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Faculty Of Engineering & Tecxhnology

Bioprocess Engineering BBT-611

Submitted By-
Namrata Singh
Department Of Biotechnology

Fermentation Medium

Most fermentations require liquid media, often referred to as **broth,**

although some solid-substrate fermentations are operated.

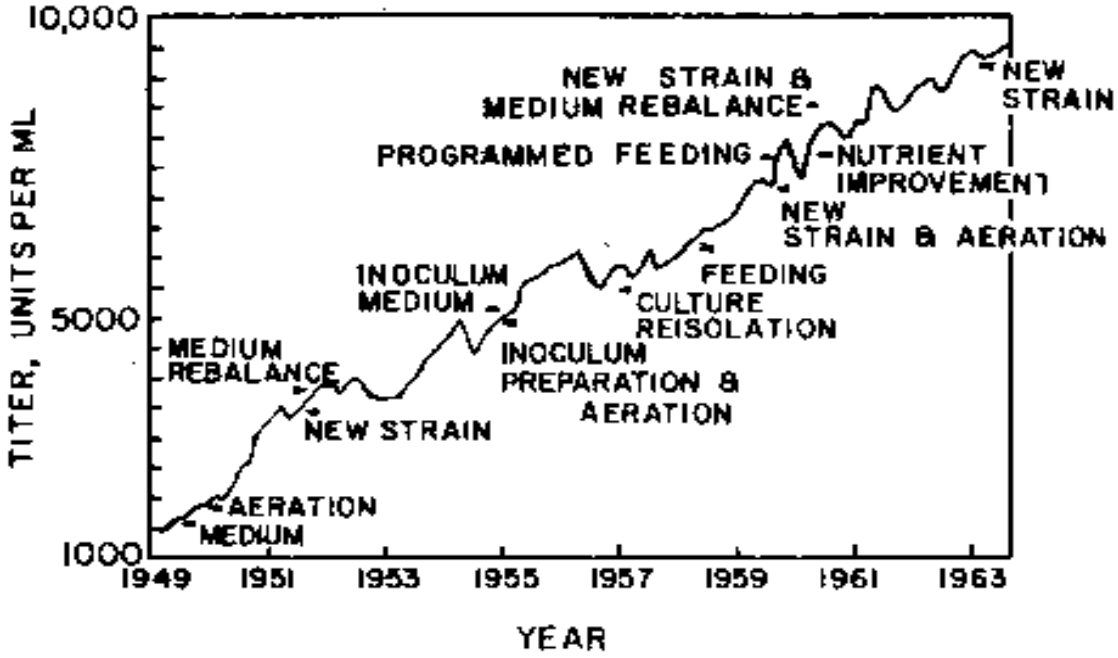


Figure 10.2 Influence of fermentation development studies on a typical antibiotic. (Courtesy of Bristol Laboratories.)

Medium improvement to what degree

- Medium designed for the initial production of antibiotic usually does not have to be developed very skillfully since the potential for antibiotic production is quite low with wild-type strains.
- Media for ultra-high antibiotic-producing strains, which have been developed through repeated genetic manipulations, must be formulated with utmost care.
- In the past, strain improvement and media development were the responsibilities of different research groups. Today we know that each higher-producing clone, after mutation and screening, requires a medium optimized for its performance.

Constituents of medium

- Water
- Carbon source / Nitrogen source / Sources of phosphorous and sulfur / Minor and trace elements / Vitamins such as biotin and riboflavin
- Oxygen: even some anaerobic fermentations require initial aeration, e.g. beer fermentations
- Buffers or controlled by acid and alkali additions
- Antifoam agents
- Precursor, inducer or inhibitor compounds

Nutritional requirements

- Nutritional requirements include elemental, specific nutrient, and energy requirements
- Elemental requirements : the stoichiometry for growth and product formation

  C-source + N-source + O_2 + minerals + specific nutrients $\rightarrow$ cell mass + product + CO_2 + H_2O + heat
- Specific nutrient requirements:

  Auxotroph: To use a complex medium or to identify the specific nutrient

Table 9. The Amounts of B Vitamins in Bacteria[a, b]

Vitamin	*Aerobacter aerogenes*		*Pseudomonas fluorescens*		*Clostridium butylicum*	
	Cells	Medium	Cells	Medium	Cells	Medium
Thiamine	11	8.9	26	48	9.3	30
Riboflavin	44	110	67	310	55	180
Nicotinic acid	240	390	210	350	250	1680
Pantothenic acid	140	640	91	220	93	225
Pyridoxin	6.8	20	5.7	70	6.2	17
Biotin	3.9	44	7.1	61	—	—
Folic Acid	14	91	8.8	66	2.8	16
Inositol	1400	—	1700	—	870	—

[a] The data (from R.C. THOMPSON, Texas Univ. Publ. 4237:87 (1972) are based on microbiological assays on cells and medium from cultures in vitamin-free media (except for *C.butylicum*, which requires biotin).
[b] All figures are in μg/gm cell dry weight.

Table 10. Average Amino Acid Distribution of Eleven Microbial Samples with Standard Deviation from Average

Amino Acid	Percent of Total Essential Amino Acids	Standard Deviation
Histidine	7.22	1.05
Arginine	11.18	0.36
Lysine	15.31	1.01
Leucine	16.57	0.53
Isoleucine	11.34	0.57
Valine	11.85	0.37
Methionine	3.81	0.31
Threonine	10.23	0.33
Phenylalanine	9.43	0.67

Source: TANNENBAUM et al. (1978)

Elemental requirements

- Main elemental formula of microbial cells $C_4H_7O_2N$ (dry weight basis 48% C, 7% H, 32% O, 14% N), e.g. Baker's yeast $C_{3.2}H_{6.2}O_{1.9}N_{0.6}S_{0.02}P_{0.03}K_{0.06}$
- Average: C 45-55%, N 6-14%, K 0.5-2%, P 1-3%, Mg 0.1-1%, S 0.02-1%, minor minerals (mg /100g cell) Cu 0.1-1, Fe 1-10, Zn ~1, Mn 0-5 (e.g. 10g/L of cell mass containing 0.4% magnesium will require at least 0.04 g/L of Mg or 0.2 g/L of $MgSO_4$ or 0.4 g/L of $MgSO_4 \cdot 7H_2O$)
- Chemical composition of fermentation product
- Typical concentration of fermentation products in the broth (dry wt / vol, %): lactic acid (13), citric acid (12), glutamic acid (10), ethanol (8), baker's yeast (5), benzyl penicillin (3), riboflavin (1), vitamin B_{12} (0.002)

Table 6. Chemical Composition of Major Fermentation Products

Antibiotics	
Bacitracin	$C_{66}H_{103}N_{17}O_{16}S$
Cephelosporin C	$C_{16}H_{21}N_3O_8S$
Erythromycin	$C_{37}H_{67}NO_{13}$
Penicillin G	$C_{16}H_{18}N_2O_4S$
Streptomycin	$C_{21}H_{39}N_7O_{12}$
Organic Acids	
Citric acid	$C_6H_8O_7$
Gluconic acid	$C_6H_{12}O_7$
Lactic acid	$C_3H_6O_3$
Solvents and Chemicals	
Acetone	C_3H_6O
Butanol	$C_4H_{10}O$
Ethanol	C_2H_6O
Vitamins and Amino Acids	
B_{12}	$C_{63}H_{88}CoN_{14}O_{14}P$
Riboflavin	$C_{17}H_{20}N_4O_6$
Glutamic acid	$C_5H_9NO_4$
Lysine	$C_6H_{14}N_2O_2$
Tryptophan	$C_{11}H_{12}N_2O_2$

Table 7. Elemental Composition of Microorganisms

Organism	% of Dry Cell Weight				
	C	H	O	N	S
Saccharomyces cerevisiae	45	6.8	30.6	9.0	
Methylomonas methanolica	45.9	7.2		14.0	2.6
Penicillium chrysogenum	43	6.9	35	8	

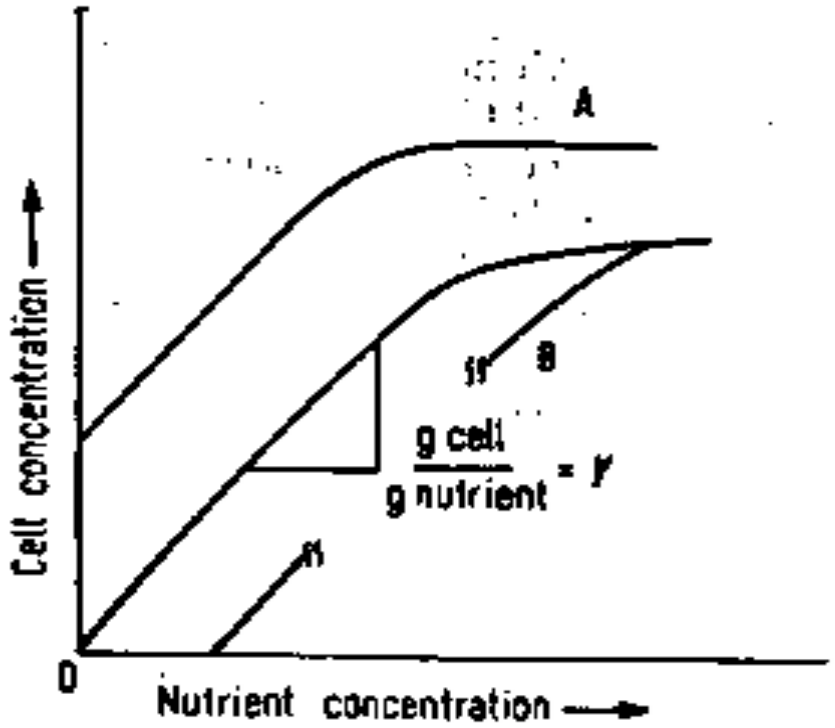


Figure 27. Dependence of final cell concentration on nutrient concentration. Curve A results when some nutrient is carried over from the seed culture. Curve B results from the need for a critical concentration of the nutrient.

