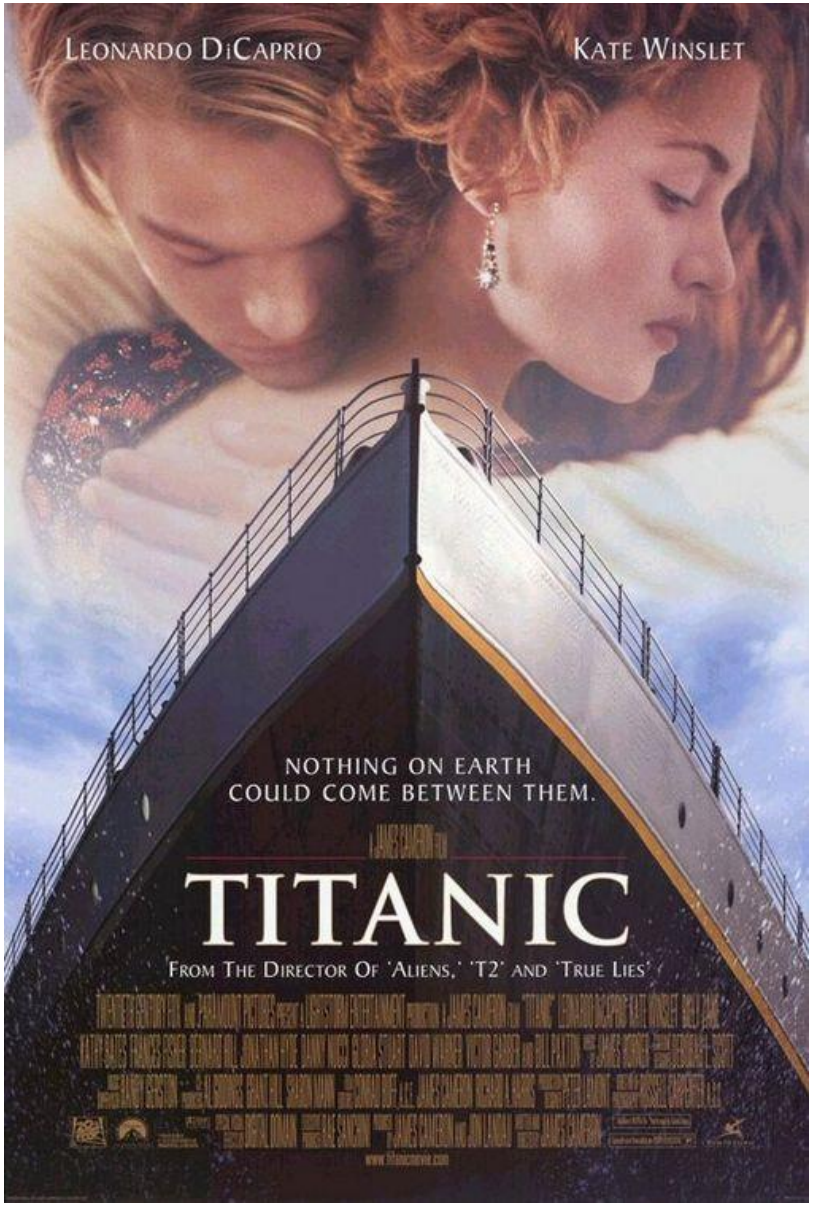


Titanic Disaster (1912)

Presentation Order

- Introduction
- O2 requirement and bacteria
- Anaerobic respiration
- Anaerobic atmospher
- Anaerobic systems
  - Anaerobic indicators
- Anaerobe ecology
- Anaerobe bakteria and infections

Introduction

- Some bacteria;
  - Energy supply via non-fermentative way
  - 1 mol glucose 38 ATP (380 kcal chemical + 308 kcal heat energy) or,
  - 15 ATP by Kreb cycle (15 kcal energy)
- During this events;
  - Flavins, cytochrome and iron containiing substances are used
  - NAD and NAD+, oxidised to NADH2
- Last electron receiver on sytoplasmic membrane is O_2
  - These are "**aerobibic bakteria**"

