


SECTION II: KINETICS AND BIOREACTOR DESIGN:

LESSON 9.4. - Enzymatic kinetics, microbial kinetics and metabolic stoichiometry –Metabolic Stoichiometry

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AIMS FOR TODAY'S LESSON

1.- **STOICHIOMETRY** applied to bioprocesses with cells.

2.- **ELEMENT BALANCE** and stoichiometric coefficient obtaining.

3.- **RESPIRATORY EXCHANGE RATIO** and when can we use it.

4.- **BALANCE of ELECTRONS** and how we can take advantage of it

5.- **YIELDS**

6.- **OTHER DIFFERENT SITUATIONS**

1.- STOICHIOMETRY

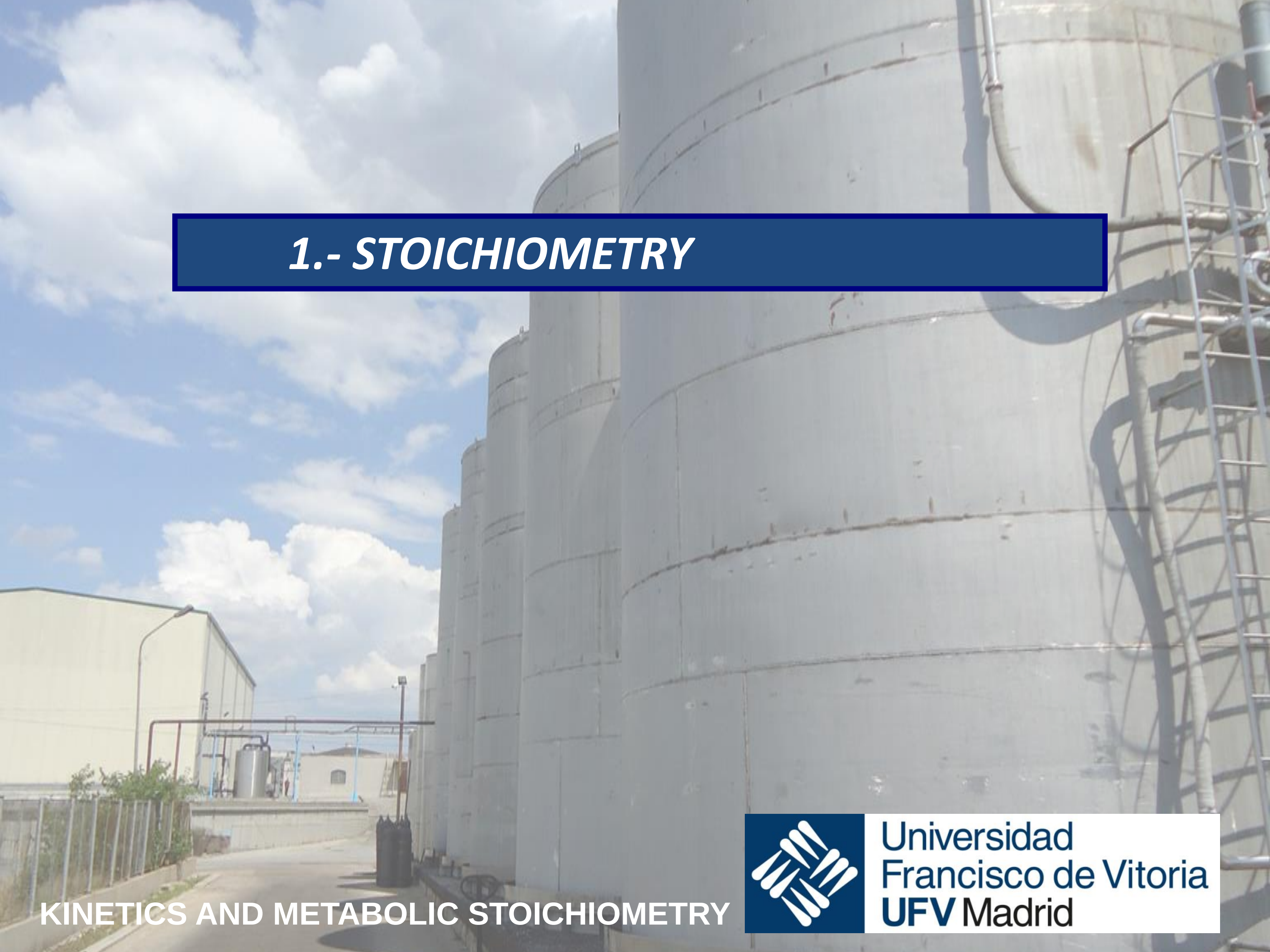
2.- ELEMENT BALANCE

3.- RESPIRATORY EXCHANGE RATIO

4.- BALANCE OF ELECTRONS

5.- YIELDS

6.- OTHER SITUATIONS



1.- STOICHIOMETRY

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1. PROCESS STOICHIOMETRY

Hundreds of reactions involved in metabolism:

- Growth.
- Generation of products.

→ Transformations within cells present **great stoichiometric complexity.**

→ **Mass Conservation Law** works.

→ Simplification of reality is possible using **pseudo-reactions**.

How are these equations written?

How are stoichiometric coefficients estimated?

1. PROCESS STOICHIOMETRY… WHAT FOR?

- Outline **Mass and Energy Balances**.
- Compare **theoretical and real yields** of a bioprocess.
- Check **consistency of experimental bioprocess data**.
- Formulate **medium for growth and / or production** when living cells are used as biocatalysts.
- Obtaining **relationships between yields** based on matter and energy balances of microbial metabolism.

1.- STOICHIOMETRY

2.- ELEMENT BALANCE

3.- RESPIRATORY EXCHANGE RATIO

4.- BALANCE OF ELECTRONS

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2.- ELEMENT BALANCE

2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