Table 11. Summary of Cellular Yield Coefficients on Selected Carbon-Energy Sources

Carbon-Energy Source	Cellular Yield	(gm cell/ gm substrate)	
Glucose	0.5	g/g C	1.3
Methanol	0.5		1.3
Ethanol	0.7		1.3
Methane	0.62		0.8
n-Alkanes ($C_{16}H_{34}$)	1.0		1.2
Cellulose	0.5		1.3
Starch	0.5		1.3
Benzene	0.6		0.6

Environmental requirements

- Effect of growth temperature on cell yield / below optimal temperature for growth
- Effect of water activity ($A_w = P_s/P_w$) on growth rate, vapor pressure of water in solution (P_s) or in pure water(P_w)
- Combined effect of temperature and pH on growth / opt pH for growth and production is not always the same
- Environmental effect of substrate

Environmental effect of substrate

- Substrate concentration: Monod equation, $\mu = \mu_m S / (K_s + S)$

  K_s for C-source 1 ~ 10 mg/L, when S = 10 ~ 100 mg/L, $\mu \approx \mu_m$; K_s for amino acid 0.003 ~ 0.2 mg/L; K_s for ammonia 0.1 ~ 1.0 mg/L
- Substrate inhibition: carbohydrate 50 to 100 ~ 150 g/L (osmotic pressure); phenol, toluene, butanol a few g/L (damage cell membrane); ammonia 3 ~ 5 g/L
- Catabolite repression
- $NO_3^- \rightarrow NO_2^-$ toxic effect
- Phosphate repression and sulfate repression

Molasses

- Byproduct of cane or beet sugar production / residues remaining after most of the sucrose has been crystallized from the plant extract
- Dark colored viscous syrup containing 50-60% (w/v) carbohydrate, primarily sucrose, with 2% (w/v) nitrogenous substances, along with some vitamins and minerals.
- Overall composition varies depending upon the plant source, the location of the crop, the climatic conditions under which it was grown, and the factory where it was processed
- The carbohydrate concentration may be reduced during storage by contaminating microorganisms
- Hydrol molasses, containing primarily glucose, is a byproduct of maize starch processing

Table 4.1 Composition of sugar beet and sugar cane molasses

Composition	Sugar beet molasses	Sugar cane molasses
Dry matter %	78 –85	77 –84
Sucrose	48.5	33.4
Raffinose	1.0	–
Invert sugar	1.0	21.2
Miscellaneous organic materials	20.7	19.6
N	0.2 – 2.8	0.4 – 1 5
P_2O_5	0.02 – 0.07	0.6 – 2.0
CaO	0.15 – 0.7	0.1 – 1.1
MgO	0.01 – 0.1	0.03– 0.1
K_2O	2.2 4.5	2.6 – 5.0
SiO_2	0.1 – 0.5	–
Al_2O_3	0.005 0.06	–
Fe_2O_3	0.001– 0.02	
Ash	4 8	7 –11
Thiamine µg/100 g dry weight	130	830
Riboflavin	41	250
Pyridoxine	540	650
Niacinamide	5100	2100
Pantothenic acid	130	2140
Folic acid	21	3 8
Biotin	5.3	120

(Rhodes and Fletcher, 1966; Imre, 1969)

Malt extract

- Concentrated aqueous extracts of malted barley to form syrups / particularly useful for the cultivation of filamentous fungi, yeasts and actinomycetes
- App. 90% carbohydrate (w/w) and some vitamins and app. 5% nitrogenous substances, proteins, peptides and amino acids / carbohydrate comprising 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (maltose and traces of sucrose), 10% maltotriose, and additionally contain 15-20% branched and unbranched dextrins, which may or may not be metabolized, depending upon the microorganisms
- Careful sterilization to prevent over-heating /Maillard reaction products (brown condensation products resulting from the reaction of amino groups and carbonyl groups) when heated at low pH / color change, loss of fermentable materials, some toxic products

Table 4.2 Typical composition of malt extract

Component	% of Dry weight
Maltose	52.2
Hexoses (glucose, fructose)	19.1
Sucrose	1.8
Dextrin	15.0
Other carbohydrates	3.8
Nitrogenous materials	4.6
Ash	1.5
Water content	2.0

pH (10% solution) = 5.5

Starch and dextrins

- Can be directly metabolized by amylase-producing microorganisms, particularly filamentous fungi
- Maize starch is most widely used
- To allow use in a wide range of fermentations, the starch is usually converted into sugar syrup, containing mostly glucose. It is first gelatinized and then hydrolyzed by dilute acids or amylolytic enzymes, often microbial glucoamylases that operate at elevated temperatures

Sulfite waste liquor

- Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of yeasts
- Waste liquors from coniferous trees contain 2-3% (w/v) sugar, 80% hexoses (glucose, mannose and galactose) and 20% pentoses (mostly xylose and arabinose) / Liquors derived from deciduous trees contain mainly pentoses
- Usually the liquor requires processing before use as it contains sulfur dioxide / The low pH is adjusted with calcium hydroxide or calcium carbonate, and these liquors are supplemented with sources of nitrogen and phosphorus

Cellulose

- Predominantly as lignocellulose (composed of cellulose, hemicellulose and lignin)
- Available from agricultural, forestry, industrial and domestic wastes
- Relatively few microorganisms can utilize it directly / The cellulose component is in part crystalline, encrusted with lignin, and provides little surface area for enzyme attack
- At present, mainly used in solid-substrate fermentations (e.g. mushrooms)
- Potentially a very valuable renewable source of fermentable sugars once hydrolyzed, particularly in the bioconversion to ethanol for fuel use

Alkanes and alcohols

- ***n*-Alkanes** (C_{10}-C_{20}): readily metabolized by certain microorganisms / industrial use is dependent upon the prevailing price of petroleum
- **Methane:** utilized by a few microorganism, but its conversion product methanol is often preferred for industrial fermentations
- High purity **methanol** is readily obtained / completely miscible with water / has a high per cent carbon content and is relatively cheap / only limited organisms will metabolize methanol / only low conc., 0.1-1% (v/v) are tolerated by microorganisms / oxygen demand and heat of fermentation are high, but this is even more problematic when growing on alkanes
- **Ethanol** is less toxic than methanol / used as a sole or cosubstrate / too expensive for general use as a carbon source / its biotransformation to acetic acid remains a major fermentation process

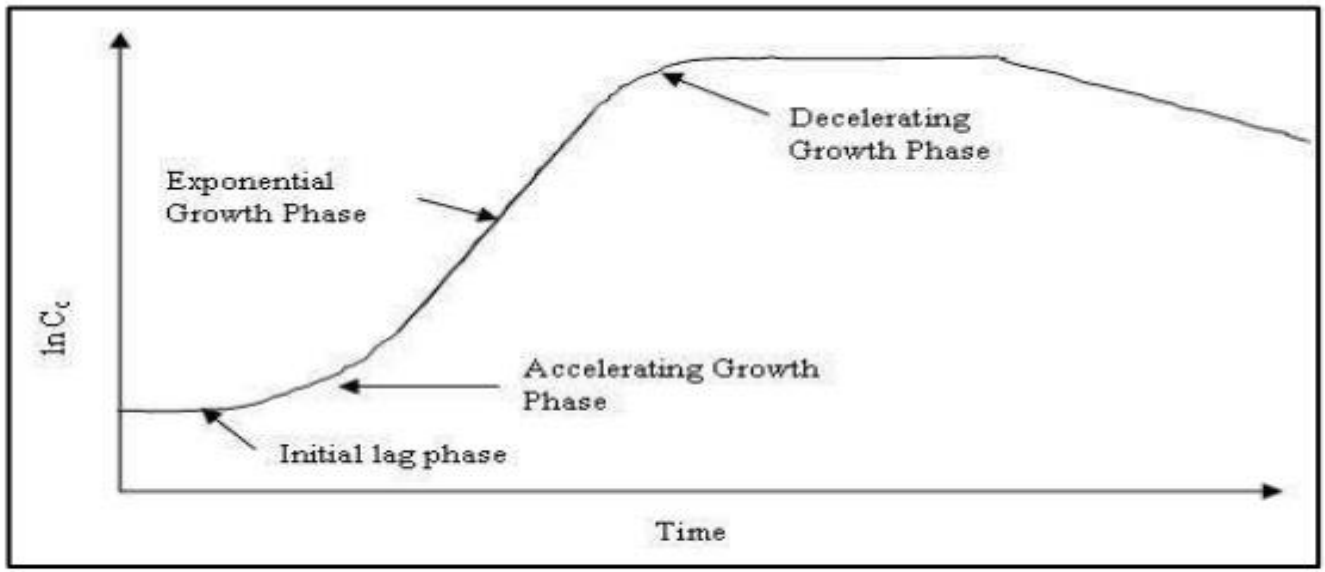
Fats and oils

- Hard animal fats (composed mainly of glycerides of palmitic and stearic acids) are rarely used in fermentation
- Plant oils (primarily from cotton seed, linseed, maize, olive, palm, rape seed and soy) and occasionally fish oil, may be used as the primary or supplementary carbon source, especially in antibiotic production / Plant oils are mostly composed of oleic and linoleic acids, but linseed and soy oil also have a substantial amount of linolenic acid
- Oils contain more energy per unit weight than carbohydrates / Oils can be particularly useful in fed-batch operations than carbohydrates (aqueous solutions less than 50%, w/v; occupy a greater volume)

Kinetics of microbial growth and product formation:

- Microbial growth is the result of both cell division and change in cell size
- Growth – variety of physical, chemical and nutritional conditions
- Conversion of nutrients into biological compounds which are used for energy production and also for biosynthesis and product formation
- Good example for *autocatalytic reaction*

Microbial Batch Growth



Phases of Growth

Lag phase:

- No increase in cell number
- Period of adaptation of cells to a new environment
- **No change in number, but an increase in mass**
- Multiple lag phases may sometimes be observed - more than one carbon source

  (Diauxic growth)....why?
- Length of the lag phase – characteristics of microbial species and in part by the media conditions

Cont….

Log Phase:

- Growth rate is higher
- Increase in cell mass and cell number with time exponentially
- This phase results in straight line… why?
- Hence, it is also known as *Exponential phase.*
- Period of balanced growth, in which all the components of a cell grow at the same rate
- Composition of biomass remains constant

Cont....

- The exponential growth rate is the first order reaction
- The rate of biomass is correlated with the specific *growth rate(μ)* and the biomass concentration or cell number, X
- A measure of the rapidity of growth has dimension T^{-1}

$$dX/dt = \mu.X$$

Integration of the eq. between the limits X_0 at the time t=0 and X at sometime t gives:

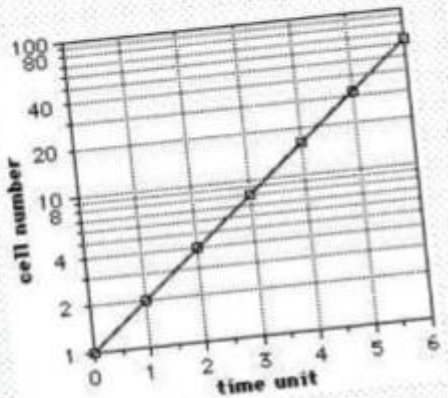
$$\ln (X/X_0) = \mu t \text{ (or) } X=X_0 e^{\mu t}$$

Taking the neutral log,

$$\ln X = \ln X_0 + \mu t$$

Generating a growth curve

By definition, bacterial growth is ***cell*** replication - i.e., growth of the culture. Most species of ***bacteria*** replicate by ***binary fission***, where one cell divides into 2 cells, the 2 cells into 4, the 4 into 8, etc. If this cell division occurs at a steady rate - such as when the cells have adequate ***nutrients*** and compatible growing conditions - we can plot numbers of cells vs. time such as on the graph at right. Before too long, we will need to extend the paper vertically as the population continues to double. For a culture where cells divide every 20 minutes, one cell can result in 16,777,216 (i.e., 2^{24}) cells after just 8 hours - barring nutrient depletion or other growth-altering conditions.



Graphing of bacterial growth with cell number on a log scale.

Cont....