Introduction

- Some bacteria;
  - 1 mol glucose 2 ATP
  - Nitrate, sulphate or inorganic nitrogen compounds are the last electron receiver on membrane
  - Fumarate, CO_2, cyanide, carbon monoxite, polyhidric alcohol or other organic compounds
  - Do not need O_2 and also O_2 inhibits growth
- These bacteria "**anaerobic bacteria**"

<u>Anaerobiosis</u>

- Atmospheric composition for anaerobic bacterial growth
- Obraining Growth conditions
- Obtaining, maintenance and monetarising reduction potential within culture
- Microbiologic systematics that cover incubation and isolation principles

Bacteria according to O_2 requirement

- **Obligate aerobe**
  - Can not grow without O_2 or small colonies
  - *Bacillus, Legionella, Pseudomonas* and *M. tuberculosis*
- **Fakültative anaerobe**
  - Originally aerobe but can grow anaerobically by an alternative metabolism
  - *Enterobacteria* and known many other bacteria family **Fakültative aerobe???**
  - Originally anerobe, but can grow aerobically by an alternative metabolism

Bacteria according to O_2 requirement

- **Microaerophilic**
  - Can tolerate 4-6 % $O_2$and can grow
  - After many days small colonies at room atmosphere (below 20% O_2)
  - Better grow at anaerobic atmosphere
  - *Lactobacillus* and *Camphylobacter*
- **Aerotolerant**
  - Originally obligate anaerobe but can grow with 2-8% O_2
  - The border between aerotolerant and microaerophilic bakteria is not clear
- **Obligate anaerobe**
  - Without oxygen or can grow on medium below 0.5 % O_2 *Clostridium haemolyticum, C. novyi* tip B, *Selenomonas ruminatum, Treponema denticola, Bacteroides gracilis*

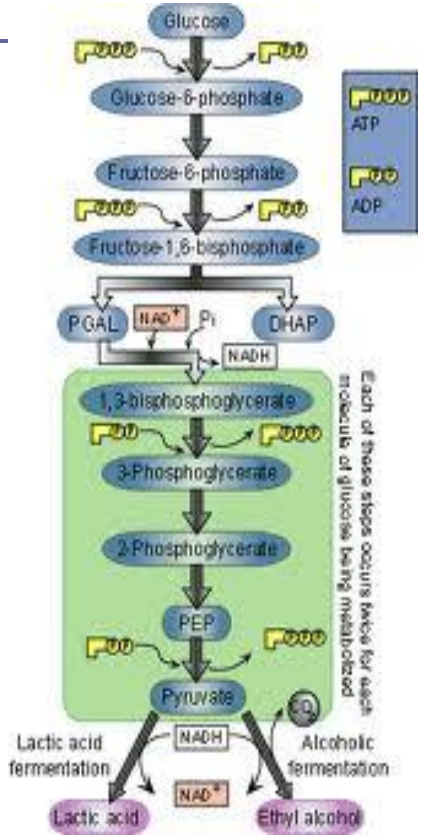
Anaerobes

- Anaerobic bakteria;
  - Do not have catalase, peroxidase and süperoxite dysmutase
- Some anaerobes have catalase enzyme
  - *Tsierella preacutus, Fusobacterium bullosum, Selenomonas sputigena, Prevotella intermedia*

"It is still unclear how these bacteria can protect themselves from oxygen that they produce"

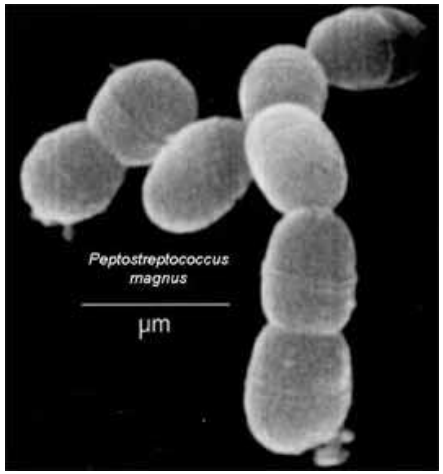
Anaerobic Respiration

- Starter electron that is necessary for electron transport chain;
  - Either from environmental H2
  - Or from pıruvate that is obtained from breaking down of hexoses (Embden-Meyerhof-Parnas Way)
- Up to know, similar to other bacteria
- Hexose breaking products is special to anaerobes



