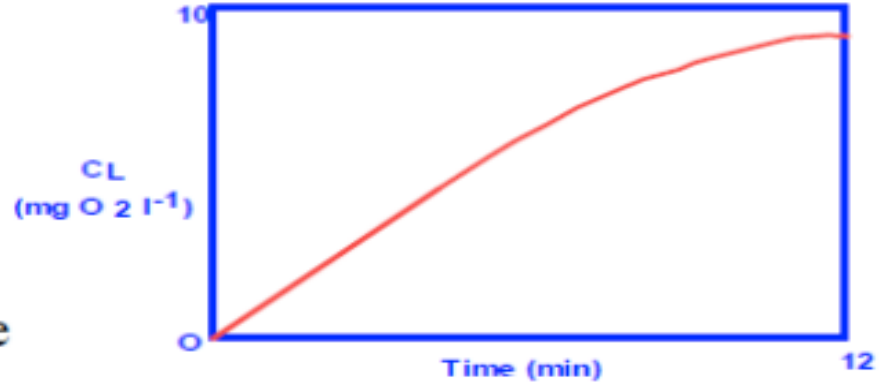


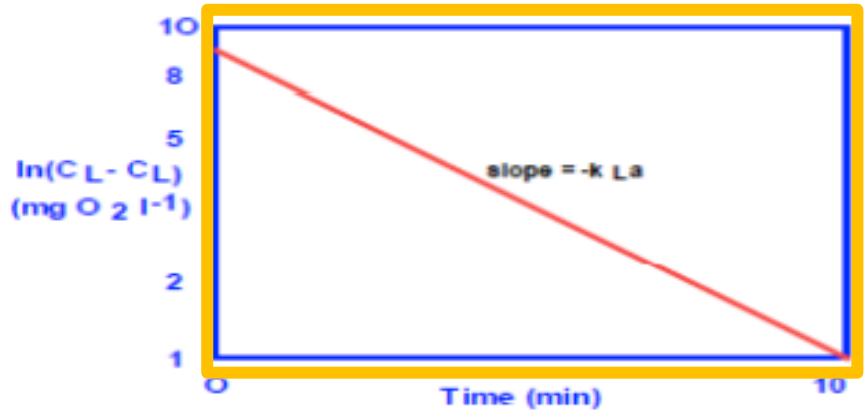
Dynamic Response Method for kLa Determination

$$Ln(C^* - C_L) = -k_L at$$

Note linear form: $y = ax + b$

Plot of $\ln(C^*-C_L)$ versus time gives slope $= k_L a$





CO_2 Mass Transfer Factors

- The sparging strategy for a bioreactor is determined by two main factors as follows:
  - Need to supply adequate O_2 to match the metabolic rate of cells
  - Need to strip off generated CO_2 so as to maintain controlled pH conditions.

CO_2 Mass Transfer Factors

- Small bubble size is good for O_2 transfer but not CO_2 removal
- The optimisation of the gas bubble size (2 - 3mm dia.) can help to minimise the gas flow rate (<0.01 VVM) while achieving the delicate O_2 and CO_2 balancing act within the bioreactor.
- For a mammalian cell application at 500L scale the following parameters are typical:
  - pCO_2: 75mm Hg
  - pO_2: 32mm Hg
  - DO: 40%, pH – 7.20
  - Temp. : 37^oC
  - Gas Flow Rate: 0.01 VVM.

