HL Paper 3

Discuss biopharming.

Markscheme

a. produces useful pharmaceuticals/drugs/proteins

b. inserts genetic material/genes into host plants/animals

c. produces more complex drugs/proteins than prokaryotic organisms

OR

no post-translation modification with prokaryotes «so no complex proteins»

d. valid example

Allow verifiable examples, eg: antithrombin/coagulation factors «in goats», development of Norwalk virus/ cholera toxin vaccines «in tomatoes»

e. issues regarding contamination of other organisms

OR

possible ecological effects

f. plants process proteins differently than humans

g. proteins produced by plants may cause allergic reaction

h. some proteins are intellectual property

i. example of ethical issue

[Max 6 Marks]

Examiners report

[N/A]

Discuss the risks of gene therapy including safety, conflict of interest and ethical arguments.

Markscheme

definition:

insertion of genes / replacement of defective genes using a vector into non-germ line cells;

safety:

the DNA/viral vector may integrate into other parts of the genome;

causing problems of gene expression;

possibly cancer;

example of SCID treatment/leukemia;

the vector may trigger an immune response;

viral vectors may recombine with other viruses;

inadvertent modification of germ line cells;

conflict of interest:

the producers of gene vectors gain financially;

clinical trials must be free of bias from commercial sponsors;

ethics:

the vectors are tested on animals and may behave differently in humans;

unethical to use healthy humans in trials;

no gene therapy has been commercialized / no valuable results as yet;

*Award **[4 max]** if responses do not address safety, conflict of interest and ethics.*

Examiners report

This part of the programme had often not been studied in detail. The candidates were not answering the question asked; especially in the area of safety which was largely not known. The only suggested answer was that gene therapy may lead to cancer. Conflicts of interest were not known and ethical arguments were very general. Indeed ethical arguments was the only area often covered but with lack of depth of expression. Many answers gave religious arguments concerning gene therapy but these were not answering the question asked about the risks of gene therapy.

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a. Beans contribute to flatulence. Alpha-galactosidase, derived from the fungus *Aspergillus niger*, is an enzyme that breaks down the fibre usually fermented by bacteria, reducing intestinal gas. Describe how alpha-galactosidase would be produced using *A. niger* in a continuous fermenter. [3]

b. Temperature is a variable that needs to be continually monitored in deep-tank batch fermentation of penicillin. List **two** other variables that need to be monitored. [2]

Markscheme

a. a. constant nutrient medium «supply» needed/maintained

   b. optimal mixing

   c. fermented in sterile bioreactor

   d. alpha-galactosidase production/general conditions assayed/screened/monitored «throughout the process»

   e. continuous removal of alpha-galactosidase/products

   [Max 3 Marks]

b. a. pH

   b. «dissolved» oxygen

Examiners report

a. [N/A]
b. [N/A]

---

a. The following base sequence represents part of a larger DNA molecule that is going to be analysed for the presence of open reading frames. [3]

5′ GTGAAACTTTTTCCTTGGTTTAATCAATAT 3′
3′ CACTTTGAAAAAGGAACCAAATTAGTTATA 5′

Explain how this DNA can have six possible reading frames.

b. State the type of codon that helps to identify open reading frames. [1]

c. Once an open reading frame is identified, explain the steps researchers would follow to determine a potential function for that sequence. [6]

Markscheme

a. a. «three» reading frames can occur in either strand

b. from 5' « to 3' »

c. reading frame can start from any of the first three nucleotides

d. from the top strand: GTG or TGA or GAA as first triplet *OWTTE*
OR
from the bottom strand: ATA or TAT or ATT as first triplet

b. start codon/AUG

OR

stop codon/UAA/UAG/UGA

c. a. use a database

b. conduct BLAST search

OR

BLASTn allows DNA sequence alignment

c. «sequence alignment software used» to identify/compare similar sequences in different organisms

d. gene function can be studied using model organisms with similar sequences with known function *OWTTE*

e. BLASTp allows protein alignment
OR
EST may be used to identify gene activity

f. can change sequence and create “knockout” study organism

g. changes in phenotype due to knockout procedure allow determination of function

h. valid example provided

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]

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a.i. Outline what is meant by the term genetic markers. [1]

a.ii. Outline **two** uses of genetic markers. [2]

b. Evaluate the use of viral vectors in gene therapy. [2]

c. Outline the use of microarrays to test for genetic disease. [3]

Markscheme

a.i. A gene/DNA sequence «with a known location on chromosome» used for identification

a.ii. a. to identify species/pathogenic organisms

OR

successful uptake of DNA in genetically modified organisms/GMOs

b. to detect disease due to variation in DNA «substitution/deletion»

c. to determine risk of developing certain disorders

d. to confer resistance to antibiotic/agent that would normally kill it

e. to make cells containing gene look different
OR
green fluorescent tag makes cells visible under UV light

b. a. gene therapy trials have used viruses to deliver un-mutated copies of genes to the «somatic» cells of the patient's body

b. examples of the use of viral vectors *eg gene therapy may provide a way to cure genetic disorders, such as severe combined immunodeficiency*

c. one of the main problems is immune response to viruses / may cause toxicity/disease

d. some viral vectors insert their genomes at a random location on one of the host chromosomes «which can disturb the function of cellular gene» / enter wrong cells «if targeting tumour» / could lead to cancer

c. a. analyze tissue/blood sample for DNA sequence

b. each spot «on microarray» has small quantity of specific DNA sequence/probe

c. reverse transcriptase used to make cDNA

d. fluorescent dye linked to cDNA

e. «cDNA» binds to/hybridizes with probes that have complementary base sequences

f. fluorescence/different colours shows probes have hybridized / which sequences were in the tissue sample

Allow specific examples of genetic diseases.

Examiners report

a.i. [N/A]
a.ii. [N/A]
b. [N/A]
c. [N/A]

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a. Metabolites that indicate disease can be detected in urine. State a metabolite found in urine and the disease it could indicate. [2]

Metabolite:

. . . . . .

Disease indicated:

. . . . . .

b. Discuss the implications of biopharming using a specific example. [4]

Markscheme

a. a. named metabolite

eg: glucose

b. associated disease

eg: diabetes

b. a. production of pharmaceuticals

OR

named example of biopharming

b. easily scaled to cover demands
OR
cheaper

c. drugs can be delivered in food «making it more attractive/easier to eat»

d. unethical/ethical aspect/OWTTE

e. allergic reactions/ side effects

f. horizontal gene transfer consideration

Examiners report

a. [N/A]
b. [N/A]

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a. Outline **one** way in which genetic sequences can be used to indicate predisposition to a disease. [3]

b. Outline the use of luminescent probes in the treatment of tumours. [2]

Markscheme

a. a. genetic markers/specific sequences can be present in people with a disease *OWTTE*

b. presence «of markers/specific sequences» indicates risk/probability of onset of condition *Allow vice versa*.

c. technique to detect the presence of the sequence *eg*: *PCR, electrophoresis, DNA sequencing, FISH, DNA databases, etc*.

d. example of predisposition *eg*: *BRCA sequence mutations indicating predisposition to breast cancer*

b. a. transferrin/other protein taken up at higher rates by tumour cells

b. transferrin/other protein can be labelled with a luminescent dye

c. different tumour cell types can be distinguished/labelled in different colours

d. can be used to highlight tumours «during surgery» *OWTTE*

Examiners report

a. [N/A]
b. [N/A]

Discuss the use of viral vectors in gene therapy including the risks involved.