➢ ALONG GROWTH:

What elements to be considered?

- Those present in significant amounts➔ **C**, **H**, **O**, **N**

- Others particularly important to describe the process.

Components not included:

-**ATP** nor **NADH** 🡐 not being exchanged with the outside, so they are included as biomass.

- **Vitamins** or **minerals** 🡐 really small uptake.

2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

ONE MOLE OF BIOLOGICAL MATERIAL:

Amount of biomass containing or being equivalent to one mole of carbon.

$$CH_{\alpha}O_{\beta}N_{\delta}$$

2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

ONE MOLE OF BIOLOGICAL MATERIAL:

TABLE 7.3 Date on Elemental Composition of Several Microorganisms

Microorganism	Limiting Nutrient	μ (h^{-1})	Composition (% by wt) C	H	N	O	P	S	Ash	Empirical Chemical Formula	Formula "Molecular" Weight
Bacteria			53.0	7.3	12.0	19.0			8	$CH_{1.666}N_{0.20}O_{0.27}$	20.7
Bacteria			47.1	7.8	13.7	31.3				$CH_2N_{0.25}O_{0.5}$	25.5
Aerobacter aerogenes			48.7	7.3	13.9	21.1			8.9	$CH_{1.78}N_{0.24}O_{0.33}$	22.5
Klebsiella aerogenes	Glycerol	0.1	50.6	7.3	13.0	29.0				$CH_{1.74}N_{0.22}O_{0.43}$	23.7
K aerogenes	Glycerol	0.85	50.1	7.3	14.0	28.7				$CH_{1.73}N_{0.24}O_{0.43}$	24.0
Yeast			47.0	6.5	7.5	31.0			8	$CH_{1.66}N_{0.13}O_{0.40}$	23.5
Yeast			50.3	7.4	8.8	33.5				$CH_{1.75}N_{0.15}O_{0.5}$	23.9
Yeast			44.7	6.2	8.5	31.2	1.08	0.6		$CH_{1.64}N_{0.16}O_{0.52}P_{0.01}S_{0.005}$	26.9
Candida utilis	Glucose	0.08	50.0	7.6	11.1	31.3				$CH_{1.82}N_{0.19}O_{0.47}$	24.0
C. utilis	Glucose	0.45	46.9	7.2	10.9	35.0				$CH_{1.84}N_{0.2}O_{0.56}$	25.6
C. utilis	Ethanol	0.06	50.3	7.7	11.0	30.8				$CH_{1.82}N_{0.19}O_{0.46}$	23.9
C. utilis	Ethanol	0.43	47.2	7.3	11.0	34.6				$CH_{1.84}N_{0.2}O_{0.55}$	25.5