Anaerobic Respiration

- *Clostridium* (*C. saccharobutyricum)* and *Butyribacterium* species,
  - Butyrate, asetate, CO_2 and H_2 by fermentation of glucose
- Some *Clostridum* species(*C. acetobutylicum* and *C. butyricum)*
  - Butanol, acetone, izopropanol, formate and ethanol
- *Propionibacterium* species;
  - Propionic acid
-
- *Veillonella* species;
  - CO2, propionate, acetate and succinate
- Other some anaerobes (*Bacteroides ruminicola* and *Peptostreptococcus* species)
  - Oxaloacetate, malate, fumarate and propionate



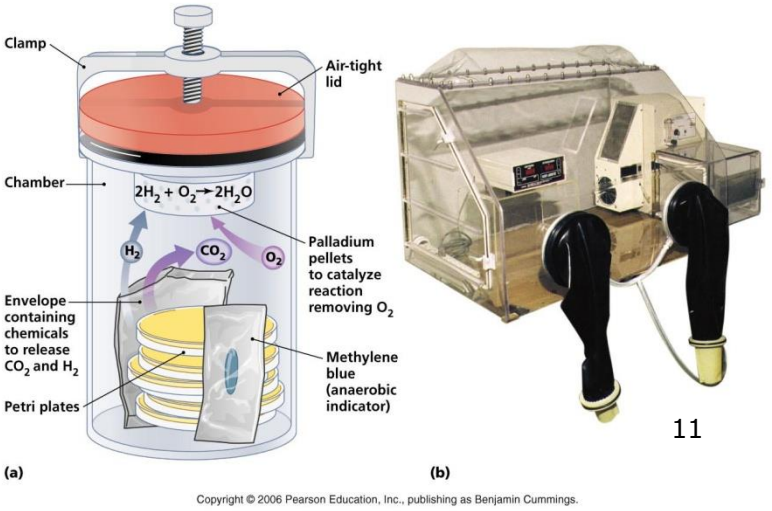
Anaerobic Respiration

- Last electron receiver for anaerobe bakteria;
- Nitrate (NO3), sulphate (SO4-2), fumarate, lactate, acetate, arsenic, CN or CO_2
- Commonly last electron receiver nitrate
- Nitrate transfer to nitrite (NO2), nitrogen gas(N2) or amonnia (NH3) 4AH2 + NO3> 4A + **NH3** + 2H2O + Energy
- Lst electron receiver of *Fusobacterium* is sulphate
  - 4AH2 + H2SO4 ---> 4A + **H2S** + 4 H2O + Energy
- Last electron receiver of *Bacteroides, Eubacterium* and *Peptostreptococcus is* fumarate transform to succinate
  - AH2 + HOOC=CH-COOH ----> A +**HOOC-CH2-CH2-COOH** + Energy
- Last electron receiver of *Capnocytophaga* and *Archaebacteria* is CO2
  - 4 AH2 + CO2 ---> **CH4** + 2H2O + Energy (2 ATP)

Anaerobic Atmosphere

- Indoor air 80 %N_2, 10 % H_2 , 10 % CO_2
  - When H_2 is given externally
  - Mix with environmental O2
  - Gas composition changes to 80 % N2, 10 % H2O, 10 % CO2

"Anaerobic atmosphere"



Anaerobic Systems

Gas-Pack 100 Anaerobic systems

- 60 min later O2 level becomes 0.2 - 0.5 %
- Eh potential become s -229 mV

- O2 tolerant anaerobic bacteria can die within this period
- Pathogen anaerobes do not die with initial contact with O2
- Some can tolerate air contact 30-60 sometimes 100 min
- Jar method is poular for this reason
- Also practical and cheap