- The exponential phase is followed by *deceleration phase,* period of unbalanced growth.
- In this phase, the growth decelerates due to either depletion of one or more essential nutrients or the accumulation of toxic by products of growth

Stationary phase:

- It starts, when the net growth rate is *zero*

  Growth rate = Death rate

- Even though the net growth rate is zero during the stationary phase, cells are metabolically active and produce secondary metabolites

Death phase

Number of cells multiplying = number of cells dying

Kinetics of death phase

Cell death is the first order process

$$r_d = K_d N \text{ , where}$$

R_d = rate of cell death

N = number of viable cells

K = specific death constant

In closed system, rate of cell death is equal to the rate of decrease in cell number. So, the above equation gives

$$r_d = dN/dt = k_d N$$

If k_d is constant, $N = N_0 e^{-kdt}$

Taking natural log, **$\ln N = \ln N_0 - k_d t$**

EFFECT OF SUSTRATE CONCENTRATION IN BATCH CULTURE

- The specific growth rate is generally found to be a function of *three parameters*

1. The concentration of *growth limiting substrate, S*
2. *The maximum specific growth rate,* μ_{max}
3. A *substrate - specific constant,* K_s

$$\mu = \mu_{max} / K_s + S \text{ (MONOD EQUATION)}$$

Specific growth rate is independent of substrate concentration as long as excess substrate is present.

Taking the reciprocal values in the monod equation and rearranging it:

$$1/\mu_{max} = K_s + S / \mu_{max}\ S \text{ (or)}$$

$$1/\mu = (K_s / \mu_{max} \cdot 1/S) + 1/\mu_{max}$$

The plot of **1/ μ against 1/S** produces a straight line with intercept on the y axis at **$1/\mu_{max}$ and slope equals to K_s / μ_{max}**

CONTINUOUS CULTURE

- Substrate concentration and other conditions remain constant, and the cells grow at a constant, fully acclimatised exponential rate on the effluent.
- Defining characteristic of continuous culture is *a perpetual feeding process.*
- The reaction variables and control parameters remain consistent, establishing a time-constant state within the reactor.

CONTINUOUS GROWTH KINETICS

- The actual growth rate depends not only on the volumetric flow rate of the medium into the reactor, but also on the dilution rate(D)

$$D = F/V$$

The net change in the cell concentration over a period of time may be expressed as:

$$dX/dt = \text{rate of growth in reactor} - \text{rate o loss from reactor}(\mu X - Dx)$$

Under steady state conditions, the rate of growth = rate of loss

$$dX/dt = 0$$

Therefore, $\mu X = DX$ & $\mu = D$

Cont....

- For any given dilution rate, under steady state conditions, the residual substrate concentration in the reactor can be predicted by substituting D for μ in the Monod equation

$$D = \mu_{max} S_r / K_s + S_r$$

where S_r = steady state residual substrate concentration in the reactor at the fixed dilution rate. Rearrangement gives,

$$D(K_s + S_r) = \mu_{max} S_r \text{ or } DK_s + DS_r = \mu_{max} S_r$$

Dividing by S gives,

$$DK_s / S_r + D = \mu_{max}$$

hence,

$$S_r = DK_s / \mu_{max} - D$$

Cont.....

- Thus growth is controlled by the availability of a *rate-limiting nutrient*
- *Chemostat* – system where the concentration of the *rate-limiting nutrient* entering the system is fixed.
- *Turbidostat* – nutrients in the medium are not limited, cell concentration is held constant(?)

Mode of operation	Advantages	Disadvantages
Batch	Versatile: can be used for different reactions every day. Safe: can be properly sterilized. Little risk of infection or strain mutation Complete conversion of substrate is possible	High labor cost: skilled labor is required Much idle time: Sterilization, growth of inoculum, cleaning after the fermentation Safety problems: when filling, emptying, cleaning
Continuous	Works all the time: low labor cost, good utilization of reactor Often efficient: due to the autocatalytic nature of microbial reactions, the productivity can be high. Automation may be very appealing Constant product quality	Often disappointing: promised continuous production for months fails due to a. infection. b. spontaneous mutation of microorganisms to non producing strain Inflexible: can rarely be used for other productions without substantial retrofitting

SOLID-STATE FERMENTATION FOR THE SYNTHESIS OF CITRIC ACID BY *ASPERGILLUS NIGER*

Abstract

- Solid-state fermentation was carried out to evaluate three different agro-industrial wastes, sugar cane bagasse, coffee husk and cassava bagasse for their efficiency in production of citric acid by a culture of *Aspergillus niger*. Cassava bagasse best supported the mould's growth, giving the highest yield of citric acid among the tested substrates. Results showed the fungal strain had good adaptation to the substrate (cassava bagasse) and increased the protein content (23 g/kg) in the fermented matter. Citric acid production reached a maximum (88-g/kg dry matter) when fermentation was carried out with cassava bagasse having initial moisture of 62% at 26°C for 120 h.

METHODS

- *Micro-organisms* - Seven strains of *A.niger*, one strain, NRRL 2001, was chosen.
- *Inoculum* - *A.niger* spores were produced in Czapeck Dox Broth with agar (50 ml) in a 250 ml Erlenmeyer flask
- *Substrate* - Three solid materials, sugar cane bagasse, co€e husk and cassava bagasse were tested
- *Fermentation* - Fermentation was carried out in vertical column fermenter
- *Analytical methods*
  Samples (5 g) were mixed well with 50 ml of distilled water to extract citric acid and sugars. The ®ltrate so obtained was subjected to high performance liquid chromatograph analysis using a Shimadzu LC-10AD HPLC. A temperature of 60°C and 5 mM H_2SO_4 as the mobile phase at a ¯ow-rate of 0.6 ml/min were used.

Table 1

Kinetics of citric acid production with cassava bagasse (120 h)

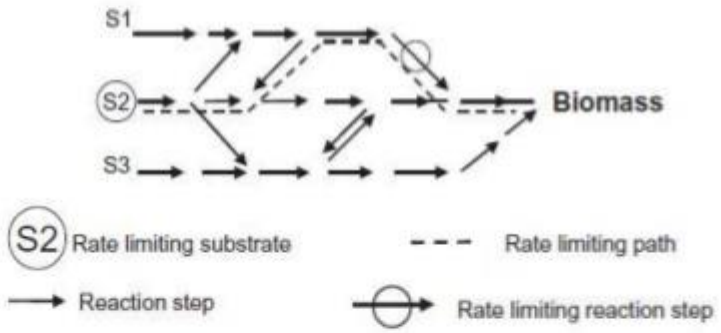
Time (h)	pH	True protein g/kg (DM)	Humidity %	Residual sugars g/kg (DM)	Residual starch g/kg (DM)	Citric acid g/kg (DM)
0	2.0	13.1	62.1	450.5	405.5	0.0
24	1.7	13.7	63.5	369.5	332.9	13.7
48	1.5	14.9	63.6	285.2	256.7	22.5
72	1.4	18.3	64.2	116.2	104.6	56.2
96	1.3	22.2	64.5	56.9	51.2	70.9
120	1.2	23.1	64.8	55.9	50.8	88.1

RESULTS

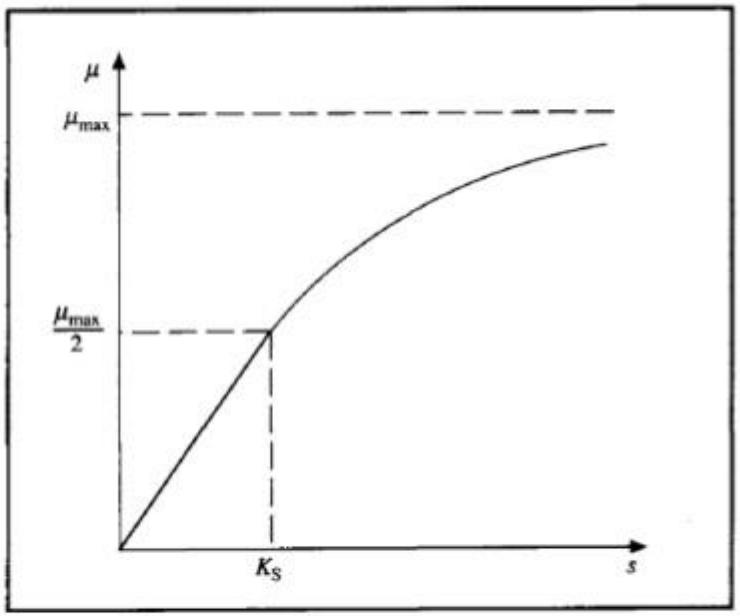
- shows the pattern of fungal growth as monitored by protein content in the fermenting substrate and change in moisture content (humidity) during the 120 h of fermentation. Protein content increased from 13 to 23 g/kg, showing more than 90% increase. There was not much change in the moisture content of the fermenting matter during the course of fermentation . The table also shows data on residual sugars and starch, available in the substrate to A. Niger. A comparison between residual sugars and starch showed that there was a good proportionate utilization pattern of starch and sugars, which indicated good efficiency of the fungal culture

Monod kinetics for growth

- Cell metabolism is made up of hundreds of sequential, branched and parallel biological reactions that are normally catalysed by enzymes.



- We can assume that growth is the result of hundreds of such enzyme-catalyzed reactions.


μ
μmax
μmax/2
KS
s

- The relationship between specific growth rate and limiting substrate concentration proposed by Monod states that:

$$\mu = \mu_{max} \frac{S}{(K_S + S)}$$

- μ and μ_{max} :specific and maximun specific growth rate respectively
- S : limiting substrate concentration
- Ks : saturation constant

- When substrate concentration is not limited, when $S >> K_s$ numerically, K_s can be ignored
- Specific growth rate approaches umax, and growth rate becomes independent of S and only proportional to cell concentration.
  - $\mu = \mu_{max}$