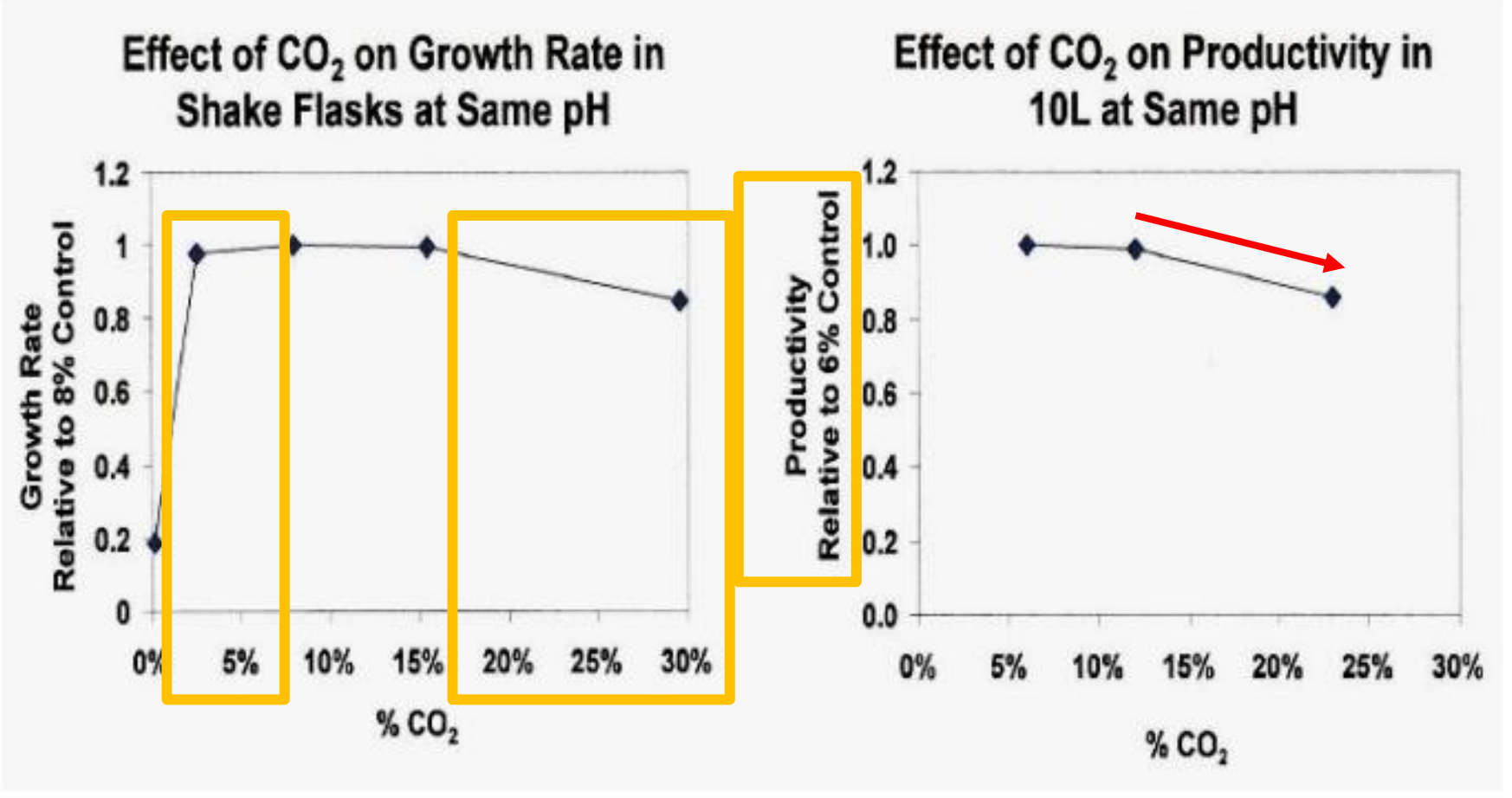
CO_2 Mass Transfer Factors Cont/d

- In a cellular metabolic reaction, there is a direct relationship between the amount of oxygen consumed and the amount of carbon dioxide generated.
- This relationship is known as the Respiratory Quotient (RQ)
  - **RQ = (CO2 produced / O_2 consumed)**
  - A dimensionless number as the units for each are the same e.g. moles of gas.

CO_2 Mass Transfer Factors Cont/d

- The range of respiratory coefficients for organisms in metabolic balance usually ranges from 1.0 (representing the value expected for pure carbohydrate oxidation) to ~0.7 (the value expected for pure fat oxidation).
  - Typical RQ values are 0.7 – 0.8 for mammalian cells.
- The phase of cell growth also has a bearing on RQ values which are higher during exponential phase growth and less during steady-state conditions.

Effects of CO_2 Levels on Cell Productivity



Summary Points

- Cells must generate energy from food to drive metabolic processes including replication and product formation
- Aerobic metabolism produces much higher volumes of ATP energy than anaerobic conditions (36 –v- 2 ATP)
- Oxygen supply to cells is critical for optimal productivity but must be balanced with CO_2 formation
- kLa measures the mass transfer rate for oxygen from gas phase to liquid phase and availability to cells
  - Becomes a critical factor during process development, scale-up and operation

Questions?

Sample Questions

- Glucose is a key nutrient for mammalian cells in culture.

  Comment on the concentrations of glucose required, and how to maximise the available energy it provides to cells during culture.

- kLa is what? Why is it so important for culturing of cells?
- How might you increase the oxygen transfer rate (OTR) in a bioreactor?