Anaerobic Systems

REDUCTION POTENTIAL (Eh):

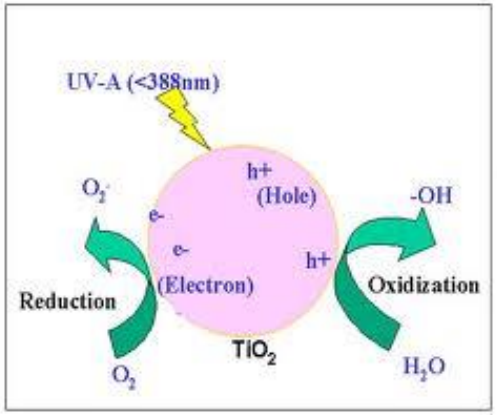
- Electron transmitter (donor, redactor or veya reductant)
  - Oxidising chemical substance within an ozidization event
  - Electron receiver (recipient, oksidant)
  - Reductor chemical substance within an oxidization event
  - This system is called **redox system**
- A redox system;
  - Electron chemicals in oxidising is called **oxidation half cell**
    Electron chemicals in reducing is called **reduction half cell**

Anaerobic Systems

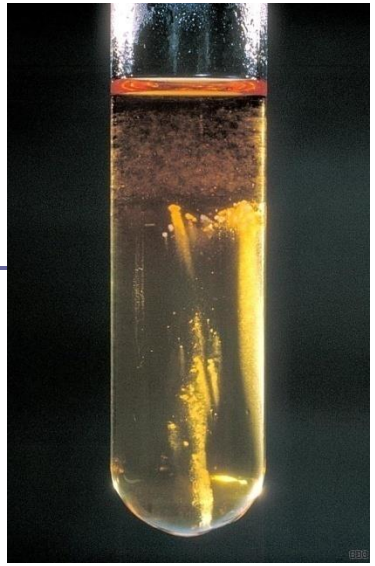
- Measurable electric charge within system ölçülebilir **Electrode potential**

- Electron potential of oxidation half cell;
- **Oxidation potential ,**positive

- Potaential of reduction half cell;
- **Reduction potential** (Eh), negative

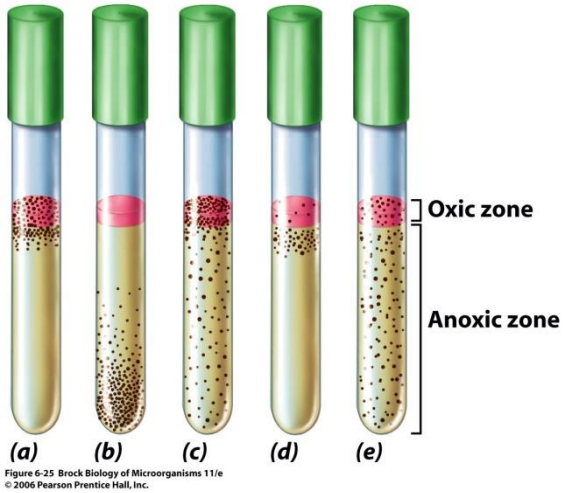


Anaerobic Systems



- Anaerobe bakteria;
  - Produce Reduction potential in low düşük environments
- Anaerobe media;
  - Adding some substances (lower reduction potential (reductase)
    - *L-cystine, cystein HCl, Na-thioglycolate, ascorbic acid, sodium bisulphide, glutathione* glukoz, metallic iron, boiled beef
- Media is sensitive to oxygen
  - Shoud be used fresh
- If stocked, closed tightly
- Boiled before used in order top remove soluble oxygen
- Anaerobe bouillons should be freezed (at least 8 ml)

Anaerobe Indicators



- During incubation;
  - Watch Eh potential of medium
  - Use Eh indicator stain if necessary
- Medium is in diffenet colour if it is oxidised or reduced
- resazurin, methylene blue and litmus
  - First they are colourless and become colourful when contact with O2
  - Resazurin become red when contact with O2 , methylene blue become colourful
- These stains become clourless if they loss O2 by boiling