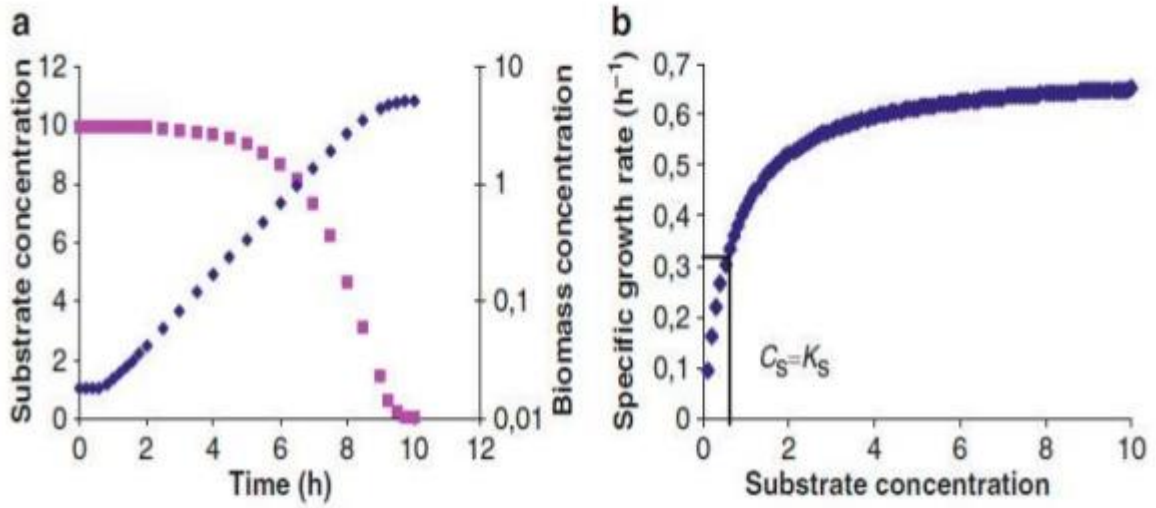
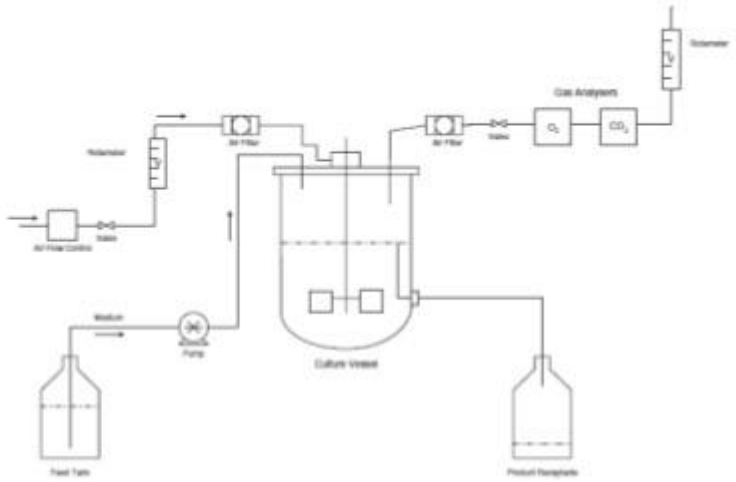


Fig. 7.2 Typical biomass and substrate concentrations profiles during a batch culture. (a) The time profile of the biomass concentration (*filled diamond*) and of the limiting substrate concentration (*filled square*). (b) A cross plot of the specific growth rate (=d(ln x)/dt) versus the substrate concentration. Corresponding values of μ and s are taken from (a). The value of K_s is indicated

Dilution rate



- The dilution rate (D) describes the relationship between the flow of medium into the bioreactor (F) and culture volume within the bioreactor (V):
  - **D=F/V**

- The change on cell concentration over a period of time, can be expressed as:

$$dx/dt = \mu x - Dx$$

- During stationary conditions, cell concetration remains constant, so $dx/dt = 0$ then:

$$\mu x = Dx$$

$$\mu = D$$

- In stationary state, specific growth rate is controlled by dilution rate (D), which is an experimental variable
- Continuos culture must be operated ath dilution rates **below** specific growth rates
- Dilution rate can be used for controlling growth of the cultrue

- Cell growth depends on growht limiting subrate, therefore, growth is expressed as:

$$\mu = \mu_{max} s / k_s + s$$

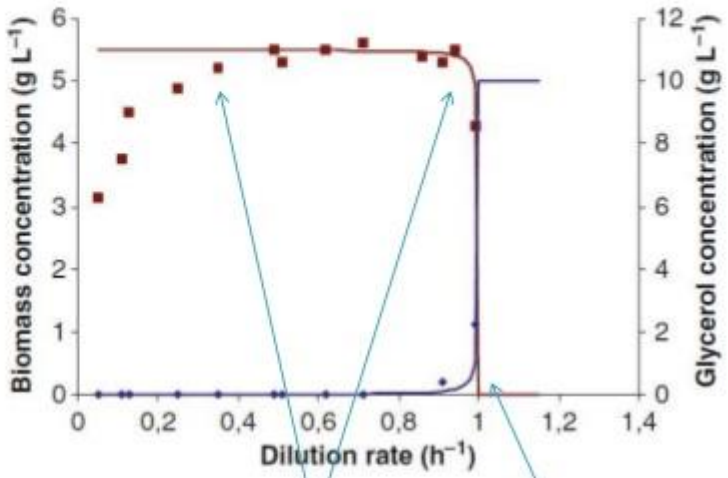
- In stationary condictions: $\mu = D$, therefore:

$$D = \mu_{max} s / k_s + \bar{s}$$

Exercise:

- A quimiostat is operating in stationary state which has a dilution rate of 0.30 h^{-1} with a limiting substrate concentration of 0.06 mM L^{-1}. Determine Monod constant if the umax for the organism is 0.25 h^{-1}.

Fig. 7.3 Growth of *Aerobacter aerogenes* in a chemostat with glycerol as the limiting substrate. The lines are model calculations using the simple Monod model, (7.16). The data are taken from Herbert (1959)



$$D_{max} = \mu_{max} \frac{s_f}{s_f + K_s}.$$

$\mu_{max} = 1.0\ h^{-1}$
$K_s = 0.01$ g glycerol L^{-1}
$Y_{sx} = 0.53$ g DW (g glycerol)$^{-1}$

Constant biomass concentration

Low glycerol concentration

Problem:

- From measurements of the residual glucose concentration in a steady-state chemostat at various dilution rates, you can find the following results:

$D\ (h^{-1})$	$s\ (mg\ L^{-1})$
0.13	11
0.19	14
0.23	18
0.36	38
0.67	85
0.73	513

- Calculate by linear regression the parameters in the Monod Model. Are any of the data points suspect?

Wash out and Critical dilution rate

- The outlet limiting substrate concentration is independent of the input limiting substrate concentration.

- At a fixed dilution rate, called the critical dilution rate, the cell concentration drops to the constraint :

$$x_{vo} = 0$$

- And the limiting substrate concentration reaches the upper constraint:

$$S_0 = S_i$$

x_{v0} = concentración de celulas viables inicial

- Después de esta velocidad de dilución crítica, se dice que las células están siendo lavadas (wash out), ya que están saliendo del bioreactor a **una velocidad mayor que el crecimiento**

- Asumiendo que el crecimiento se comporte de acuerdo a la cinética de Monod, la velocidad de dilución crítica se encuentra usando la serie de condiciones de lavado:

$$S_0 = S_i \quad cuando \quad D = D_{crit}$$

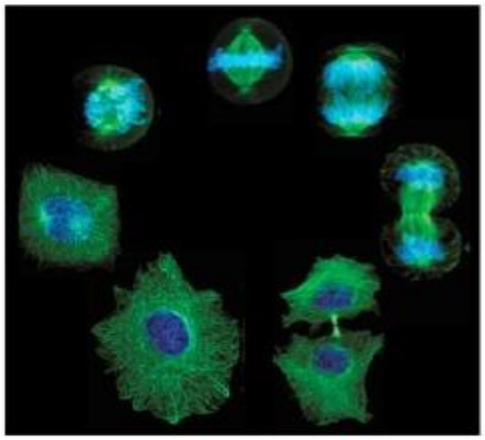
- Then, in monod expression:

$$D_{crit} = \frac{\mu_{max} S_i}{K_s + S_i}$$

- Since S_i is usually very much greater than K_s, D_{crit} is approximately equal to μ_{max}

Stoichiometry of cell growth and product formation

Introduction



- Growth is a result of 2 things:
  - Replication (Mitosis)
  - Change in cell size
- Growth occurs in response to the physiochemical environment
- Nutrients are taken up and used for:
  - Energy Production
  - Biosynthesis & Product Formation → Increase Mass

Introduction

- Batch Growth
  - How do we quantify cell concentration?
  - How do we determine cell number density?
- Growth Patterns
- Cell Growth Kinetics
- **Will need to add/change this once all slides are submitted**

What is Batch Growth…

- the culturing of cells in a vessel with a predetermined amount of medium that is not altered by further nutrient addition or removal.

- Simple & widely used process
  - Laboratory
  - Industry

Quantifying Cell Concentration

Why ?

- To determine the kinetics & stoichiometry of microbial growth
- Basically we're trying to figure out how much stuff we can make given the starting amounts (yields)

How ?

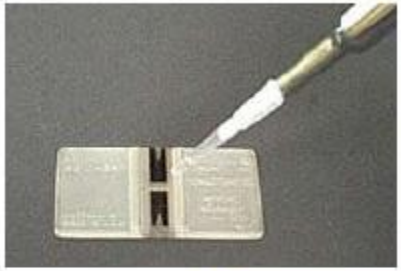
- The method used is classified as either:
  1.) Direct or 2.) Indirect
- Direct method is usually not feasible due to the presence of suspended solids or interfering compounds in the medium.

Quantifying Cell Concentration
Indirect Methods

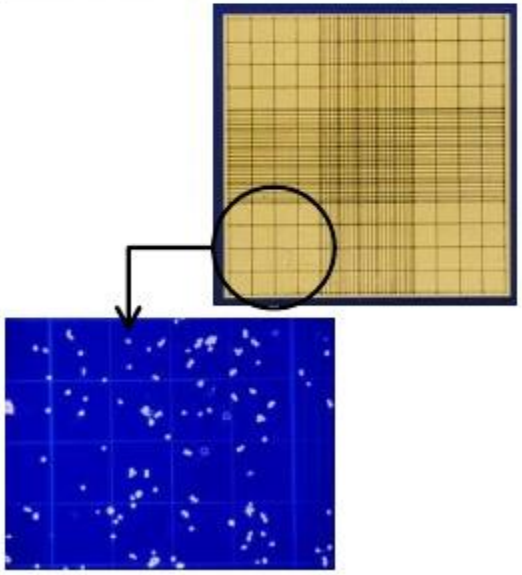
- Petroff-Hausser slide or a hemocytometer
- Plate Counts from agar plates
- Ring-mounted microscope slide
  (miniture culture dish)
- Commercial particle counters

Cell Number Density - Hemocytometer

- A Petroff-Hausser slide or a hemocytometer is often used for cell counting.



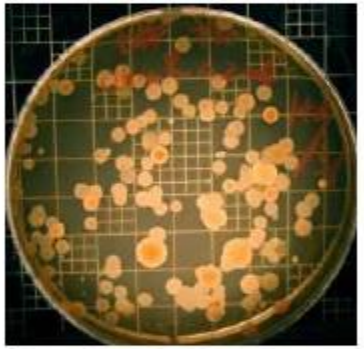
- A calibrated grid is placed over the culture chamber and cells per grid square are counted using a microscope.
  - *To be statistically reliable at least 20 grid squares must be counted.*



- Suitable for non-aggregated cultures.
- Stains can be used to distinguish between live and dead cells.

Cell Number Density – Plate Counts

- Plates with growth medium and agar gel are used for counting **viable** (capable of reproduction) cells.
- Samples are diluted, spread on agar and incubated.
- Colony-forming units (CFU's)
  CFU/mL for liquid
  CFU/g for solids
- More suitable for bacteria and yeast compared to mold.