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2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

ONE MOLE OF BIOLOGICAL MATERIAL:

$$CH_{\alpha}O_{\beta}N_{\delta}$$

CELLS	MICROORGANISM	EMPIRICAL CHEMICAL FORMULA
Prokaryote	*Escherichia coli*	$CH_{1,77}O_{0,49}N_{0,24}$
	Klebsiella aerogenes	$CH_{1,75}O_{0,43}N_{0,22}$
		$CH_{1,73}O_{0,43}N_{0,24}$
	Pseudomonas C12 B	$CH_{2,00}O_{0,52}N_{0,23}$
Eukariote	*Saccharomyces cerevisiae*	$CH_{1,64}O_{0,52}N_{0,16}$
		$CH_{1,81}O_{0,51}N_{0,17}$
	Candida utilis	$CH_{1,83}O_{0,54}N_{0,10}$
		$CH_{1,83}O_{0,46}N_{0,19}$

Source: Research Team FQPIMA Dpto. Ingeniería Química UCM

2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

Average Empirical Chemical Formula:

$CH_{1,8}O_{0,5}N_{0,2}$

Molecular weight: 24,6 g/mole dry biomass

(5-10% ashes)

- General equation for aerobic process:

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{células} cCH_{\alpha}O_{\beta}N_{\delta} + dCO_2 + eH_2O$$

CARBON SOURCE

NITROGEN SOURCE

BIOMASS

2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

CARBON SOURCE

NITROGEN SOURCE

BIOMASS

5 STOICHIOMETRIC COEFFICIENTS

- Equation written on the basis of 1 mole of carbon substrate.
- Simplification of reality.
- Good tool for the thermodynamic analysis of the process.

2. STOICHIOMETRIC COEFFICIENTS FROM ELEMENT BALANCES

- Microbial Composition ← elemental analysis.
- Material balance of the elements: C, H, O and N.

PROBLEM: Five unknowns and four balance equations → One more equation is needed.

1. STOICHIOMETRY
2. ELEMENT BALANCE
3. RESPIRATORY EXCHANGE RATIO
4. BALANCE OF ELECTRONS
5. YIELDS
6. OTHER SITUATIONS

3.- RESPIRATORY EXCHANGE RATIO

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3. RESPIRATORY EXCHANGE RATIO

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

C Balance: $w = c + d$

H Balance: $x + b\cdot g = c\cdot\alpha + 2\cdot e$

O Balance: $y + 2\cdot a + b\cdot h = c\cdot\beta + 2\cdot d + e$

N Balance: $z + b\cdot i = c\cdot\delta$

RESPIRATORY EXCHANGE RATIO ← microorganism composition ←

← elemental analysis.

$$\text{RER} = \frac{\text{generated } CO_2}{uptaken\ O_2} = \frac{d}{a}$$

3. RESPIRATORY EXCHANGE RATIO

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{\text{células}} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

CARBON SOURCE | NITROGEN SOURCE | BIOMASS

C Balance: $w = c + d$

H Balance: $x + b \cdot g = c \cdot \alpha + 2 \cdot e$

O Balance: $y + 2 \cdot a + b \cdot h = c \cdot \beta + 2 \cdot d + e$

N Balance: $z + b \cdot i = c \cdot \delta$

- **PROBLEM: water within the medium** ➔ hydrogen and oxygen balances cannot be verified.

