EN2002 AY17/18 S1

Question 1

I. d : Archaea lack peptidoglycan in cell walls, Gram negative-> pink
II. c
III. b
IV. a : Catabolism=energy-generating; Krebs not part of fermentation
V. c
VI. a :benefits one species but has little beneficial or harmful effect on the other
VII. d
VIII. b
IX. b : growth rate= $(N_f - N_i) / (t_f - t_i)$=1.38/h, doubling time = Ln2 / 1.38=0.5h
X. d : speed Z < X < Y so size Z > X > Y

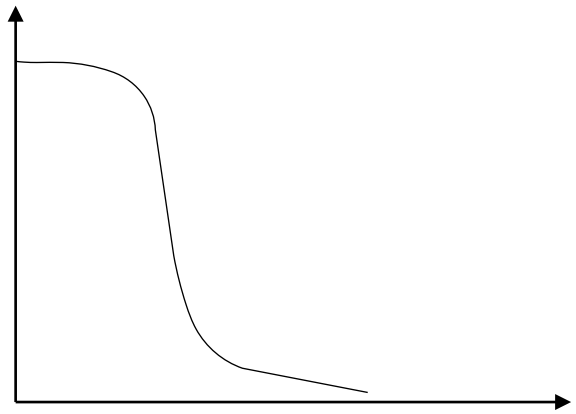
Question 2

a. P limits algal production. For 1 mol of algae, 1 mol of P and 16 mol of N needed.
Ratio N/P needed= 16.
No of moles of N in water: 2.0/14= 0.143mmol/L
No of moles of P in water: 0.1/31= 0.00323 mmol/L
Ratio N/P in water: 44.3, hence N is in excess => P is limiting factor

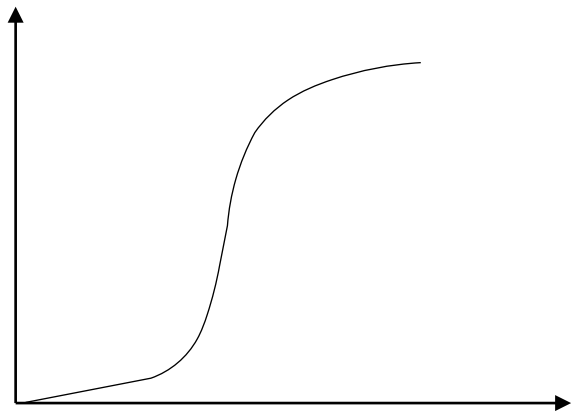
bi. Electron donor is phenol, electron acceptor is O.

ii. 1-C, 2-B, 3-C

c. [Lactate] vs t and $[Fe^{3+}]$ vs t:



$[Fe^{2+}]$ vs t:



Question 3

a. Respiration: Glycolysis, Krebs cycle, Electron transport chain, “outside” electron acceptor and Chemiosmosis (ATP synthesis)
Fermentation: Glycolysis, fermentation

Differences: Release energy from sugars or other organic molecules; No oxygen required; fermentation does not involve Krebs cycle or electron transport chain, uses “inside” organic molecules as final electron acceptor; Produces varying amounts of ATP 38 for respiration vs 2 for fermentation

b. There has been genetic exchange in bacteria. Mechanisms are:
Transduction — DNA exchange mediated by bacteria and bacteriophages in sludge
Transformation – free DNA released by one cell is taken up by another
Conjugation – DNA transfer from one bacteria to another through a direct contact cytoplasmic mating bridge

c. N is nutrient for all organisms. N needs to be processed into usable forms so that organisms can assimilate. Soil bacteria and plan-root associated bacteria are capable of converting N_2 into ammonium and nitrate. These aerobes or anaerobes are important for assimilatory or dissimilatory nitrate reductions in the biogeochemical cycle.

P is required for nucleic acids, lipids and some polysaccharides. Bacteria is needed to mineralise P into phosphate, and to immobilise it to P where necessary. Soil bacteria act as both sinks and sources of available phosphorus in the biogeochemical cycle. There is also enhanced biological P removal in water treatment, where phosphorus accumulating organisms take in amounts exceeding normal requirements.

d. Microbial ecology studies the microbial interactions and their interactions with the environment. Microorganisms in the air usually do not have metabolic interactions with each other. In contrast, the microbial mixture in aerosols is a random collection of microorganism than a functional microbial community.

e. The air is an unfavourable environment because there is a lack of nutrients for growth, poses threats of desiccation and has solar radiation. To survive, microorganisms become dormant, produce biomolecules with high moisture retention, form spores, produce dyes or protective barriers.

Question 4

4a. Good dilutions give 30-100 CFU per plate, so use second row.

Microbial concentration (CFU/mL) = $\{[(50+56+68)/3]*10^4\}/0.1 = 5.8 \times 10^6$ CFU/mL

b. Different order of magnitude. It could be due to the Great Plate Count Anomaly, a discrepancy between the number of microbial cells observed microscopic examination and the no of colonies that can be cultivated from the same natural sample (CFU count). Possible reasons are there could be dead cells that are observable but do not form colonies, some cells are viable but not culturable. Some are obligate symbiotic and thus grow only when favourable partners are present. It may also be that conditions such as nutrients, temperature and pH are not suitable for growth.

c. The enrichment process is carried out to be selective for the target bacteria, and counter selective for the other organisms. For isolation, it is necessary to streak plate, transfers colonies to new agar plates, and perform inoculation from agar to broth. The first step in the spread-plate

method is to pipette the sample onto surface of agar plate, and spread evenly using sterile glass spreader. Thereafter, the agar will be incubated for a period of time, eventually producing surface colonies. After acquiring cells, the DNA has to be isolated. Polymerase chain reaction is used for amplification, followed by DNA sequencing. The sequence analysis will then be used to construct the phylogenetic tree.

d. Direct count, viable count methods and genomics can also be used. For example, the Fluorescent in-situ hybridisation method uses an oligonucleotide probe targeting 16S rRNA gene of all Bacteria and all ammonia-oxidisers containing the ammonia monooxygenase gene. Microbial activity can be measured in terms of total cell protein, enzyme activity and metabolic activity. Genomics enables identification by comparative analysis, pinpointing sequence similarity to genes found in other organisms.

Meta-genomics can also better address this question, as it is a study of the collective genome of a microbial community in a given environment. After taking environmental samples, the microbial community is isolated and the genomic DNA acquired. High-throughput sequencing will generate the raw sequences, which after assembling and analysing the genomes of a community will answer this question with greater detail, including metabolism and transport, occurrence of lateral gene transfer and phylogeny.

Question 5

a. High loading rate increase substrate concentration, which favours growth of floc-forming bacteria. Use higher nitrate concentration and regulate amount of oxygen in the treatment plant.

bi. DBPs are formed by reactions of disinfectants with natural organic matter (NOM) and microbial extracellular polymeric substances (EPS).

ii. Chlorine: trihalomethanes (THMs), haloacetic acids (HAAs)
Choramine: N-nitrosodimethylamine (NDMA)

iii. Biofilms of microorganisms will react with the disinfectants, thus forming DBPs and protecting pathogens from the action of residual disinfectant in water distribution networks.

ci. Ideal FIB:
- Suitable for analysis of all types of water
- Present whenever enteric pathogens are present
- Survives longer than hardiest enteric pathogen
- Does not reproduce in contaminated water
- Detected by highly specific test: test should be easy to do and sensitive
- Harmless to humans
- Its level in water reflects degree of fecal pollution

ii. Coliforms and Fecal streptococci.

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