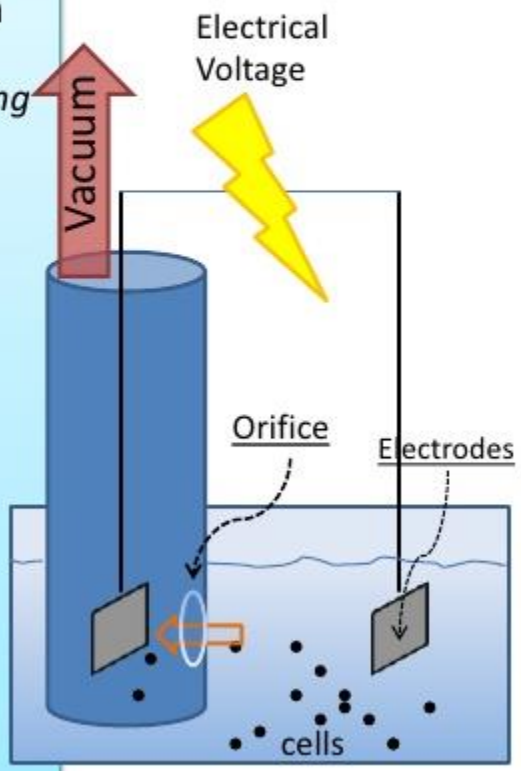
- Viable count may vary depending on the composition of the growth medium and culture conditions chosen.
- A large number of colonies must be counted in order to obtain a statistically reliable value.

Cell Number Density - Ring Mounted Slides

- Agar-gel medium is placed in a small ring mounted on a microscope slide
- Cells are spread on this miniature culture dish.
- Cells are incubated and then examined under a microscope.
- Much quicker than plate count with the same limitation.

Cell Number Density – Particle Counters

- Relatively high electrical resistance of cells
- Uses 2 electrodes and an electrolyte solution
  One electrode is placed in a tube with an orifice, a vacuum is applied to this tube causing the electrolyte solution (which contains the cells) to be sucked through the orifice
- Electrical potential is applied across the electrodes
- As cells pass through the orifice, electrical resistance increases and causes pulses in electrical voltage
- # of pulses = # of particles
- Height of the pulse = a measure of cell size



Determining Cell Mass Concentration

- Direct vs. Indirect Methods
  1.) Direct
    a) Dry Weight
    b) Packed Cell Volume
    c) Optical Density
  2.) Indirect
    a) Measurements of substrate consumption
    b) Measurements of product formation

Determining Cell Mass Concentration

1.) Direct

Dry Weight: most commonly used, only used for cells grown in solids-free medium, process may involve centrifuging, filtering, washing & drying

Ex.) Sometimes cellulose, molasses or corn steep are present in which case dry weight would measure these as well and therefore be inaccurate

Packed Cell Volume: used for rough but rapid estimates of fermentation broth, process involves centrifuging under standard condition and measuring volume.

Optical Density: based on light absorption of suspended cells, uses a spectrometer, fast, inexpensive and simple.

Determining Cell Mass Concentration

1.) Indirect

Measurement of Substrate Consumption or Product Formation

Useful for molds and other fermentation processes

- Intracellular components of cells that change with time during the growth cycle:
  - -DNA, RNA & Protein (kits available)
  - -ATP concentration (luciferase activity)
- Nutrients used for production but not in product formation
  - - Nitrate, phosphate, sulfate
  - -Utilization of carbon or oxygen uptake rates
- Products produced that are growth associated
  - -Production of ethanol, lactic acid
- Changes in Physiochemical Properties
  - - pH changes
  - -viscosity of broth

STOICHIOMETRIC BALANCE

- Stoichiometric equation $\sum_{k=1}^{S} \alpha_k C_k = 0$

- S = total number of components
- α_k = stoichiometric coefficient
- C_k = molecular formula of component k
- Sign Convention: α_k + for products & - for reactant

$2SO_2 + (1)O_2 \rightarrow 2SO_3$

$2SO_3 - 2SO_2 - (1)O_2 = 0$

$N_2 + 3H_2 \rightarrow 2NH_3$

$2NH_3 - N_2 - 3H_2 = 0$

STOICHIOMETRIC BALANCE

- Material balance
  Total mass of reactants = mass of products in Stoich. Equation
  Conservation of mass

$$\sum_{k=1}^{N} \alpha_k M_k = 0$$

M_k = MW of component k

$$2SO_2 + (1)O_2 \rightarrow 2SO_3$$

$$2M_{ST} - 2M_{SD} - (1)M_O = 0$$

$$2(64) - 2(48) - (1)(32) = 0$$

$$N_2 + 3H_2 \rightarrow 2NH_3$$

$$2M_A - M_N - 3M_H = 0$$

$$2(17) - (28) - 3(2) = 0$$

STOICHIOMETRIC BALANCE

- Elemental balance= total element in reactants is equal to the total element in the product in the stoichiometric equation
- $$\sum_{k=1}^{S} \alpha_k m_{kj} = 0$$
- m_{kj}= number of element atom in a molecule of komponent k

$2SO_2 + (1)O_2 \rightarrow 2SO_3$

Balance on S: $2m_{S1S} - 2m_{S0S} - (1)m_{0S} = 0$

$2(1) - 2(1) - (1)(0) = 0$

Balance for o2: $2m_{S1O} - 2m_{S0O} - (1)m_{0O} = 0$

$2(3) - 2(2) - (1)(2) = 0$

STOICHIOMETRIC BALANCE

$N_2 + 3H_2 \rightarrow 2NH_3$

Balance of N: $2m_{AN} - m_{NN} - 3m_{HN} = 0$

$2(1) - 1(2) - 3(0) = 0$

Balance of H: $2m_{AH} - m_{NH} - 3m_{HH} = 0$

$2(3) - 1(0) - 3(2) = 0$

Element balance can be used to determine the stoichiometric coefficients provided that both the reactants and the products are known

- If L elements are involved in the stoichiometric equations, then there are L independent element balance equation a
- If S components and L elements are involved the stoichiometric equations , degree of freedom = $S - L$

EXAMPLE

Example 3.2 Balance the stoichiometric equations of a reaction between As_2S_5 and HNO_3.

$$-\alpha_1 As_2S_5 - \alpha_2 HNO_3 \rightarrow \alpha_3 H_3AsO_4 + \alpha_4 H_2SO_4 + \alpha_5 H_2O + \alpha_6 NO_2$$

The stoichiometric equation is rewritten as:

$$\alpha_1 As_2S_5 + \alpha_2 HNO_3 + \alpha_3 H_3AsO_4 + \alpha_4 H_2SO_4 + \alpha_5 H_2O + \alpha_6 NO_2 = 0$$

There are 6 species & 5 elements. Degree of freedom= 6 – 5 = 1

Balance of each element

As	$2\alpha_1 + \alpha_3$	$= 0$
S	$5\alpha_1 + \alpha_4$	$= 0$
H	$\alpha_2 + 3\alpha_3$	$+2\alpha_4 + 2\alpha_5 = 0$
N	$\alpha_2 + \alpha_6$	$= 0$
O	$3\alpha_2 + 4\alpha_3 + 4\alpha_4 + \alpha_5 + 2\alpha_6$	$= 0$

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Biotechnological products are produced in fermentation proses involving cell growth and bioproduiction
- Bioreactor/fermentor
- Biochemical transformation processes involved thousands of biochemical reaction in the cell.
- Its stoichiometry is represented by a simple pseudochemical reaction equation
- Stoichiometric balance of pseudochemical reaction:
  - Elemental balance = ordinary chemical reactions
  - Electron balance = different from ordinary chemical reactions = involves energy transition
  - Yield coefficient of biomass
  - Yield coefficient of product

BIOREACTOR/FERMENTOR

Bioreactor/fermentor

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Biochemical reaction involves

  Substrate = glucose (CH_mO_n), oxygen & ammonia

  Products: cell mass ($CH_\alpha O_\beta N_\delta$), biochemical product ($CH_xO_yN_z$), water and & carbon dioxide

$$-\alpha_1 CH_mO_n - \alpha_2 O_2 - \alpha_3 NH_3 \rightarrow \alpha_4 CH_\alpha O_\beta N_\delta + \alpha_5 CH_xO_yN_z + \alpha_6 H_2O + \alpha_7 CO_2$$

- Value of coefficients m and n depends on substrate
- Example: glucose $m = 2$ and $n = 1$.
- Value of coefficients α, β and δ depends on microbe
- Example: yeast, $\alpha = 1.66$, $\beta = 0.13$ and $\delta = 0.40$.
- Divide the stoichiometric equations with α_1

$$-CH_mO_n - \alpha'_1 O_2 - \alpha'_2 NH_3 \rightarrow \alpha'_3 CH_\alpha O_\beta N_\delta + \alpha'_4 CH_xO_yN_z + \alpha'_5 H_2O + \alpha'_6 CO_2$$

- $\alpha'_j = \alpha_{j-1} / \alpha_1$ and $j = 1, 2, 3, \ldots 6$.
- Number of elements = 4; Number of components/stoichiometric coefficients = 6
  Degree of freedom of biochemical Stoichiometry = 6 – 4 = 2

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Biochemical transformation involves electron transfer determined by an electron balance
- Additional independent balance equations!
- Degree of reduction of component k, γ_k is used in the electron balance
- Degree of reduction γ_k = number of equivalents of available electrons per atom C
- Available electrons = electrons transferred to oxygen after organic compound is oxidized to carbon dioxide, water and ammonia in biochemical reactions
- Degree of reduction of organic compounds = sum of all the product of element valency and element atomic number divided by the number of C atoms in the compound

$$\gamma_k = \sum_{i=1}^{I} v_i m_{ki} \Big/ m_{kC}$$

V_i = element valency in component i, m_{kC} = number of carbon atoms in component

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Degree of reduction of several common organic materials: :

Methane CH_4 $\gamma = [1(4) + 4(1)]/1 = 8$

Glucose $C_6H_{12}O_6$ $\gamma = [6(4) + 12(1) + 6(-2)]/6 = 24/6 = 4$

Ethanol C_2H_5OH $\gamma = [2(4) + 6(1) + 1(-2)]/2 = 12/2 = 6$

Glucose CH_mO_n $\gamma_s = [1(4) + m(1) + n(-2)]/1 = 4 + m - 2n$

Cell mass $CH_\alpha O_\beta N_\delta$ $\gamma_b = [1(4) + \alpha(1) + \beta(-2) + \delta(-3)]/1 = 4 + \alpha - 2\beta - 3\delta$

Product $CH_xO_yN_z$ $\gamma_p = [1(4) + x(1) + y(-2) + z(-3)]/1 = 4 + x - 2y - 3z$

- The degree of reduction of water, ammonia & carbon dioxide = 0
- The degree of reduction of oxygen = -4
- Electron balance equation:
- Additional independent equations!

$$\sum_{i=1}^{5} \alpha_i \gamma_i = 0$$

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Respiratory quotient ***RQ*** molar basis
- $$RQ = -\alpha_7/\alpha_2 = -\alpha'_6/\alpha'_1$$
- Yield of cell biomass mass basis
- $$Y_{X/S} = \alpha'_3 M_B/M_S = -\alpha_4 M_B/(\alpha_1 M_S)$$
- M_b = formula MW of biomass & M_s = formula MW of substrate
- Yield of product mass basis
- $$Y_{P/S} = \alpha'_4 M_P/M_S = -\alpha_5 M_P/(\alpha_1 M_S)$$
- M_p = formula MW of product
- Value of ***RQ*** is obtained from experiment