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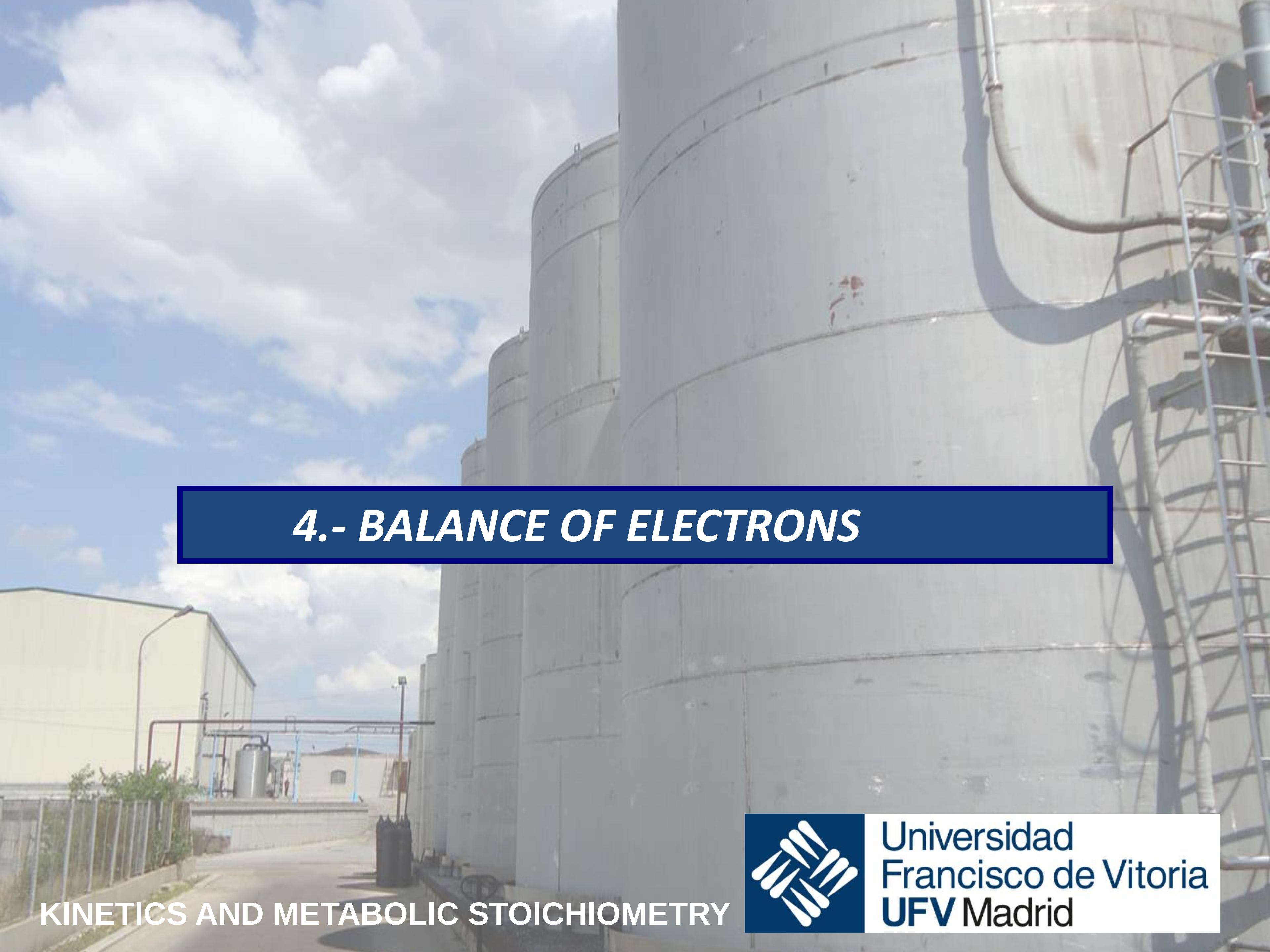

4.- BALANCE OF ELECTRONS

4. BALANCE OF ELECTRONS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

➢ **SOLUTION:**

Perform an ELECTRONIC BALANCE

(or balance of degree of reduction γ)

DEGREE OF REDUCTION γ is the number of equivalents of electrons per mole of carbon.

➔ number of electrons that can be transferred to oxygen by combustion of a hydrocarbon substance, producing carbon dioxide, water and nitrogen-containing products.

4. BALANCE OF ELECTRONS

$$\mathrm{C_wH_xO_yN_z} + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

VALENCES:

C➔ 4; H➔ 1; N➔ (0 (N_2);-3 (ammonium); 5 (nitrate)); O➔ -2; P➔ 5;…

DEGREE OF REDUCTION:

- For an element its degree of reduction is its valence.