Markscheme

viral vector used to replace defective gene in somatic cell;

virus genetically engineered to carry normal copy of gene;

valid example; (*e.g. SCID (severe combined immunodeficiency*))

cause of disease; (*e.g. lack of enzyme/adenosine deaminase/ADA in bone marrow cells*)

technical issues need to be solved / ensure correct amount of gene product/at the right time/in the right place;

need to be sure insertion of therapeutic gene does not harm other necessary cell functions;

viral vectors may infect healthy cells;

causing illness/disease/cancer;

virus may revert to original form and cause disease;

newly introduced DNA may affect reproductive cells causing genetic changes;

immune system may attack newly introduced viruses causing inflammation/toxicity/ organ failure;

Examiners report

Answers failed to include enough of the range of risks needed for a high score.

The dye Reactive Black 5 (RB5) is widely used for dyeing in textile industries. Removal of the dye from factory waste-water is important not only for aesthetic reasons but also because the dye can lead to mutations that may lead to cancer. *Paenibacillus* is a bacterium that can metabolize the dye.

a. Suggest **one** way in which organisms such as *Paenibacillus* metabolize toxic substances. [1]

b. The decontamination system for the removal of the dye uses a surface to which *Paenibacillus* can attach. Suggest **one** advantage of providing a surface for attachment. [1]

c. Outline another **named** example of a microorganism used in bioremediation. [3]

Markscheme

a. Uses it as an energy source/carbon source/electron acceptor

Do not accept "metabolizes" it.

b. To allow for the formation of a biofilm

OR

Does not get washed away/can be re-used

Increases surface area which increases contact between bacteria and dye

c. Name of microorganism *eg: Pseudomonas. Must use at least Genus name*.

Name of pollutant *eg: oil*

Outline of action *eg: uses oil as energy/carbon source* ***OR*** *degrades oil*

Allow other valid checked examples

Examiners report

a. Some candidates had difficulty with this but many were able to give one way the dye was used by the bacterium.

b. Some were able to achieve the mark by using the term 'biofilm' while others understood the concept but did not seem to be familiar with the term.

c. This was a relatively easy 3-mark question on bioremediation but a few missed out on details or confused organisms and actions.

Distinguish between the structure of Gram-positive and Gram-negative bacteria.

Markscheme

a. Gram-negative bacteria have a thinner peptidoglycan cell wall / Gram-positive bacteria have a thicker peptidoglycan cell wall

b. Gram-negative bacteria have an additional membrane of «lipopolysaccharide and protein» outside the wall «whereas Gram-positive bacteria do not»

Examiners report

[N/A]

Explain how infection by a pathogen can be detected by an ELISA test for antigens.

Markscheme

Infected individual will have pathogens/antigens in bodily fluids

Test contains immobilized/fixed antibodies to the pathogen/antigen

If present, antigen binds to «immobilized» antibody/capture molecule

Solution is washed and only fixed antigen-antibody complexes persist

Then a detection antibody linked to an enzyme is added

Reacts with fixed antigen-antibody complex

A chromogenic/potentially fluorescent substance is added

Enzyme changes substance to a different/detectable colour

The presence of colour is a positive response/absence of colour a negative response

Examiners report

Most candidates had a fairly good idea of how to use an ELISA test to detect antigens from a pathogen although the order of steps, and antigen and antibody, were sometimes confused. This discriminated well between candidates.

Explain how BLAST searches are carried out and the applications of different types of these searches.

Markscheme

Process **(max [5])**:

a. BLAST «Basic Local Alignment Search Tool» search enables comparison of an unknown sequence with databases of sequences

b. «software» finds similar sequences / aligns sequences by locating matches between two sequences

c. carries out statistical calculations «to find matches with other sequences»

d. BLASTn used to align/show similarities in nucleotide sequences in nucleic acids

e. BLASTp used to align/show similarities in amino acid sequences in proteins

f. used to identify the gene of a protein

Application **(max [2])**:

g. one application of BLAST

h. second application of BLAST

eg BLAST can be used for identifying species / locating domains / establishing phylogeny / DNA mapping / other verifiable examples

Examiners report

[N/A]

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a. Outline the diversity of Eubacteria according to cell wall structure. [2]

b. State the role of *Rhizobium* and *Nitrobacter* in the nitrogen cycle. [2]

Rhizobium: .....

Nitrobacter: .....

d. Explain the use of bacteria in bioremediation. [2]

Markscheme

a. can be Gram-positive or Gram-negative;

Gram-negative have a thinner wall/less peptidoglycan/*converse*;

Gram-negative have an outer layer of lipopolysaccharide and protein;

b. *Rhizobium*: converts atmospheric nitrogen to ammonia / nitrogen fixation;

Nitrobacter: oxidizes nitrite into nitrate / nitrification;

d. bacteria remove contaminants from the environment;

by using them as energy sources;

(or) by converting them to a soluble/ harmless form;

example of bioremediation (*e.g. Pseudomonas* is used to clean up oil spills);

Examiners report

a. Most were able to get a mark for indicating that bacteria can be Gram-positive or Gram-negative. Many were able to obtain a second mark for indicating the different compositions of their cell walls.

b. Candidates tended to either get the roles of both bacteria in the nitrogen cycle correct or both wrong.

d. While many had a general idea of the use of bacteria in bioremediation, few were able to give an actual example.

Describe the consequences of releasing raw sewage into rivers.

Markscheme

a. pathogens/toxins may be released/contaminate drinking water;

b. (saprotrophic) bacteria live off sewage;

c. decrease dissolved oxygen/DO / increase the oxygen demand/BOD;

d. animals/other (aerobic) organisms may die;

e. decomposition causes increase in ammonia/nitrates/phosphates/CO_2;

f. high levels of nitrate/phosphate can stimulate algal growth/blooms / eutrophication;

g. block light for other algae/plants below;

h. which die and decompose, releasing more nutrients;

i. promote more algal growth;

Examiners report

Most candidates provided good, but often only partial answers about the release of raw sewage into rivers; many omitted to mention the saprotrophic and/or pathogenic nature of bacteria present in sewage and to make references to specific substances such as phosphates, nitrates and ammonia.

Explain the use of bacteria in the bioremediation of water.

Markscheme

a. example of where this has been used *e.g.* Exxon Valdez spill, Alaska / other correct example;

b. indigenous/existing bacteria can break down oil;

c. bioremediation is the use of nutrients to enhance the activity of existing organisms / the addition of non-indigenous microorganisms;

d. converts the toxic compounds of oil to non-toxic products;

e. bioremediation is used after other cleanup methods have been used;

f. bioaugmentation is when bacteria are added to supplement the existing microbial population;

g. biostimulation is when nutrients are added to stimulate the growth of the existing oil-degrading bacteria;

h. bacteria added seem to compete poorly with the indigenous population;

i. nutrient concentrations have to be sufficient to support the maximal growth rate of the bacteria throughout the clean-up operation;

Examiners report

Few candidates answered this question well. It is important to note that this option, in many cases, seemed to be answered by the occasional candidate within a school where the majority of candidates answered other options. Some understood that bacteria could decompose contaminants, but with no mention of oil, and certainly no differentiation between methods or details of any sort.