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Element balance equations (4 equations) plus

  Electron balance equation

  Respiratory quotient equation

  Yield of biomass

  Yield of product

- 8 equations & 6 unknown variables
- Degrees of freedom = 6-8 = -2
- Two equations are not independent and can be used to check the balance stoichiometry
- Balance of elements

C $-1+a'_3+a'_4+a'_6 = 0$

H $-m+3a'_2+\alpha a'_3+xa'_4+2a'_5 = 0$

N $a'_2+\delta a'_3+za'_4 = 0$

O $-n+2a'_1+\beta a'_3+\gamma a'_4+a'_5+2a'_6 = 0$

STOICHIOMETRY OF BIOCHEMICAL REACTIONS

- Electron balance
- $-\gamma_s - 4\alpha'_1 + \gamma_b\alpha'_3 + \gamma_p\alpha'_4 = 0$
- γ_s = degrees of reduction of substrate
- γ_b = degrees of reduction of biomass
- γ_p = degrees of reduction of product
- H and O element balances involve water and there is so much water
- Both balances are difficult to use
- Only the C, N and electron balances are used

C $\quad -1+\alpha'_3 + \alpha'_4 + \alpha'_6 = 0$

N $\quad \alpha'_2 + \delta\alpha'_3 + z\alpha'_4 = 0$

$\gamma_s \quad -4\alpha'_1 + \gamma_b\alpha'_3 + \gamma_p\alpha'_4 = 0$

EXAMPLE

Example 3.3 Aerobic growth of S. cerevisiae (yeast) on ethanol

$$-CH_3O_{0.5} - \alpha'_1 O_2 - \alpha'_2 NH_3 \rightarrow \alpha'_3 CH_{1.704}O_{0.408}N_{0.149} + \alpha'_4 H_2O + \alpha'_5 CO_2$$

- Determine the values of α'_1, α'_2, α'_3, and α'_5 if RQ = 0.66, Yield of biomass on substrate & Yield of biomass on oxygen
- Degree of reduction of substrate & biomass

Ethanol $CH_3O_{0.5}$ $\quad \gamma_S = [1(4) + 3(1) + (0.5)(-2)]/1 = 6$

Biomass $CH_{1.704}O_{0.408}N_{0.149}$ $\quad \gamma_B = [1(4) + 1.704(1) + 0.408(-2) + 0.149(-3)]/1 = 4.441$

- Element balance of C & N, electron balance and *RQ*.
- C $\quad -1 + \alpha'_3 + \alpha'_5 = 0$
- N $\quad \alpha'_2 + 0.149\alpha'_3 = 0$
- Electron $\quad -6 - 4\alpha'_1 + 4.41\alpha'_3 = 0$
- RQ $\quad \alpha'_5 = -0.66\alpha'_1$

EXAMPLE

4 unknowns & 4 equations & degree of freedom = 4 – 4 = 0

Substitute last equation with first equation

$$\alpha'_3 - 0.66\alpha'_1 = 1$$

and

$$4.41\alpha'_3 - 4\alpha'_1 = 6$$

$$\begin{bmatrix} 1 & -0.66 \\ 4.41 & -4 \end{bmatrix} \begin{bmatrix} \alpha'_3 \\ \alpha'_1 \end{bmatrix} = \begin{bmatrix} 1 \\ 6 \end{bmatrix}$$

Then $\alpha'_3 = \dfrac{(1)(-4)-(6)(-0.66)}{(1)(-4)-(4.41)(-0.66)} = 0.0367$ $\alpha'_2 = (1-0.0367)/(-0.66) = -1.4595$

and $\alpha'_2 = -(0.149)(0.0367) = -0.0055$ $\alpha'_4 = -(0.66)(-1.4595) = 0.96327$

Formula biomass MW $M_B = (12 + 1.704(1) + 0.149(14) + 0.408(16)) = 22.318$

Formula ethanol MW $M_S = (12 + 3(1) + 0.5(16)) = 23$

Yield of biomass on substrate

$$Y_{X/S} = \alpha'_3 M_B / M_S = (0.0367)(22.318)/23 = 0.0356 \text{ g g}^{-1}$$

Yield of biomass on oxygen

$$Y_{X/O2} = -\alpha'_3 M_B / (\alpha'_1 M_O) = -(0.0367)(22.318)/[(-1.4595)(32)] = 0.0175 \text{ g g}^{-1}$$

MATERIAL BALANCE WITH SINGLE REACTION

COMPONENT MATERIAL BALANCE FOR REACTING SYSTEMS

Molar

$$\sum_{i=1}^{M} N_{i,j} x_{ik,j} - \sum_{i=1}^{L} N_{i,j} x_{ik,j} = r_k$$

Mass

$$\sum_{i=1}^{M} F_{i,j} w_{ik,j} - \sum_{i=1}^{L} F_{i,j} w_{ik,j} = M_k r_k$$

Rate of chemical reaction of component k r_k

Ammonia synthesis reaction $N_2 + 3H_2 \rightarrow 2NH_3$

If rate of reaction of nitrogen = $-r_N$ (negative: nitrogen is used)

Rate of reaction of hydrogen = $-r_H = (\alpha_H / \alpha_N)(-r_N) = (-3/-1)(-r_N)$

Rate of reaction of ammonia = $r_A = (\alpha_A / \alpha_N)(-r_N) = (2/-1)(-r_N)$

Then $$\frac{r_A}{\alpha_A} = \frac{r_H}{\alpha_H} = \frac{r_N}{\alpha_N} = \frac{r_i}{\alpha_i} = r$$

Rate of reaction r is fixed for a given reaction stoichiometric equation

Rate of reaction of component $r_i = \alpha_i r$

COMPONENT MATERIAL BALANCE FOR REACTING SYSTEMS

Molar

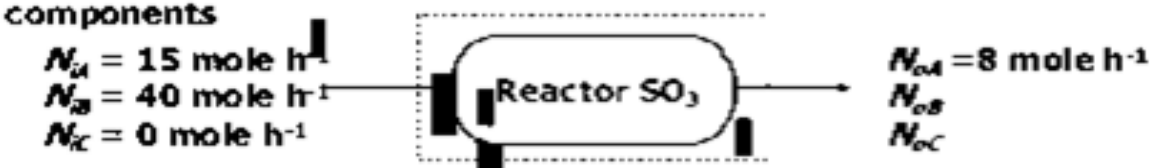
$$\sum_{i=1}^{M} N_{i,j} x_{ik,j} - \sum_{i=1}^{L} N_{i,j} x_{ik,j} = \alpha_k r$$

Mass

$$\sum_{i=1}^{M} F_{i,j} w_{ik,j} - \sum_{i=1}^{L} F_{i,j} w_{ik,j} = M_k \alpha_k r$$

MATERIAL BALANCE WITH SINGLE REACTION

Example 3.4 Lets say for the SO_3 synthesis reaction, 15 mole h^{-1} O_2 (A) 40 mole h^{-1} SO_2 (B) and 0 mole h^{-1} SO_3 (C) is fed into a reactor. If the flow rate out of O_2 is 8 mole h^{-1} calculate the flow rates of other components



Basis 55 mole j^{-1} feed $\quad O_2 + 2SO_2 \rightarrow 2\,SO_3$

3 components & 3 independent material balance equations

Degree of freedom = 3 – 3 = 0

Choose component mole balance that has most information to get r: O_2

$$N_{oA} = N_{iA} - \alpha_A r \qquad 15 - 8 - (-1)r \qquad r = 7 \text{ mole h}^{-1}$$

SO_2 mole balance $\quad N_{oB} = N_{iB} - \alpha_B r \qquad 40 - N_{oB} - (-2)r$

SO_3 mole balance $\quad N_{oC} = N_{iC} - \alpha_C r \qquad 0 - N_{oC} - (2)r$

Substituting r in SO_2 & SO_2 balances:

$$N_{oB} = 40 - 2r = 40 - 2(7) = 26 \text{ mole h}^{-1}$$

$$N_{oC} = 2r = 2(7) = 14 \text{ mole h}^{-1}$$

Example

Example 3.5 Growth of *S. cerevisiae* on glucose is described by the following equation

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \rightarrow 0.48C_6H_{10}NO_3 + 4.32H_2O + 3.12CO_2$$

In a batch bioreactor of volume 10^5 l, the yeast concentration required is 50 g dry mass L^{-1}.

Calculate the yield of biomass/substrate $Y_{X/S}$ Yield of biomass /oxygen $Y_{X/O}$ and respiratory quotient RQ. Calculate the required concentration and total amount of glucose and $(NH_3)_2SO_4$ in the nutrient media.

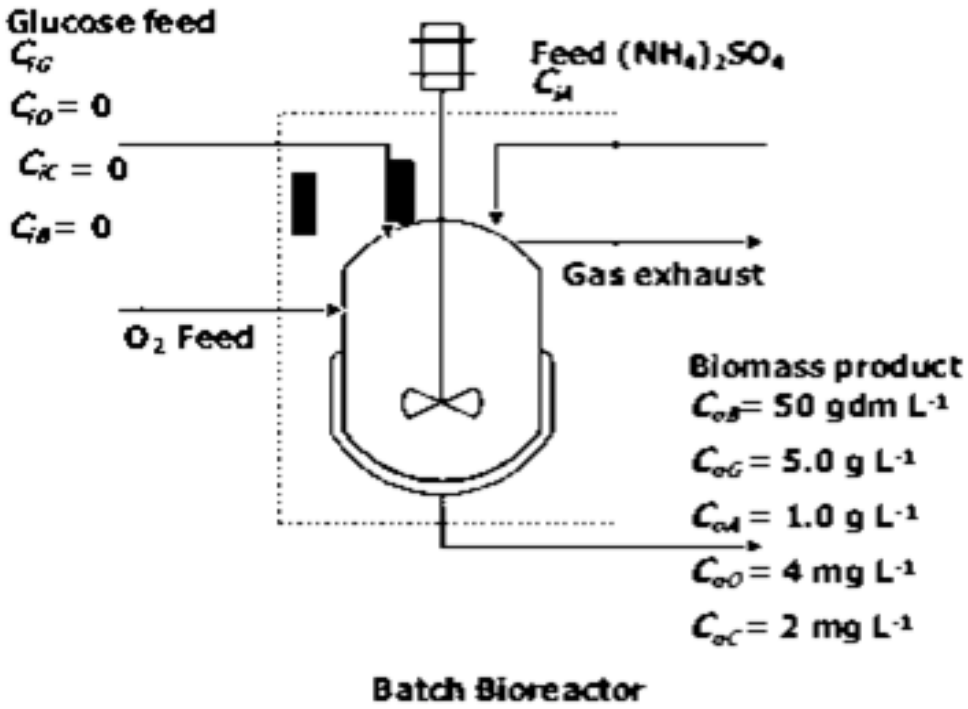
How much oxygen is required and carbon produced by the bioreaction ?