- Within a molecule the degree of reduction need to be referred to its number of carbon atoms.

4. BALANCE OF ELECTRONS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

DEGREE OF REDUCTION:

-Methane $CH_4\ \gamma_{met} = \dfrac{1(4)+4(1)}{1} = 8$

-Glucose $C_6H_{12}O_6\ \gamma_{glu} = \dfrac{6(4)+12(1)+6(-2)}{6} = 4$

- Ethanol $C_2H_5OH\ \gamma_{EtOH} = \dfrac{2(4)+6(1)+1(-2)}{2} = 6$

- Substrate $C_wH_xO_yN_z\ \gamma_S = \dfrac{w(4)+x(1)+y(-2)+z(-3)}{w}$

- Biomass $C\ H_\alpha O_\beta N_\delta\ \gamma_b = \dfrac{(4)+\alpha(1)+\beta(-2)+\delta(-3)}{1}$

4. BALANCE OF ELECTRONS

TABLE 7.4 Degree of Reduction and Weight of One Carbon Equivalent of One Mole of Some Substrates and Biomass

Compound	Molecular Formula	Degree of Reduction, γ	Weight, *m*
Biomass	$CH_{1.64}N_{0.16}O_{0.52}$ $P_{0.0054}S_{0.005}$[a]	4.17 (NH_3) 4.65 (N_2) 5.45 (HNO_3)	24.5
Methane	CH_4	8	16.0
n-Alkane	$C_{15}H_{32}$	6.13	14.1
Methanol	CH_4O	6.0	32.0
Ethanol	C_2H_6O	6.0	23.0
Glycerol	$C_3H_8O_3$	4.67	30.7
Mannitol	$C_6H_{14}O_6$	4.33	30.3
Acetic acid	$C_2H_4O_2$	4.0	30.0
Lactic acid	$C_3H_6O_3$	4.0	30.0
Glucose	$C_6H_{12}O_6$	4.0	30.0
Formaldehyde	CH_2O	4.0	30.0
Gluconic acid	$C_6H_{12}O_7$	3.67	32.7
Succinic acid	$C_4H_6O_4$	3.50	29.5
Citric acid	$C_6H_8O_7$	3.0	33.5
Formic acid	CH_2O_2	2.0	46.0
Oxalic acid	$C_2H_2O_4$	1.0	45.0

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4. BALANCE OF ELECTRONS

DEGREE OF REDUCTION:

- Substrate $C_wH_xO_yN_z \; \gamma_S = \dfrac{w(4)+x(1)+y(-2)+z(-3)}{w}$
- Biomass $C\;H_\alpha O_\beta N_\delta \; \gamma_b = \dfrac{(4)+\alpha(1)+\beta(-2)+\delta(-3)}{1}$

$$\mathrm{C_wH_xO_yN_z} + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

-As a result:

$$w\cdot(\gamma_S) + 2a(-2) = c\cdot(\gamma_b)$$

4. BALANCE OF ELECTRONS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

FIVE UNKNOWNS; How many equations?

1 **C Balance:** $w = c + d$

2 **N Balance:** $z + b \cdot i = c \cdot \delta$

3 **RESPIRATORY EXCHANGE RATIO:** $RQ = \dfrac{d}{a}$

4 **REDUCTION POWER BALANCE:** $w \cdot (\gamma_S) + 2 \cdot a(-2) = c \cdot (\gamma_b)$

We still need One equation

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5.- YIELDS

5. YIELD FROM SUBSTRATE TO BIOMASS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

FIVE UNKNOWNS; How many equations?