Explain how methane can be generated from biomass.

Markscheme

processes:

a. generated from waste animal/plant material;

b. material placed in a digester/fermenter/bioreactor;

c. bacteria/microorganisms convert organic waste into organic acids and alcohol;

d. all processes are anaerobic / fermentation;

e. other bacteria act on waste to convert to acetate/carbon dioxide and hydrogen;

f. methanogenic bacteria produce methane anaerobically;

g. from acetate/carbon dioxide and hydrogen;

conditions:
h. digester needs to be damp/warm for reactions to occur;
i. pH conditions not too acidic / in absence of oxygen;
j. temperature rise needs to be controlled to prevent killing the useful bacteria;

Examiners report

This was probably the most successful third question of the whole paper, perhaps because it was based on factual information, but also because candidates were able to incorporate precise details and present the process in a logical order. Most candidates, obviously well prepared, gained many if not all the marks for this, but some irrelevant answers were also seen.

List **two** roles for microbes in ecosystems.

Markscheme

producers/nitrogen fixers/decomposers/parasites/pathogens/nitrifiers/denitrifiers (*accept other correct roles*)

*Award **[1]** for any two.*

Examiners report

Many candidates could name two roles of microbes but a surprising number were only able to give one correct role for microbes in ecosystems and thus did not get the mark.

Outline how bacteria can be classified by Gram staining.

Markscheme

a. Gram stain permits classifying bacteria according to structure of their cell walls;

b. Gram-positive bacteria have thick peptidoglycan layer and Gram-negative a thin layer with an outer membrane;

c. giving different pathogenic/resistance properties;

d. Gram-positive bacteria appear purple / Gram-negative pink/reddish;

Examiners report

Most knew about Gram staining, but some confused the characteristics between $Gram^{+}$ and $Gram^{-}$.

Outline **one** example of the use of a marker gene in genetic engineering.

Markscheme

a. marker gene inserted into DNA containing target gene

b. recombinant DNA «with marker gene and target gene» inserted into cell/organism

c. named example of marker and target gene *eg*: *ampicillin resistance with BT gene for glyphosate resistance*

d. further details of how the marker gene works *eg*: *culture cells in ampicillin and if the cell grows into a callus, uptake has occurred*

Examiners report

[N/A]

Explain how bacteria are used in bioremediation of soil.

Markscheme

a. bioremediation uses bacteria to remove contamination;

b. suitable bacteria may already be present in the environment;

c. bacteria may use contaminants in metabolism/as an energy source;

d. end products (of metabolism) are less toxic than (inorganic) contaminants;

e. bacteria may concentrate/isolate it within the microbial cells;

f. can be carried out in situ or ex situ;

g. suitable example (eg degradation of solvents / oil spill cleanup / oxidation of selenium / can be used to deal with pesticides);

h. second suitable example;

i. useful bacterial strains obtained by genetic modification;

Examiners report

This was the least popular of the HL options but it was encouraging to note some schools studying it with some good standards seen.

Many candidates knew that bioremediation used bacteria to remove contamination from soil, such as happens during an oil spill. Only better candidates were able to actually explain how bacteria used the contaminants in their own metabolism and what the source of these bacteria was. Many were incorrectly describing the nitrogen cycle. Perhaps candidates had been taught bioremediation of high nitrate content ground water by bacteria but expressed it, incorrectly, as the nitrogen cycle.

State, giving **one** specific example, how individual bacteria change their characteristics when they form aggregates.

Markscheme

example;

change;

e.g.:

Vibrio fischeri;

emit light when part of high density population/high concentration of regulatory substance;

Accept other verifiable example.

Examiners report

The majority of candidates exhibited knowledge and gained marks in part (a), but some failed to gain all of them because of imprecision in their answers; a certain number of candidates, especially in French scripts, confused between the type of clusters characteristic of certain bacteria seen under the microscope (e.g. grape-like clusters in *Staphylococcus sp*.), and aggregates causing a change in properties.

The table shows a comparison of DNA base sequences in several yeast (*Saccharomyces*) genomes.

Species	Number of DNA base sequences	Percentage of coding sequences
S. paradoxus	728	88
S. cariacanus	867	88
S. mikatae	1136	84
S. bayanus	851	80
S. castellii	2290	70
S. kluyveri	2145	70
S. unisporus	2357	69

[Source: P. F. Cliften et al. (2001) 'Surveying Saccharomyces Genomes to Identify Functional Elements by Comparative DNA Sequence Analysis', Genome Research, 11, pp. 1175–1186. © Cold Spring Harbor Laboratory Press. Reproduced with permission.]

a. Identify the species that has the lowest percentage of coding sequences. [1]

b. State how similar nucleotide sequences can be identified. [1]

c. The yeast *Saccharomyces cerevisiae* was the first eukaryotic organism to have its entire genome sequenced. Suggest reasons for the choice of yeast as a study organism. [3]

d. Outline possible medical applications of the polymerase chain reaction (PCR). [1]

Markscheme

a. *S. unisporus*

b. BLASTn/sequence alignment software

"n" required in BLASTn

c. a. easy to grow

OR

easy/cheap to produce large amounts

OR

fast generation time

b. genomes are small/easy to manipulate

c. metabolically diverse

d. industrial applications/biopharming

e. no ethical issues «with yeast»

[Max 3 Marks]

d. a. identify different viral/influenza strains

b. genetic testing/testing for genetic disease mutations

c. tissue typing

d. vaccine development

[Max 1 Mark]

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]
d. [N/A]

Discuss methods used in gene therapy, including the risks involved.

Markscheme

somatic cell therapy methods alter the genetic material of somatic cells/nongametes;

could cure the individual treated but disease can still be passed to offspring;

germ-line therapy methods alter the genetic material of sex-cells/gametes/sperm/eggs;

disease would be absent in future offspring;

retroviruses/viruses are used (as vectors);

to insert (normal) gene/allele (in host cells);

verified application, *e.g.* treatment of SCID (severe combined immunodeficiency);

no fully successful cases since relatively new / technical issues need to be solved *e.g.* ensure correct amount of gene product/at the right time/in the right place;