If the growth rate at exponent phase is $r = 0.7$ gdm L^{-1} h^{-1}, determine the rate of oxygen utilization.

MW glucose = 180, MW oxygen = 32, MW ammonia = 17, MW $(NH_4)_2SO_4$ = 116, MW biomass = 144, MW carbon dioxide = 44 and MW water = 18.

Example

E


Glucose feed
C_{fG}
$C_{fO} = 0$
$C_{fC} = 0$
$C_{fB} = 0$
Feed $(NH_4)_2SO_4$
C_{fA}
Gas exhaust
O_2 Feed
Biomass product
$C_{oB} = 50$ gdm L^{-1}
$C_{oG} = 5.0$ g L^{-1}
$C_{oA} = 1.0$ g L^{-1}
$C_{oO} = 4$ mg L^{-1}
$C_{oC} = 2$ mg L^{-1}
Batch Bioreactor

EXAMPLE

6 components and six independent mass balance equations

Water balance is not used because the presence of a lot of water

Degrees of freedom = 6 − 5 = 1

Yield of biomass/glucose $Y_{X/S}$

$$Y_{X/S} = \frac{\alpha_B M_B}{\alpha_G M_G} = \frac{(0.48)(144)}{(1)(180)} = 0.384 \text{ g g}^{-1}$$

Yield of biomass/oxygen $Y_{X/O2}$.

$$Y_{X/O2} = \frac{\alpha_B M_B}{\alpha_O M_O} = \frac{(0.48)(144)}{(3)(32)} = 0.72 \text{ g g}^{-1}$$

Respiratory quotient

$$RQ = \frac{\alpha_C}{\alpha_O} = \frac{3.12}{3} = 1.04 \text{ mole mole}^{-1}$$

Choose Basis =500 kg dry biomass = 50 gdm L^{-1} in a 10^5 L bioreactor

Choose component balance with the most information to get *r*

Biomass balance

$$N_{iB} = N_{oB} - \alpha_B r V$$

EXAMPLE

Biomass balance

$$\frac{C_{bi}V}{M_b} = \frac{C_{bo}V}{M_b} - \alpha_b rV$$

$$0 = \frac{(50)(10^3)}{144} - (0.48)(10^3)$$

Hence the rate of reaction

$$r = \frac{50}{(144)(0.48)} = 0.772 \text{ mole L}^{-1}$$

Glucose balance

$$N_{sc} = N_{so} - \alpha_s rV$$

$$\frac{C_{si}V}{M_s} = \frac{C_{so}V}{M_s} - \alpha_s rV$$

$$\frac{C_{si}(10^3)}{180} = \frac{(5)(10^3)}{180} + \frac{(50)(10^3)}{(144)(0.48)}$$

$$C_{si} = 5 + \frac{(50)(180)}{(144)(0.48)} = 5 + 130.208 = 135.208 \text{ g L}^{-1}$$

Total amount of glucose required = 13,520.8 kg

EXAMPLE

One mole NH_3 requires 1/2 mole of $(NH_4)_2SO_4$.

$(NH_4)_2SO_4$

$$N_A = N_{A0} - \frac{a_A}{2} r V$$

$$\frac{C_A V}{M_A} = \frac{C_{A0} V}{M_A} - \frac{a_A}{2} r V$$

$$\frac{C_A (10^3)}{116} = \left(\frac{50}{1000}\right) - \frac{(0.45)(150)(10^3)}{2(144)(0.45)}$$

$$C_A = 1 \cdot \frac{(50)(116)}{2(144)} = 1 \cdot 20.139 = 21.139 \text{ g/L}$$

Total $(NH_4)_2SO_4$ required = 2,113.9 kg

O_2 balance

$$N_{O_2} = N_{O_2 0} - \frac{C_{A0} V}{M_A} a_{O_2} r V$$

$$N_{O_2} = N_{O_2 0} - \frac{(0.004)(10^3)}{32} \cdot \frac{3(50)(10^3)}{(144)(0.45)} = 217.026 \times 10^3$$

Total O_2 utilization $N_{O_2} - N_{O_2 0}$ **= 217.026 kmole oxygen**

Example

CO_2 balance $$N_c - N_{c_0} + \frac{C_c V}{M_c} \alpha_c r V$$

$$N_c \quad N_{c_0} \cdot \frac{(0.002)(10^3)}{44} \quad \frac{(3.12)(50)(10^3)}{(144)(0.48)} \cdot 225.689 \times 10^3$$

N_{oCO2} = 225.689 kmole carbon dioxide = 9930.316 kg carbon dioxide

The dissolved gas concentrations are very small and will be neglected in fermenter balances

CONVERSION & LIMITING REACTANT

- Common measure of course of reaction is the fractional conversion / conversion of the limiting reactant

$$X_j = \frac{N_{j,0} - N_j}{N_{j,0}} \qquad N_j X_j = N_{j,0} - N_j \qquad N_j X_j = \alpha_j r$$

- Conversion links the outlet flow rate with the inlet flow rate of the same component = additional independent equation!
- Reaction rate r

$$r = \frac{N_j X_j}{\alpha_j}$$

- The limiting reactant finishes first if the reaction is left to react by itself
- If the reaction is left to react, the rate of reaction r increases to reach the value of $r_{limiting}$ when $N_{ok} = 0$

$$r_{limiting} = \frac{N_{k,0}}{\alpha_k}$$

- Reactant with the lowest value for $N_{k,0}/(-\alpha_k)$ finishes first
- Limiting reactant= reactant that has the lowest $N_{k,0}/(-\alpha_k)$
- Other reactants= excess reactant
  Excess fraction of component k

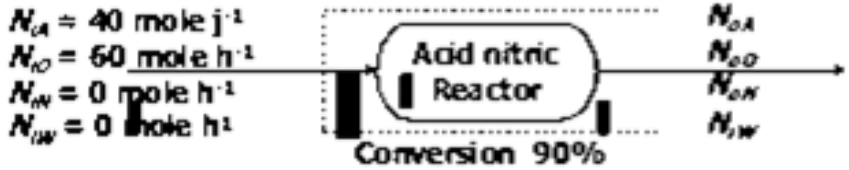
$$E_k = \frac{N_{k,0} - \alpha_k N_{j,0} / \alpha_j}{\alpha_k N_{j,0} / \alpha_j}$$

EXAMPLE

Example 3.6 The reaction between ammonia (A) and oxygen (O) on Pt catalyst produces nitric acid and water (W). The stoichiometric equation is given by

$$4NH_3 + 5O_2 \leftrightarrow 4NO + 6H_2O$$

Under certain conditions, conversion of NH_3 into NO (N) can achieve 90% at ammonia flow rate NH_3 40 mole h^{-1} and O_2 60 mole h^{-1}. Calculate the other flow rate



Basis is 100 mole h^{-1} feed.

4 components & 4 independent material balance equations.

Degree of freedom = 4 - 4 = 0

Example

Stoichiometric coefficient

NH_3 $\alpha_A = -4$ O_2 $\alpha_O = -5$

NO $\alpha_N = 4$ H_2O $\alpha_W = 6$

Use conversion of ammonia to get Rate of reaction *r*

$$r = \frac{N_{A0}X_A}{-\alpha_A} = \frac{(40)(0.9)}{-(-4)} = 9 \text{ mole/h}$$

Component mole balance

NH_3 $N_A = N_{A0} - \alpha_A r$ $40 - N_A - (-4)(9)$ $N_A = 4$ mol/h

O_2 $N_O = N_{O0} - \alpha_O r$ $60 - N_O - (-5)(9)$ $N_O = 15$ mol/h

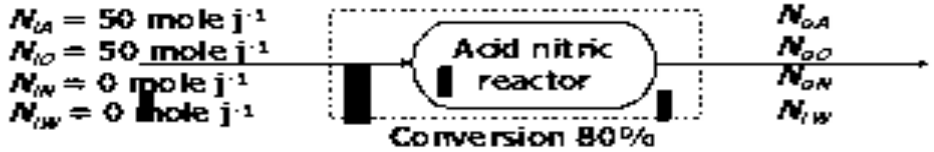
NO $N_N = N_{N0} - \alpha_N r$ $0 = N_{N0} - (4)(9)$ $N_N = 36$ mol/h

H_2O $N_W = N_{W0} - \alpha_W r$ $0 = N_W - (6)(9)$ $N_W = 54$ mol/h

EXAMPLE

Example 3.7 If the reaction in Example 3.6 achieves 80% conversion with equimolar ammonia and oxygen feed that is fed at 100 mole h^{-1}. Calculate the flow rate out of all components

- Stoichiometric equation is given by
- $$4NH_3 + 5O_2 \rightarrow 4NO + 6H_2O$$
- Choose basis 100 mole h^{-1} feed



Determination of the limiting reactant

$$\frac{N_{iA}}{-\alpha_A} = \frac{50}{-(-4)} = 12.5 \qquad \frac{N_{iO}}{\alpha_O} = \frac{50}{(5)} = 10$$

Limiting reactant= Oxygen because it has the smallest $N_{ik}/(-\alpha_k)$

- Conversion information is for conversionm of oxygen!

Example

- **Use conversion of oxygen to get the rate of reaction:**

$$r = \frac{N_{O_2,0} X_{O_2}}{-\alpha_{O_2}} = \frac{(50)(0.8)}{-(-5)} = 8 \text{ mole h}^{-1}$$

Component balance

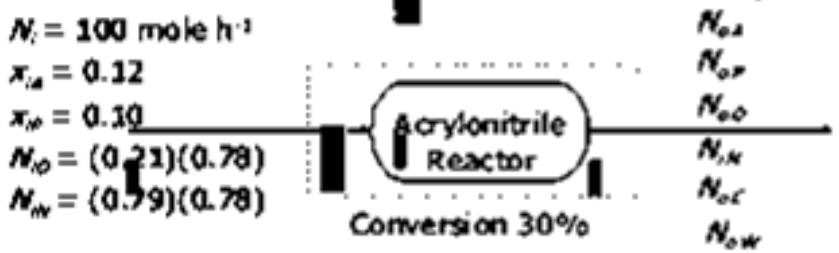
- **NH_3** $N_A = N_{A,0} - \alpha_A r$ $N_{A,2} = 50 + (-4)(8) = 18$ mole h^{-1}
- **O_2** $N_{O_2} = N_{O_2,0} - \alpha_{O_2} r$ $N_{O_2,2} = 50 - (-5)(8) = 10$ mole h^{-1}
- **NO** $N_N = N_{N,0} - \alpha_N r$ $N_{N,2} = 0 + (4)(8) = 32$ mole h^{-1}
- **H_2O** $N_W = N_{W,0} - \alpha_W r$ $N_{W,2} = 0 - (6)(8) = 48$ mole h^{-1}

Example

Example 3.8 Acrylonitrile (C) is produced by the following reaction:

$$C_3H_6 + NH_3 + (3/2)O_2 \rightarrow C_3H_3N + 3H_2O$$

The feed contains 10% mole propylene (P), 12% mole ammonia (A) and 78% mole air. Conversion of limiting reactant is 30%. By choosing 100 mole h^{-1} feed as the basis, determine the limiting reactant, fractional excess of other reactants and flow rate out of all components.