1 **CARBON BALANCE:** w= c + d

2 **NITROGEN BALANCE:** z + b·i = c·δ

3 **RESPIRATORY EXCHANGE RATIO:** $RQ = \frac{d}{a}$

4 **REDUCTION POWER BALANCE:** $w\cdot(\gamma_S) + 2\cdot a(-2) = c\cdot(\gamma_b)$

5 **YIELD FROM SUBSTRATE TO BIOMASS, $Y_{x/s}$**

5. YIELD FROM SUBSTRATE TO BIOMASS

$$\mathrm{C_wH_xO_yN_z} + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

YIELD FROM SUBSTRATE TO BIOMASS, $Y_{x/s}$

It depends on:

- The composition of the culture medium.
- The nature of the carbon source and the source of nitrogen.
- The operating variables: pH, T, aeration, ...
- Yx/s is bigger under aerobiosis than under anaerobiosis

🡰 ≠ final acceptor of electrons.

5. YIELD FROM SUBSTRATE TO BIOMASS

$$\mathrm{C_wH_xO_yN_z} + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

YIELD FROM SUBSTRATE TO BIOMASS, $Y_{x/s}$

- If biomass yield can be considered constant during growth.
- If we know the molecular mass of the substrate.
- If we know the average molecular mass of biomass.

$$Y_{X/S} = \frac{g\ biomass}{g\ substrate} = \frac{c \cdot Mm_{(Biomass)}}{Mm_{(Substrate)}}$$

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5. YIELD FROM SUBSTRATE TO BIOMASS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{cells} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

FIVE UNKNOWNS; How many equations?

1 **CARBON BALANCE:** w= c + d

2 **NITROGEN BALANCE:** z + b·i = c·δ

3 **RESPIRATORY EXCHANGE RATIO:** $RQ = \frac{d}{a}$

4 **REDUCTION POWER BALANCE:** $w \cdot (\gamma_S) + 2 \cdot a(-2) = c \cdot (\gamma_b)$

5 **YIELD FROM SUBSTRATE TO BIOMASS, $Y_{x/s}$** $Y_{X/S} = \frac{c \cdot Mm_{(Biomass)}}{Mm_{(Substrate)}}$

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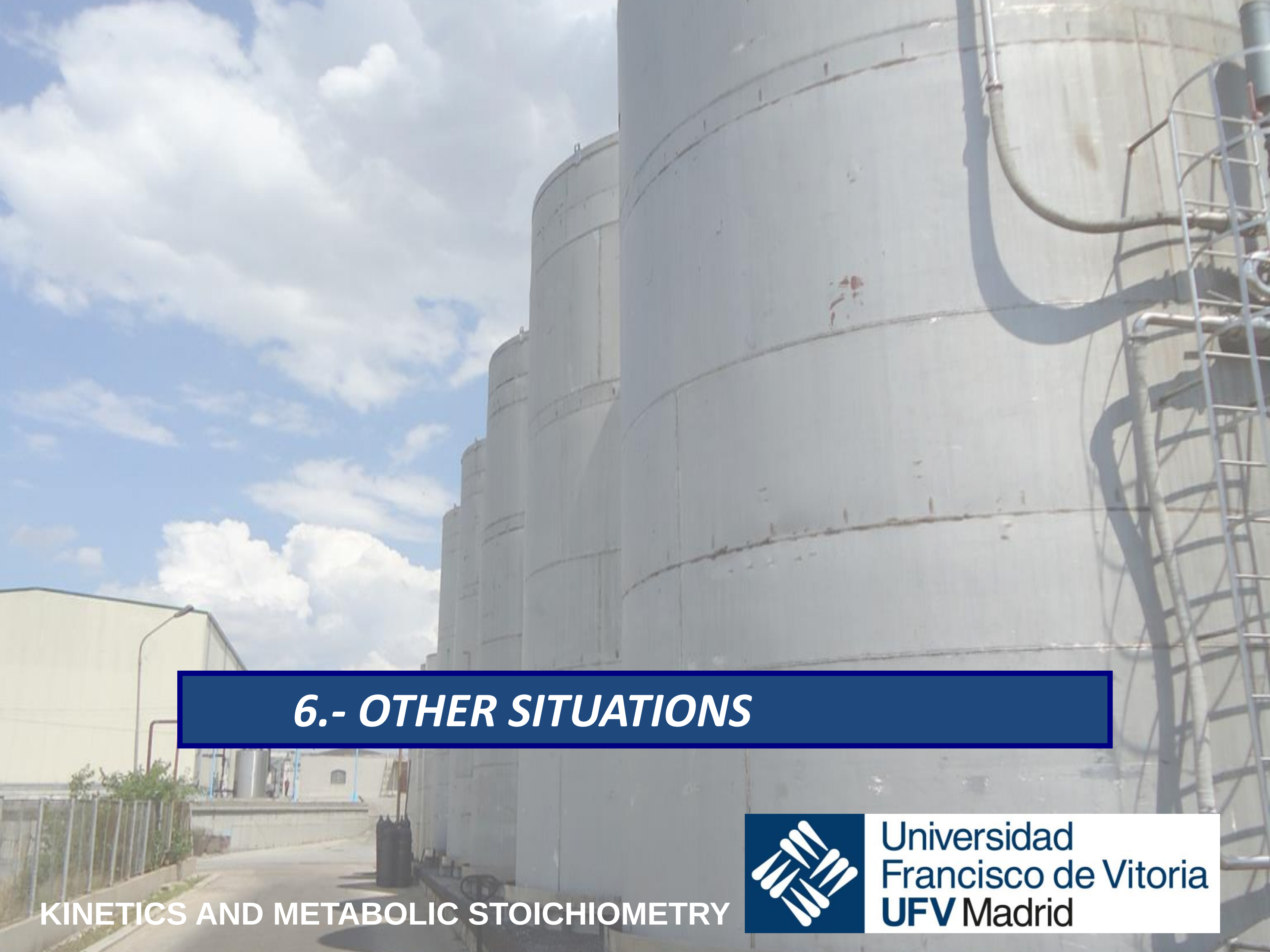