Anaerobic Ecology

- Good bleeding tissue;
  - Generally do not produce anaerobic infection
  - Becouse, kanın Eh potential of blood is approximately +150 mV
- For an anaerobe infection;
  - Tissue should lose oxygen
- Anaerobic infections generally;
  - Can occur in necrosis, non bleeding tissue
  - In such tissu Eh voltage become lower than -250 mV and become an anerobic condition

Anaerobic Ecology

- Mesentery, colons, appendix, bursa, ovarium, muscular tissue and peritoneum;
  - Anatomically no oxygen reach region
  - Any injury, sensitive to anaerobic infections

Anaerobic peritonitis

Question

Can obligate abaerobic bacteria grow on condition with plenty of oxygen such as respiratory epithelial, conjuctiva or gingiva mucosa?

Anaerobic Ecology

- Within and surface of tissue;
  - Regions that anaerobes can protect from O2
- Hiding from O2
  - Cilia on mucosa
  - Regions where host tissue secretions cover mucosa
  - Mucosa on nasal passage
- Teeth and saliva
- Surfactant like secretions in lung alveoli
- Crypt on tonsils
- Produce oxygen free surfaces

Anaerobic Ecology

- Anaerobe bacteria;
  - More common in periapical lesions
  - But limited numbers in marginal gingivitis flora

    Becouse gingiva is not as close tissue as root canal and contact with O2
- Anaerobe bacteria colonization;
  - Depth of Gingiva
  - Bones that have periodontal tissue loss doku
  - Approximal surfaces of teeth

Anaerobic Ecology

- Also;
  - Advanced periodontitis
  - Bad prothetic restoration
  - Relaxed or krone with hole and bridges
  - Surfaces of total and partial prothesis on mucosa
  - Tongue papilla
  - Tartar and decay floor

  Oxygen fail to reach and anaerobe ecology can produce
- Bad mouth hygiene and insufficient cleaning trigger anaerobic ecology
- *Fusobacterium produce* Vincent stomatiti and necrosis ulcerative gingivitis

Anaerobic Ecology

- Other factors for anerobic ecology in host tissue;
  - Obligate aerobic bacteria of flora can use limited oxygen quickly
  - Thus, conditions will be suitable for anaerobic bacteria

- Necrotic tissues;
  - Deep injuries,
  - Crushed and ripped injuries
  - İnjuries with foreign substances in it
  - Decubitus ulcers

Anaerobic Bacteria and Anaerobic Infections

Clinically important anaerobes;

- Spore forming, Gram pozitive basilli:
  - *Clostridium*, *Desulfotomaculum*.
- Non spore forming, Gram pozitive basilli
  - *Actinomyces*, , *Lactobacillus*, *Propinibacterium*
  - *Acetobacterium, Arcanobacterium, Bifidobacterium, Eubacterium, LachnospiraMethanobacterium,*
- Non spore forming, Gram pozitive cocci:
  - *Peptococcus, Peptostreptococcus, Caprococcus, Gemmiger, Ruminococcus, Sarcinia, Gamella*

Anaerobic Bacteria and Anaerobic Infections

- Non spore forming, Gram negative basilli:
  - *Bacteroides*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Acetivibrio*, *Acidaminobacter*, *Anaerovibrio*, *Anaerorhabdus*, *Anaerobiospirillum*, *Anaerobacter*, *Bilophila*, *Capnocytophage*,
  - *Desulfobacter*, *Desulfobulbus*, *Desulfococcus*, *Desulfosarcinia*, *Desulfomonas*, *Desulfuromonas*, *Desulfovibrio*, *Dichelobacter*, *Fibrobacter*,
  - *Leptotrichia*, *Megamonas*, *Mitsuokella*, *Mobilincus*, *Pelobacter*, *Pectinatus*, *Progiogenium*, *Propionispira*, *Rikenella*, *Roseburia*, *Ruminobacter*, *Sebaldella*, *Selenomonas*, *Serpula*, *Spirochaeta*, *Succinomonas*, *Succinovibrio*, *Tisierella*, *Wolinella*.
- *Non spore forming, Gram negative cocci :*
  - *Veillonella*, *Acidaminococcus*, *Megasphera*