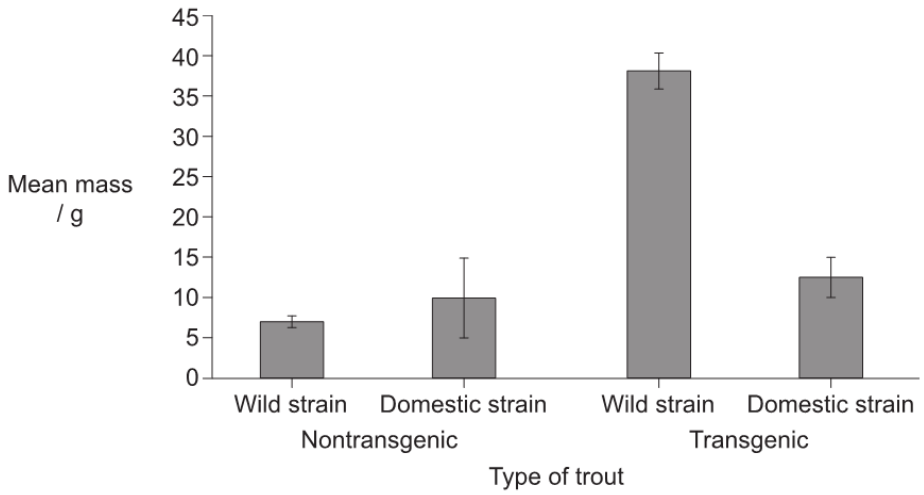
cases of gene therapy causing cancer in patients / infect healthy cells causing illness / harm other cell functions / *e.g.* (two) children treated for SCID developed leukaemia;

immune system may attack newly introduced viruses causing inflammation/toxicity/organ failure;

Examiners report

Points were awarded for use of viruses as vectors and for a possible risk of gene therapy. Some better candidates were able to give one verified example such as SCID. Very few were able to discuss somatic cell therapy and germ-line therapy. Candidates tended to confuse gene therapy with other genetic topics.

Transgenic rainbow trout (*Oncorhynchus mykiss*) were produced from both wild strain and domestic strain trout, using a gene coding for growth hormone from coho salmon (*Oncorhynchus kisutch*). The graph shows the mean mass of the nontransgenic and transgenic trout at 8 months post-fertilization.



[Source: Reprinted by permission from Macmillan Publishers Ltd: Nature, 409, Growth of domesticated transgenic fish, R H Devlin et al., pp. 781–782, copyright 2001]

a. Analyse the data for the growth of nontransgenic trout and transgenic trout. [2]

b. Suggest a reason for the growth differences between the nontransgenic trout and transgenic trout. [1]

c. Describe the use of marker genes in the development of transgenic organisms such as trout. [2]

d. Outline the possible environmental impact associated with the accidental release of transgenic trout. [2]

Markscheme

a. a. both transgenic «strains» show more growth/mean mass than nontransgenic

Allow vice versa

b. wild nontransgenic «strain» showed less growth than wild transgenic

Allow vice versa

OR

greatest difference between wild nontransgenic and transgenic «strains»

OR

wild «strain» showed less growth/mean mass in nontransgenic but reverse in transgenic

c. mean mass/growth in domestic nontransgenic «strain» lower than «domestic» transgenic

d. error bars overlap for domestic nontransgenic and transgenic «strains»

[Max 2 Marks]

b. gene for growth hormone has been assimilated/is expressed in the transgenic trout

OR

more growth hormone produced/expressed in transgenic trout

c. a. indicates successful uptake of recombinant DNA

b. identifies transgenic organisms

c. example of a marker gene

eg: antibiotic resistance gene in bacteria

[Max 2 Marks]

d. a. transgenes may be transferred to other species/organisms

b. may alter ecosystem/food chain

c. may outgrow other species

OR

decrease biodiversity

OR

outcompete nontransgenic individuals/trout

[Max 2 Marks]

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]
d. [N/A]

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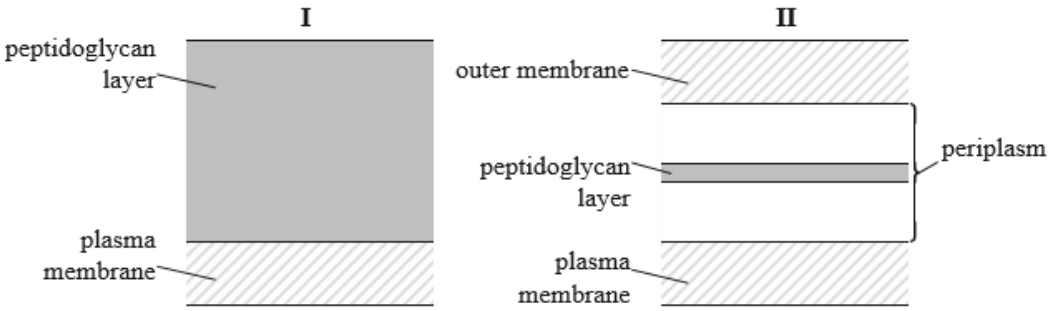
b. The diagram below represents the cell walls of two different bacteria. State, with a reason, which cell wall (I or II) is Gram-positive. [1]



c. Microorganisms play many roles in ecosystems. List **two** of these roles. [2]

1. .....
2. .....

Markscheme

b. I (is Gram-positive) because it has thick/outer layer of peptidoglycan/does not have outer layer (of lipids) external to peptidoglycan layer

c. producers / food source in aquatic environments;

nitrogen fixers / denitrifiers;

decomposers;

methane producers;

Examiners report

b. Many candidates were able to correctly indicate that the diagram was of a Gram-positive bacterium and why.

c. It was surprising that many candidates did not get the two marks for listing two roles of microorganisms in the environment as this was very basic.

Explain the formation of biofilms and the problems associated with their formation.

Markscheme

Formation

a. biofilm is a group of microorganisms embedded in a «exopolysaccharide/EPS» matrix

b. microorganisms adhere on a surface/to each other

c. cells are able to communicate/cooperate via quorum sensing
OR
secrete molecules that facilitate the aggregate adhering to the surface
OR
facilitate individual cells sticking together/OWTTE

d. phenotypic shift in behaviour
OR
emergent properties appear
OR
differential regulation of genes

Problems
e. «formation of biofilms» in the body facilitates infections/OWTTE

Accept any verifiable health problem caused by biofilms, e.g. dental plaque causing caries, lung infection in cystic fibrosis patients, etc.

f. clogging/corrosion of pipes in water systems

g. transfer of microorganisms in ballast water

h. contamination of surfaces in food production

i. highly resistant to antimicrobial agents/antibiotics

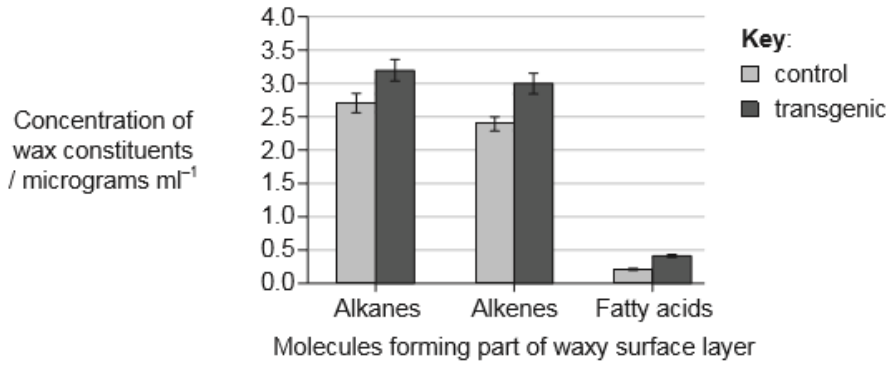
j. EPS provides a physical barrier to the entry of the antibiotic «into the colony»

*Award **[5 max]** if only problems are mentioned.*

Examiners report

[N/A]

Crop genetic engineering was performed to improve drought tolerance in tomato plants (*Solanum lycopersicum*) by adding a gene from an edible fungus (*Flammulina velutipes*). The cotyledons of tomato plants were cut and co-cultivated with *Agrobacterium tumefaciens* containing the transgenic Ti plasmid. Plates containing kanamycin were used to select for transgenic cotyledons. The graph shows concentrations of three constituents of the wax that coats wild type plants (control) and transgenic tomato plants.