6.- OTHER SITUATIONS

6. AEROBIOSIS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{\text{células}} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

1 **C BALANCE:** w= c + d

2 **N BALANCE:** z + b·i = c·δ

3 **RER:** $RQ = \dfrac{d}{a}$

4 **REDUCTION POWER BALANCE:** $w \cdot (\gamma_S) + a(-2) = c \cdot (\gamma_b)$

5 **BIOMASS YIELD, $Y_{x/s}$** $Y_{X/S} = \dfrac{c \cdot Mm_{(Biomass)}}{Mm_{(Substrate)}}$

7. PRODUCTION DURING AEROBIOSIS

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \xrightarrow{células} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O + fC_jH_kO_lN_m$$

PRODUCT

1 **C BALANCE:** w= c + d+ f·j

2 **N BALANCE:** z + b·i = c·δ+ f·m

3 **RER:** $RQ = \frac{d}{a}$

4 **REDUCTION POWER BALANCE:** $w\cdot(\gamma_S) + 2\cdot a(-2) = c\cdot(\gamma_b) + f\cdot j\cdot(\gamma_p)$

5 **BIOMASS YIELD, $Y_{x/s}$** $Y_{X/S} = \frac{c\cdot Mm_{(Biomass)}}{Mm_{(Substrate)}}$

6 **PRODUCT YIELD, $Y_{p/s}$** $Y_{P/S} = \frac{f\cdot Mm_{(Product)}}{Mm_{(Substrate)}}$

8. GROWTH AND PRODUCTION DURING ANAEROBIOSIS

$$C_wH_xO_yN_z + bH_gO_hN_i \xrightarrow{\text{células}} cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O + fC_jH_kO_lN_m$$