6 unknown & 6 independent material balance equations

Degree of freedom = 6 − 6 = 0

Determine the limiting reactant

$$\frac{N_{iA}}{-\alpha_A} = \frac{12}{-(-1)} = 12 \qquad \frac{N_{iP}}{-\alpha_P} = \frac{10}{-(-1)} = 10 \qquad \frac{N_{iO}}{-\alpha_O} = \frac{16.38}{-(-1.5)} = 10.92$$

Propylene is the limiting reactant

EXAMPLE

Fractional excess of other reactants

NH_3

$$E_A = \frac{N_A - \alpha_A N_F/\alpha_F}{\alpha_A N_F/\alpha_F} = \frac{12 - (10)(10)/(1)}{(10)(10)/(1)} = 0.2$$

O_2

$$E_O = \frac{N_O - \alpha_O N_F/\alpha_F}{\alpha_O N_F/\alpha_F} = \frac{16.38 - (1.5)(10)/(1)}{(1.5)(10)/(1)} = 0.092$$

From conversion, calculate rate of reaction

$$r = \frac{X_F N_F}{-\alpha_F} = \frac{(0.3)(10)}{-(-1)} = 3 \text{ mole h}^{-1}$$

NH_3

$$N_A = N_{A0} - \alpha_A r \qquad N_A = 12 + (-1)(3) = 9 \text{ mole h}^{-1}$$

O_2

$$N_O = N_{O0} - \alpha_O r \qquad N_O = 16.38 - (-1.5)(3) = 11.88 \text{ mole h}^{-1}$$

C_3H_3N

$$N_A = N_{A0} - \alpha_A r \qquad N_A = 0 - (1)(3) = 3 \text{ mole h}^{-1}$$

H_2O

$$N_W = N_{W0} - \alpha_W r \qquad N_W = 0 + (3)(3) = 9 \text{ mole h}^{-1}$$

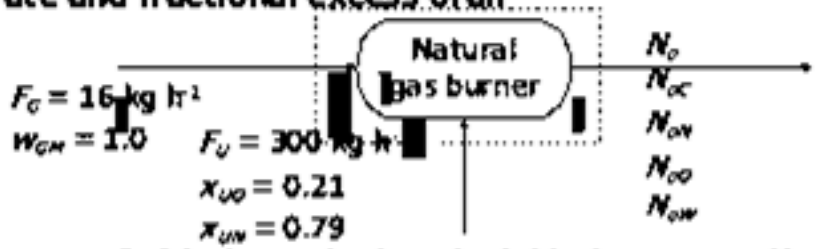
N_2

$$N_A = N_A = 61.62 \text{ mole h}^{-1}$$

Example

Example 3.9 Natural gas containing methane only is burnt in an incinerator $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$

Calculate all the outgoing molar flow rate of components, total molar flow rate and fractional excess of air.



4 unknown & 4 independent material balance equations

Degree of freedom = 4 – 4 = 0

- Convert the flow rate units into mole units.
- $F_G \rightarrow N_G$ $\quad \frac{16 \text{ kg}}{\text{h}} \left| \frac{1 \text{ kmole } CH_4}{16 \text{ kg } CH_4} \right. = 1 \text{ kmole h}^{-1} \text{ } CH_4$
- Air MW = 0.21(32) + 0.79(28) = 28.84
- $F_U \rightarrow N_U$ $\quad \frac{300 \text{ kg}}{\text{h}} \left| \frac{1 \text{ kmole air}}{28.84 \text{ kg air}} \right. = 10.40 \text{ kmole h}^{-1} \text{ air}$

$N_{UO} = 0.21(10.4) = 2.184$ kmole h^{-1}

$N_{UN} = 0.79(10.4) = 8.216$ kmole h^{-1}

EXAMPLE

Basis is 1.0 kmole h^{-1} natural gas

Stoichiometric coefficients

CH_4 $\alpha_{CH4} = -1$ O_2 $\alpha_{O2} = -2$ CO_2 $\alpha_{CO2} = 1$ H_2O $\alpha_{H2O} = 2$

Assume complete combustion = all methane reacted.

Rate of methane reaction

$$r = \frac{N_c}{-\alpha_M} = \frac{(1.0)}{-(-1)} = 1.0 \text{ kmole h}^{-1}$$

Component mole balances

N_2 $N_{1N} = N_{2N} = 8.216$ kmole h^{-1}

O_2 $N_{1O} = N_{2O} - \alpha_O r$ $\quad N_{2O} = 2.184 - (-2)(1) = 0.184$ kmole h^{-1}

CO_2 $N_{2C} = N_{1C} - \alpha_C r$ $\quad N_{2C} = 0 - (1)(1) = 1.0$ kmole h^{-1}

H_2O $N_{2W} = N_{1W} - \alpha_W r$ $\quad N_{2W} = 0 - (2)(1) = 2.0$ kmole h^{-1}

Total flow rate out N_o = 11.4 kmole h^{-1}

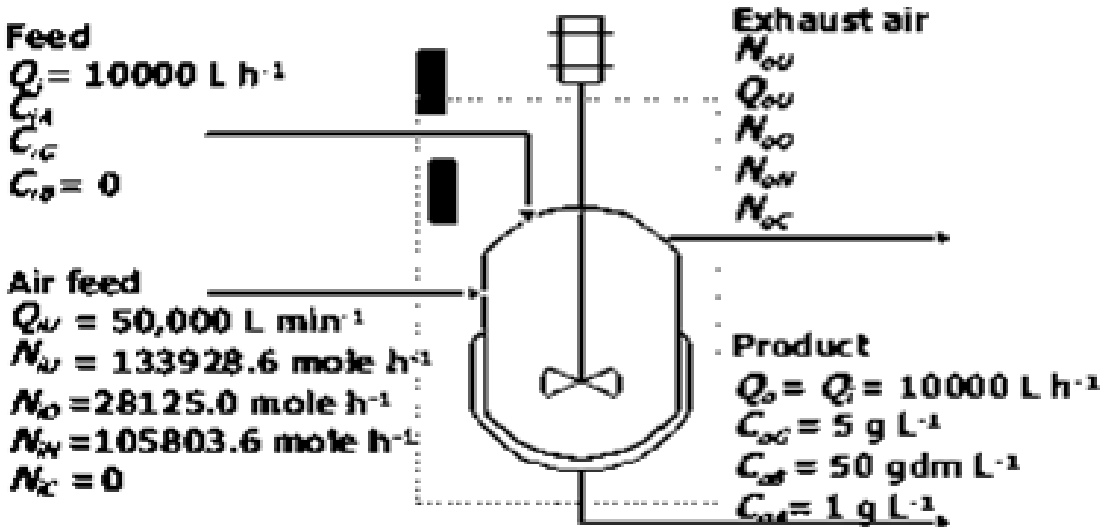
Air fractional excess:

$$E_a = \frac{N_{1O} - \alpha_O N_c/\alpha_M}{\alpha_O N_c/\alpha_M} = \frac{2.184 - (-2)(1.0)/(-1)}{(-2)(1.0)/(-1)} = 0.092$$

or 9.2%

Example

Example 3.10 Yeast in example 3.2 reacts with glucose, oxygen and ammonium sulfate according to the same stoichiometry in a chemostat bioreactor with volume $V = 10^5$ L in the figure below. The rate of ventilation is 50,000 L min^{-1}. Dilution rate $D = Q_i/V = 0.1\ j^{-1}$.



EXAMPLE

Chemostat = continuous bioreactor

At steady sate, substrate, other nutrient and oxygen are fed and products are withdrawn at the same volumetric flow rate

Volumetric flow rate $Q_i = VD = (10^5)(0.1) = 10000$ **L** h^{-1}

5 equations for 5 unknowns & Degree of freedom= 5 – 5 = 0

Basis 10000 L h^{-1} **volumetric flow rate**

Chose component balance with most information to get r:
Biomass

$$N_{i,b} = N_{i,b} - \alpha_b r V$$

$$Q_i \frac{C_{i,b}}{M_b} = Q_i \frac{C_{i,s}}{M_b} - \alpha_b r V$$

$$0 = (10^4)\frac{(50)}{144} - (0.48)r(10^5)$$

Then $r = (50)/[(10)(144)(0.48)] = 0.072338$ **mole** L^{-1} h^{-1}

Example

- **Glucose** $N_{iG} = N_{oG} - \alpha_G r V$

$$Q\frac{C_{iG}}{M_G} = Q\frac{C_{oG}}{M_G} - \alpha_G r V$$

$$(10^3)\frac{C_{iG}}{188} = (10^3)\frac{(5)}{188} + (0.072338)(10^1)$$

Then $C_{iG} = 5 + (188)(10)(0.072338) = 140.99\ g\ L^{-1}$

- **One mole NH_3 requires 1/2 mole of $(NH_4)_2SO_4$. Then $(NH_4)_2SO_4$ balance** $N_{iA} = N_{oA} - \frac{\alpha_A}{2} r V$

$$Q\frac{C_{iA}}{M_A} = Q\frac{C_{oA}}{M_A} - \frac{\alpha_A}{2} r V$$

$$(10^3)\frac{C_{iA}}{116} = (10^3)\frac{(1)}{116} + \frac{(0.48)(0.072338)(10^1)}{2}$$

Then $C_{iA} = 1 + (116)(0.48)(10)(0.072338)/2 = 21.14\ g\ L^{-1}$

- **Molar flow rate of air feed**
- **Then** $Q_{air} = 50000\ \frac{L}{min}\left|\frac{60\ min}{1\ h}\right|\frac{mol}{22.4\ L} = N_{air} = 133928.571\ mole\ h^{-1}$

EXAMPLE

- Hence $N_{iO} = (0.21)(133928.571) = 28125.0$ mole h^{-1}
- $N_{iN} = (0.79)(133928.571) = 105803.6$ mole h^{-1}
- Oxygen $N_{oi} = N_{ei} - \alpha_i r V$

$$28125.0 = N_{oi} - (-3)(0.072338)(10^3)$$

$N_{oO} = 28125.0 - 21701.4 = 6423.6$ mole oxygen h^{-1}

- Nitrogen: $N_{oN} = N_{iN} = 105803.6$ mole h^{-1}
- Carbon dioxide $N_{oi} = N_{ei} - \alpha_i r V$

$$0 = N_{oi} - 3.12(0.072338)(10^3)$$

- Rate of CO_2 production $N_{oCO2} = 22569$ mole carbon dioxide h^{-1}
- Rate of gas out

$N_{oV} = N_{oO} + N_{oN} + N_{oC} = 6423.6 + 105803.6 + 22569.0 = 134795$ mole h^{-1}

or $$Q_{oi} = 134795 \frac{\text{mole}}{\text{h}} \left| \frac{1\text{ h}}{60\text{ min}} \right| \frac{22.4\text{ L}}{\text{mole}} = 50323.47\text{ L min}^{-1}$$

-