[Source: Reprinted by permission of Nature Publishing Group. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3517979/) Reprinted by permission from Macmillan Publishers Ltd: *Nature*, 'Expression of a fungal sterol desaturase improves tomato drought tolerance, pathogen resistance and nutritional quality' by Ayushi Kamthan *et al*. 2, p. 951. (2012).]

a. Outline the use of kanamycin in the selection of transgenic cotyledons. [2]

b. State how the sequence of the target gene from the fungus could be identified using a bioinformatics tool. [1]

c. Suggest whether the results of this experiment show that these transgenic tomato plants are more resistant to drought. [2]

Markscheme

a. a. kanamycin resistance as marker gene

b. when organisms grown in kanamycin, only resistant survive
OR
those that took up resistance/cloned ones survive

b. database/NCBI search to find target gene/OWTTE

OR

search for target gene in other/related organisms

Allow other named database.
Please check unfamiliar names for authenticity.

c. a. more wax deposition constituents «in leaves» of transgenic than control plants

b. wax is waterproof

c. less evaporation from «waxy» leaves «protects from drought»

Examiners report

a. [N/A]
[N/A]

b. [N/A]

Outline how a defective gene can be replaced using viral vectors.

Markscheme

a. viral vector modified to include healthy gene;

b. virus is taken up by cells;

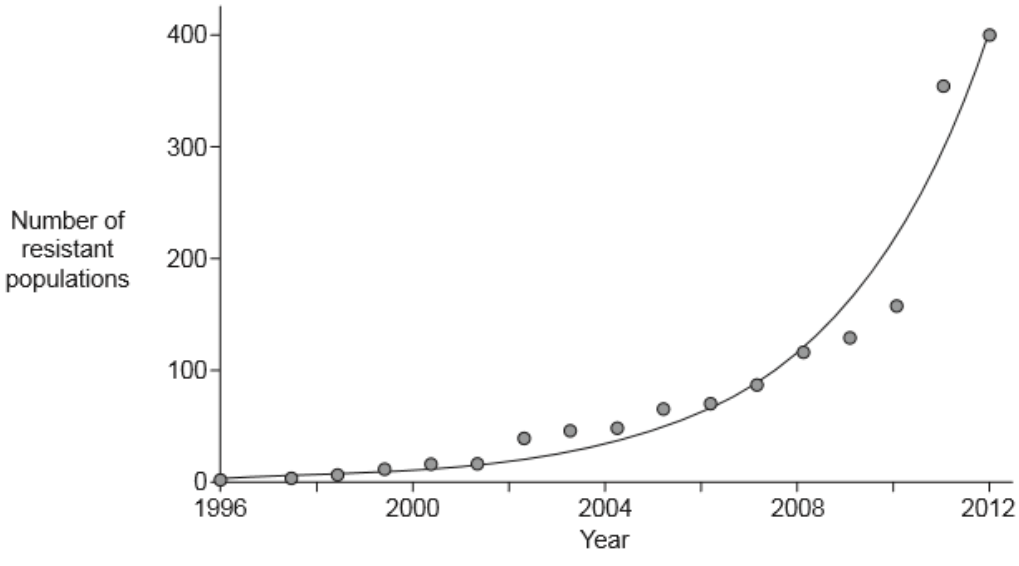
c. inserts normal gene into chromosome;

d. white blood cells / bone marrow/other cells replaced into patient;

Examiners report

There was a range of answers about using viral vectors: although some provided clear and complete answers, many did not use appropriate terminology or were incomplete; others showed only a vague understanding of the process or confused it with other processes related to biotechnology.

Annual ryegrass (*Lolium rigidum*) is a weed species that has been successfully controlled by the application of the herbicide glyphosate. The graph shows the number of confirmed cases of glyphosate resistant ryegrass across Australia between 1996–2012.



[Source: adapted from www.grdc.com.au]

a. (i) Outline the pattern of change in resistant populations of ryegrass over time in Australia. [2]

(ii) Suggest **one** reason for the pattern.

b. State **two** environmental benefits from the use of genetically modified glyphosate resistant soybeans. [2]

c. Explain the role of the *Agrobacterium tumefaciens* Ti plasmid in genetic modification. [3]

Markscheme

a. (i) Resistance is «exponentially» increasing (*OWTTE*)

(ii) Increased growth/differential survival of resistant population
OR
Natural selection for glyphosate resistance

Evolution of resistance is possible due to pre-existing variability

«Strong» selective pressure «by application of glyphosate»

b. Increased crop yields

OR

Reduced demand for more land

Less application of other herbicides «to control weeds»
OR
Glyphosate can replace more toxic/persistent herbicides

Less need for tilling/plowing/ploughing
OR
Less soil erosion

«Less plowing/herbicide application» uses less fuel/less emissions produced

c. *Agrobacterium tumefaciens* «Ti» plasmid causes tumours in the plant it infects

Donor gene inserted into Ti plasmid

Along with antibiotic resistance gene

Construct/recombinant Ti plasmid re-inserted into an *A. tumefaciens* bacterium

Infect leaf with bacterium «and grow on antibiotic medium»

«Some surviving» cells contain the gene/are glyphosate resistant

Examiners report

a. Most were able to identify the pattern of change in the graph in (i) but suggesting a reason for the pattern (ii) was more discriminating with fewer candidates able to express clearly why this was happening.

b. Most seemed able to give two environmental benefits and thus earned the 2 marks.

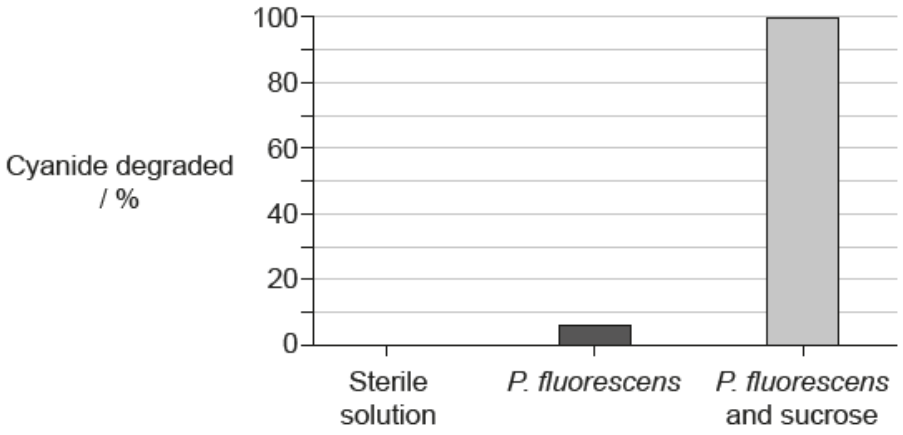
c. Most candidates were also able to explain the use of Ti plasmids in genetic modification, although some steps were left out, such as the reinsertion of the Ti plasmid in the bacterium or the role of the antibiotic resistant gene.