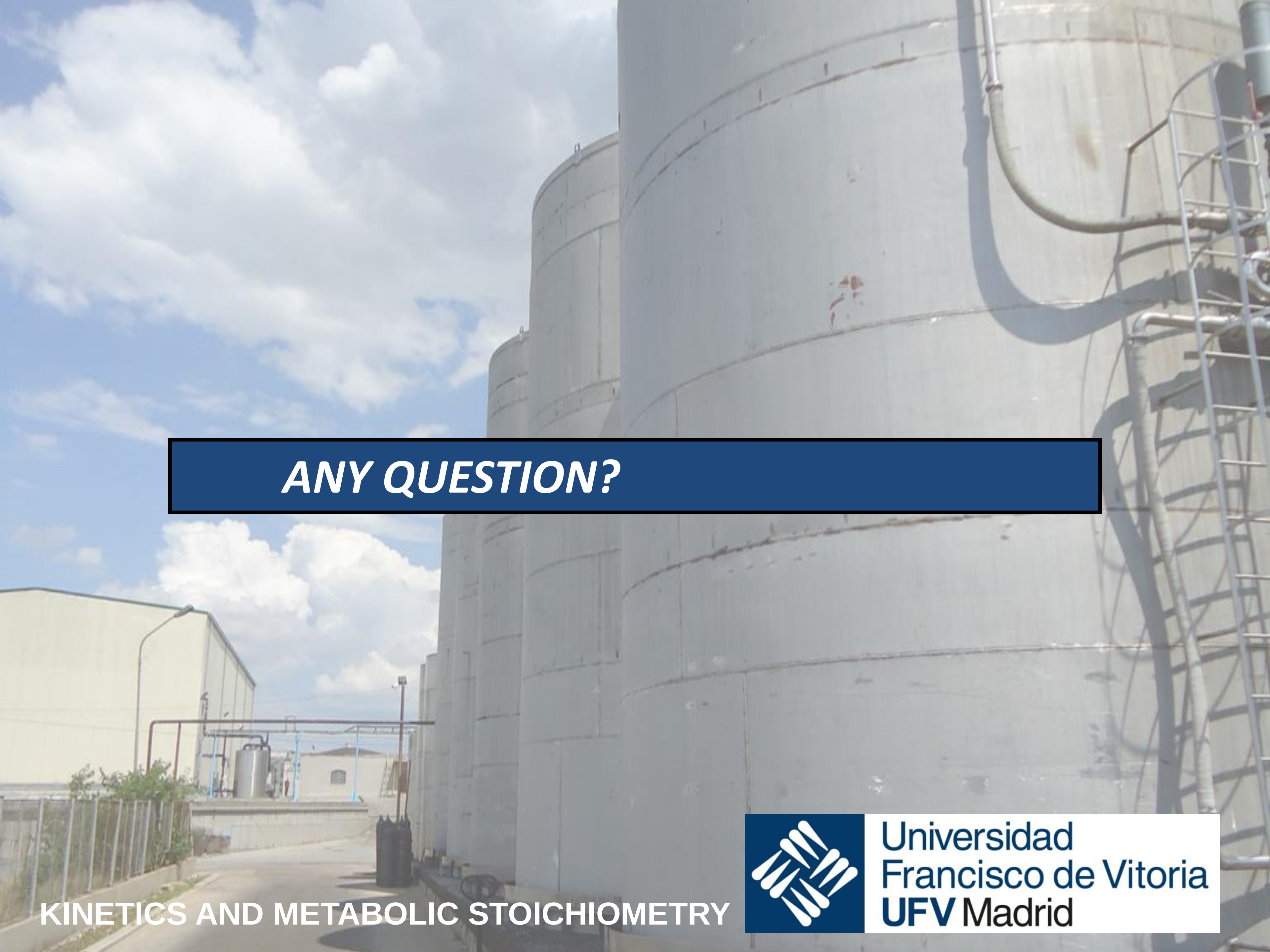
1 **C BALANCE:** w= c + d + f·j

2 **N BALANCE:** z + b·i = c·δ + f·m

3 **REDUCTION POWER BALANCE:** $w\cdot(\gamma_S) + \cancel{2\cdot a(-2)} = c\cdot(\gamma_b) + f\cdot j\cdot(\gamma_p)$

4 **BIOMASS YIELD, $Y_{x/s}$** $$Y_{X/S} = \frac{c\cdot Mm_{(Biomass)}}{Mm_{(Substrate)}}$$

5 **PRODUCT YIELD, $Y_{p/s}$** $$Y_{P/S} = \frac{f\cdot Mm_{(Product)}}{Mm_{(Substrate)}}$$



ANY QUESTION?

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SECTION II: KINETICS AND BIOREACTOR DESIGN:

LESSON 9.4. - Enzymatic kinetics, microbial kinetics and metabolic stoichiometry –Metabolic Stoichiometry

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