Anaerobic Infections

- Reasons of Anaerobe infections

  Low reduction potential

  Sufficient blood can not be found in tissues

- Anaerobe infections;
  - Nosocomial diarrhea, botulismus, diarrhea, clostridial myonecrosis, septic abortus, gas gangrene, actinomiycose apses, closed organ apses, aspiration pneumonia, apandicitis, cholecystitis, cellulitis,
  - Diş kökü ve dişeti infeksiyonları, stomatit, endokardit, beyin apsesi, menenjit, osteomyelit, orta kulak iltihabı, peritonit, septik artrit, kronik sinüzit, ampiyem ve tetanoz

Anaerobic Infections

Infection Type	Anerobe (%)
Aspiration pneumonia, lung apses and necrotic pneumonia	85-93
Bacteremia	**9-20**
Sinusitis	50-100
Thoracic empyemata	76
Brain apses	83
Root canal infections	99
Gingivitis and Periodontitis	**84**
Appandicitis and colon surgical injuries	79-95
Subcutaneous apses	60
Nonclostridial crepitan cellulitis	75
Pilonidal sine	73
Diabetes ulcerand gangrene	63-85
Urinary system Infections	**1**

Anaerobic Infections

- Most common anaerobes
- *B. fragilis, B. thetaiotaomicron*

  (Penicillin and analogs, tetracyclines, 3. generation s-cefalosporin, quinolon and aminoglycoside resistance)

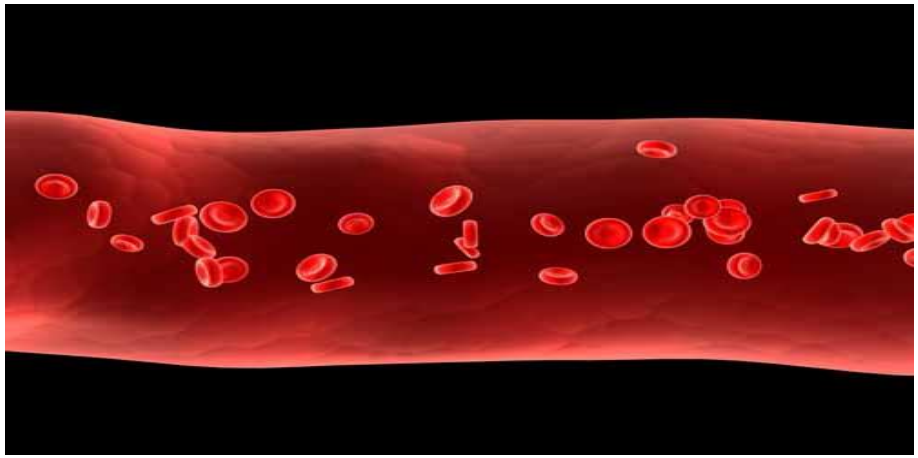
- *Clostridium* species, and *Fusobacterium;*
  - Assertive anaerobic infections

- Less common anaerobe infections;
- Actinomycosis (cronic, granulomatous and suppuratibe apses)
  - Most common agnets: *A. israelii, A. naeslundii, A. viscosus, A odontoliticus, A. meyeri.*

Anaerobic Infections

- *Propinobacterium propionicum* infections
  - Especially endocarditis in implant prothesis users

- Gram pozitive anaerobe cocci
  - Especially bone, joint and abdominal material (10%)
  - *Peptostreptococcus magnus, P. asaccharolyticus, P. prevotii, P. anaerobius* ve *Streptococcus intermedius*



Anaerobic Infections

- Infective tooth root canal anaerobic pathogens;
- *Fusobacterium nucleatum, Porphyromonas , Prevotella, Peptostreptococcus, Eubacterium, Lactobacillus, Wolinella recta, Streptococcus anginosus, Actinomyces israelii, Capnocytophage ochracea, Selenomonas sputigena, Veillonella parvula, Treponema denticola, Propinobacterium propionicum* ve *Acidaminococcus*