Compounds containing the cyanide group (CN) are used to help extract gold from gold-containing rocks called ore. The process results in heaps of rocks that are contaminated with cyanide, a toxin that can inhibit cellular respiration. The bacterium *Pseudomonas fluorescens* degrades cyanide to ammonia (NH_3), which is less toxic.

cyanide + oxygen + organic carbon source → carbon dioxide + ammonia + nitrates

In an effort to explore the conditions that lead to maximum degradation of cyanide, researchers sprayed different samples of cyanide-processed ore with one of three solutions:

- a sterile solution
- a solution containing a culture of *P. fluorescens*
- a solution containing a culture of *P. fluorescens* and sucrose.



[Source: adapted from C White and J Markweise, (1994) *Journal of Soil Contamination*, 3, pages 271–283. http://www.informaworld.com]

a. Outline the evidence that *P. fluorescens* can degrade the cyanide. [2]

b. Suggest how the addition of sucrose promotes the degradation of cyanide. [1]

c. With respect to the degradation of cyanide by *P. fluorescens*, explain what is meant by bioremediation. [2]

Markscheme

a. a. in sterile solution/control there is no degradation of cyanide but there is in the solutions with *P. fluorescens* *OWTTE*

b. in solution containing *P. fluorescens* and sucrose degradation of cyanide higher than without sucrose

c. control with sucrose «only» missing to establish causality *OWTTE*

b. «organic» carbon source «necessary for the reaction to degrade cyanide»

OR

sucrose provides the energy source

c. a. bioremediation is the use of organisms to degrade pollution/toxins in the environment

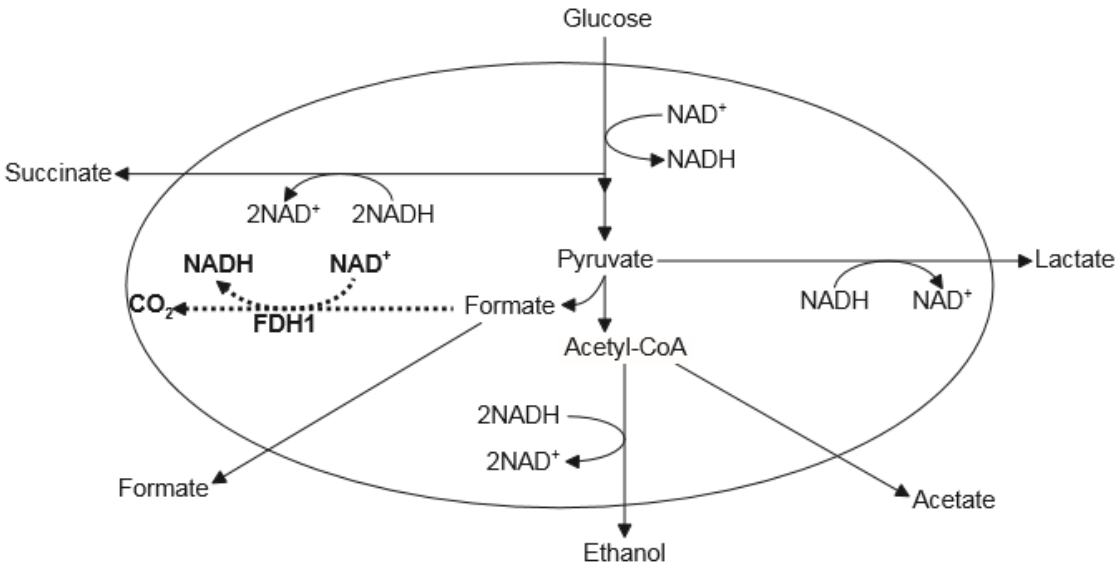
b. *P. fluorescens* necessary to degrade cyanide which is toxic to the environment *OWTTE*

c. often involves supplementing with nutrients/carbon source/aeration

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]

Succinate is industrially produced by continuous fermentation. It is used as a raw material in the production of flavour enhancers, drugs and industrial chemicals. One method of increasing the production of succinate is to genetically modify *E. coli* to express high levels of formate dehydrogenase (FDH1). This results in the production of higher concentrations of NADH. The engineered pathway is shown as a bold dotted line in the image.



[Source: Ka-Yiu San, E. D. Butcher Professor of Bioengineering, Professor of Chemical Engineering, Rice University.]

a. Using the diagram, suggest a reason for high concentrations of NADH favouring the production of succinate. [1]

b. Predict **one** metabolite other than succinate that will be produced in greater amounts if the amount of NADH available is increased. [1]

c. Outline the process of continuous culture fermentation. [2]

d. Outline **one** reason this process, to increase the production of succinate, represents pathway engineering. [1]

Markscheme

a. NADH is required as a reducing agent/electron donor/hydrogen donor/co-enzyme/limiting factor for the production of succinate

   High levels of FDH1 produce greater quantities of NADH which is required for conversion of glucose «via intermediates» to succinate

b. Lactate/ethanol

   CO_2 «favoured by high FDH1 levels»

c. Raw materials are supplied in continuous amounts

   Products/wastes are continuously extracted

   Conditions are monitored/regulated to keep variables at a steady state

d. Genetic processes/rate limiting chemicals/regulatory processes are being optimized

Examiners report

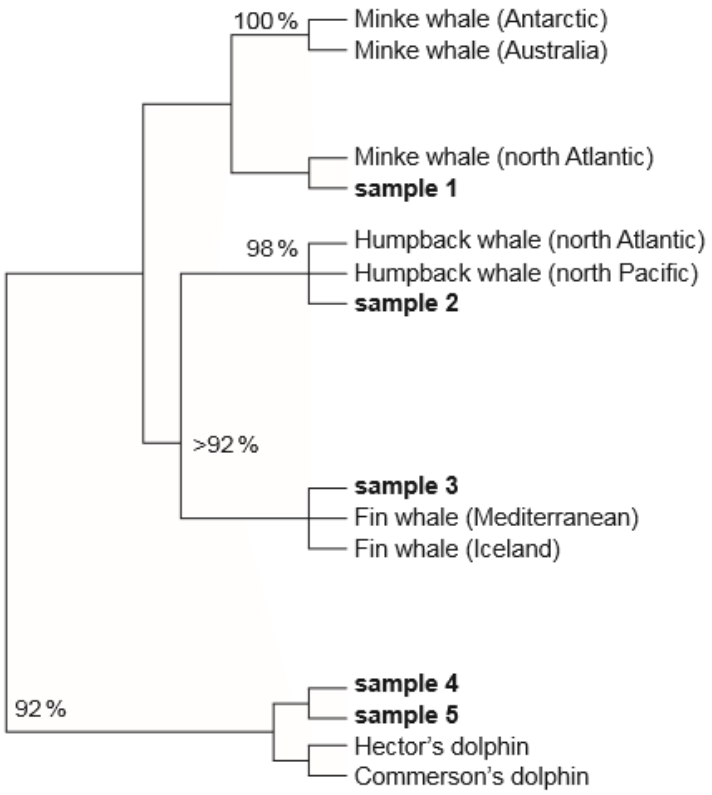
a. This was fairly well answered with many able to achieve the mark, usually for noting that NADH was a reducing agent or electron donor.

b. This was an easy question with almost all candidates able to predict one metabolite using the diagram.

c. The process of continuous culture fermentation seemed to be familiar to all candidates selecting this option with many scoring the 2 marks.

d. This was discriminating as many found it difficult to clearly show why the given process represented pathway engineering.

International agreement limits the hunting of whales. Only the meat of the Minke, Fin and Humpback whales from Southern Hemisphere populations is allowed to be sold on the domestic market in Japan. Scientists obtained five samples of food that were being sold as “whale meat” in a Japanese market place. They identified the species and probable geographic origin of the meat using genetic analysis. The results were used to construct the cladogram.



a. Using the data in the cladogram, state the reason for sale of Sample 1 meat being illegal in Japan. [1]

b. Using the data in the cladogram, state the reason for sale of Sample 4 meat being illegal in Japan. [1]

c. Outline how the polymerase chain reaction (PCR) might have been used in this study. [3]

d. Explain how sequence alignment software might have been used in this study. [2]

Markscheme

a. It is from a North Atlantic population

b. It is from dolphin/species that is not on the list

c. Collected DNA sample is small/too minute to study

DNA from tissue sample amplified «by PCR»
OR
Obtain increased yields/quantities of specific DNA sequences

Test primers «from legal species» are used

Example of genes used «to construct test primers» *eg: 18S ribosome, cytochrome C oxidase*

If no amplification occurs, then test is negative

d. Sequence a sample of DNA/protein from the tissue

Run a BLAST/sequences alignment software search for similar sequences «of DNA/protein»

A match can be used for species identification
OR
Percentage similarity of sequences used to build up cladogram

BLASTp is used if protein sequenced ***AND*** BLASTn if DNA sequenced (*Both needed*)

Examiners report

a. Almost all candidates seemed familiar with reading cladograms and were able to answer this question.

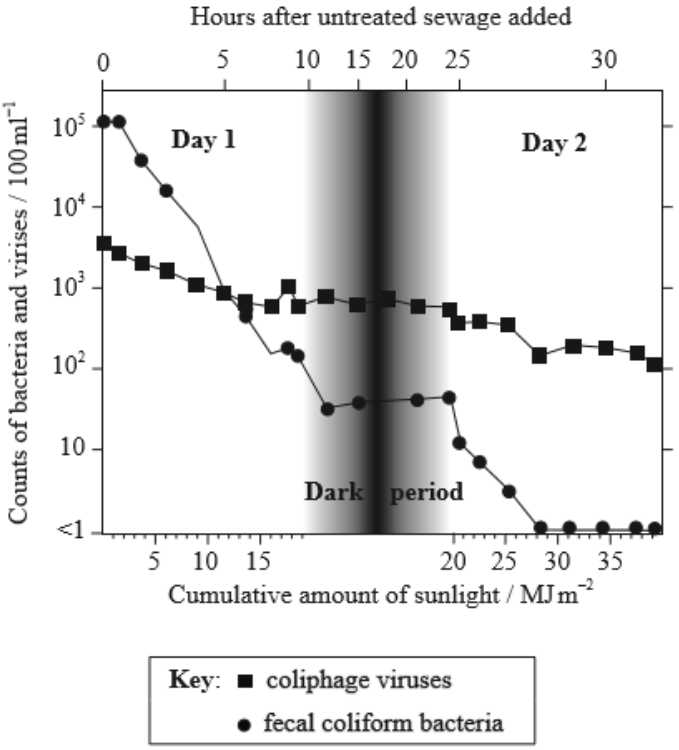
b. Again almost all candidates were able to answer this question.

c. This was a more discriminating question. Many were able to score one mark for indicating that DNA from the tissue sample was amplified using PCR but few were able to give additional details needed to receive 2 or 3 marks. They tended to confuse the answer to this question with that of the following one.

d. This question on sequence alignment software was often confused with that on PCR. Better candidates were able to explain the use of BLAST software for DNA and protein sequences.

Release of sewage in marine waters is a common practice but it can cause water contamination with pathogens. A series of experiments were conducted to compare inactivation rates of two different groups of microbes with different sunlight exposures. One group were fecal coliform bacteria and the other were coliphage viruses. Experiments were conducted outdoors using 300-litre mixtures of sewage-seawater in open-top tanks.

A two-day experiment was carried out with untreated sewage added to seawater. Both days were sunny with no clouds. The figure below shows the inactivation of the microbes in seawater as a function of the cumulative amount of sunlight and time. The survival curves of the two microbes are plotted against sunlight exposure (lower x axis) during daylight periods and against time during the overnight period (upper x axis). The y axis gives counts of bacteria and viruses per 100 ml.



[Source: adapted from L W Sinton, *et al*., (1999), *Applied and Environmental Microbiology*, **65** (8), pages 3605–3613]

a. Identify the time at which fecal coliform bacteria counts fell below 1 unit per 100 ml. [1]

b (i) Deduce, using the data in the graph, the effect of sunlight on fecal coliform bacteria. [2]

b (ii) Deduce, using the data in the graph, the effect of sunlight on coliphage viruses. [2]

c. For an accidental sewage spill, suggest, giving a reason, which of the two microbes may be most useful as a fecal indicator two days after the spill. [1]

Markscheme

a. 28 hours after untreated sewage added (*Allow answers in range 27–29 hours*)

b (i) sunlight reduces counts of fecal coliform bacteria significantly;

fecal coliform bacteria fall below 1 count per 100 ml on day 2;

no reduction during dark period;

significant drop on day 1 / bacteria count drops from 10^5 per 100 ml to less than 10^2 per 100 ml on day 1;

b (ii) sunlight causes small reduction of coliphage viruses;

coliphage virus counts never fall below 10^2 counts per 100 ml;

no reduction during dark period;

c. coliphage viruses because they are less affected by the sun / numbers do not decrease much in two days

Examiners report

a. Most were able to correctly read the graph to identify the time as 28 hours after sewage was added.

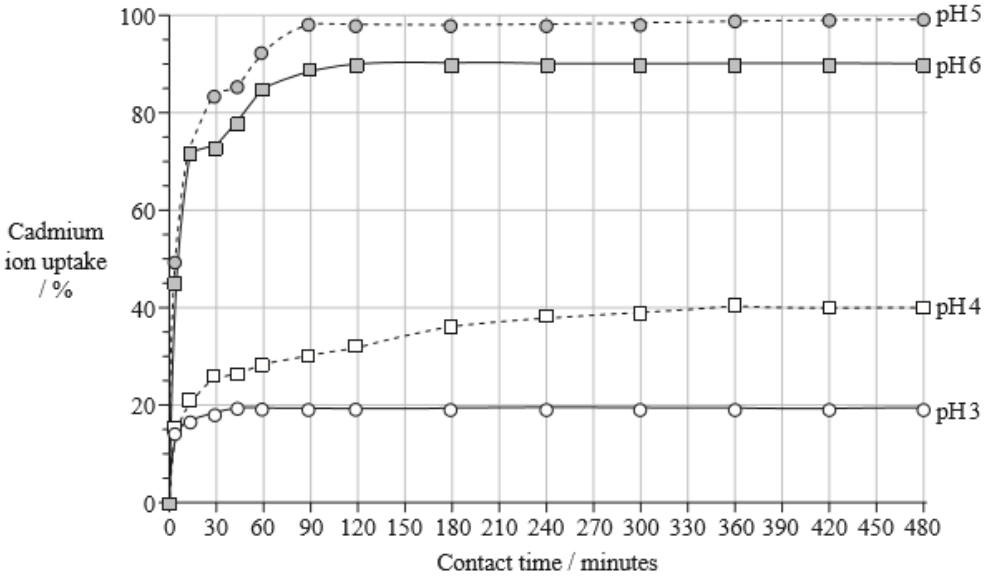
b (i) Many were able to correctly deduce the effect of sunshine on (i) coliform bacteri.

b (ii) Many were able to correctly deduce the effect of sunshine on (ii) coliphage viruses.

c. Only some were then able to correctly suggest that coliphage viruses were the most useful fecal indicator as they were less affected by the sun.

Removal of toxic heavy metals from industrial waste water is essential in order to control environmental pollution. Industrial waste water near Yanbu City, Saudi Arabia was found to contain 19 species of microorganisms that could tolerate heavy metals. The accumulation of cadmium ions in the most common of these microorganisms, *Aspergillus fumigatus*, was investigated.

The graph below shows the effect of pH on the ability of *A. fumigatus* to absorb cadmium ions from an aqueous solution.



[Source: adapted from S Al-Garni, *et al.*, (2009), *African Journal of Biotechnology*, **8** (17), pages 4163–4172]

a. Describe the cadmium ion uptake by *A. fumigatus* at pH 6. [2]

b. Calculate the difference in cadmium ion uptake between pH 4 and pH 5 at 60 minutes. [1]

.....%

c. Discuss the use of *A. fumigatus* for the removal of cadmium ions in polluted waters. [2]

d. The investigation found that both living and dead *A. fumigatus* cells were able to absorb cadmium ions. Suggest an advantage of using dead *A. fumigatus* cells. [1]

Markscheme

a. rapid initial uptake (to approximately 75 % uptake);

rate of uptake slows and plateaus (at approximately 85 % uptake after 90 minutes);

only 90 % of cadmium ions absorbed (however long the contact time) / reaches maximum at 120 min;

b. 64 (%) (*allow responses in the range of 62 to 66* %)

c. can remove almost 100 %/98 % cadmium ions at pH 5 therefore very efficient;

A. fumigatus able to remove cadmium ions at pH values tested;

removal of cadmium ions more efficient at higher pH/weak acid;

strongly acidic/very low pH may inhibit/reduce uptake of cadmium ions by *A. fumigatus*;

pollution causing acidification of water may make removal more difficult;

A. fumigatus common therefore may be convenient/easy to use / OWTTE;

cadmium is not actually removed as it may pass along food chains / be released when *A. fumigatus* dies / unknown impact on environment;

d. easier to store/collect/transport dead/dried material;

prevents overgrowth of *A. fumigatus*;

reduce BOD and allow other organisms to use more resources/live in water;

Examiners report

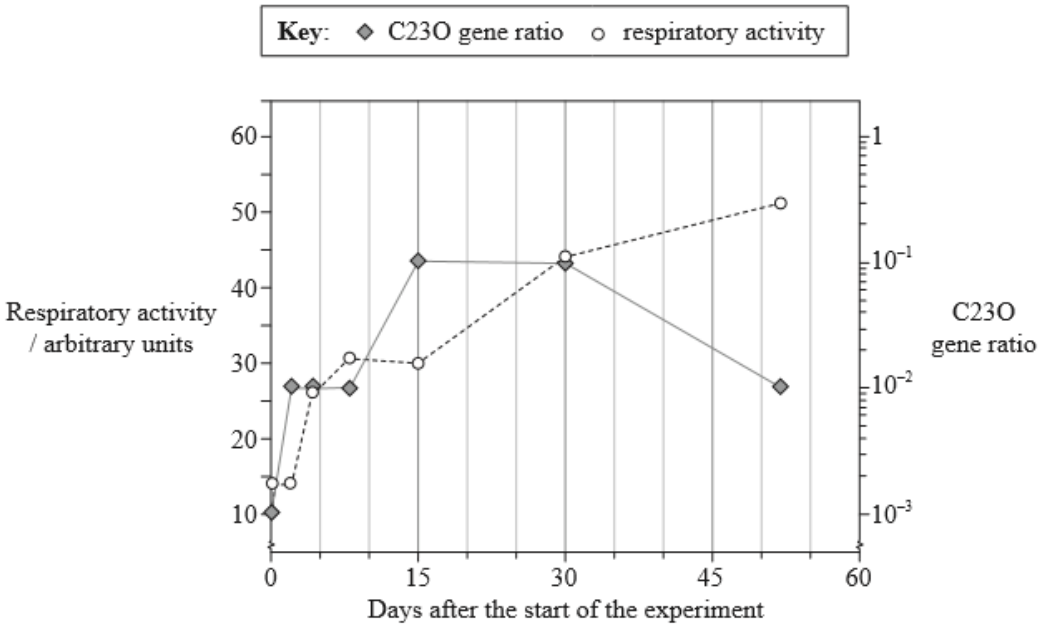
a. Well answered in general.

b. Well answered in general although many candidates failed to calculate the difference in cadmium ion uptake.

c. Little use of the data available. Only 2 of the mark scheme choices seen in answers.

d. Mostly well answered.

Soil contaminated with crude oil contains a very high amount of hydrocarbons, which may be an environmental hazard. In order to understand how bacteria could be helpful to remedy such a situation, scientists created laboratory samples of soil contaminated with crude oil and analysed the bacteria growing in it by measuring the respiratory activity and C23O gene ratio. The respiratory activity is an indication of the total amount of live bacteria in soil. The C23O gene ratio is an indication of the proportion of soil bacteria capable of hydrocarbon degradation compared to the total amount of bacteria.



[Source: adapted from M. Zucchi, L. Angiolini, S. Borin, L. Brusetti, N. Dietrich, C. Gigliotti, P. Barbieri, C. Sorlini and D. Daffonchio (2003) 'Response of bacterial community during bioremediation of an oil-polluted soil.' *Journal of Applied Microbiology*, 94 (2), pp. 248–257. Published by Wiley Blackwell. Reprinted with permission.]

a . State the respiratory activity when the C23O gene ratio first reached its highest level. [1]

b. Describe the respiratory activity as the soil treatment progresses. [2]

c. The data in the graph indicates that hydrocarbon degradation occurred during the first 30 days of the experiment. Explain the evidence for this conclusion. [2]

e. Scientists are interested in inserting the C23O genes into bacteria to clean up oil spills in the sea. State the term used to qualify the bacteria that are able to survive in a saline habitat. [1]

Markscheme

a . 30 (arbitrary units) (*accept answer in the range of 29 to 31 (arbitrary units)*)

b. rapid increase at the beginning/up to around day 8;

stable phase between days 7/8 to 15;

keeps increasing (not as much) after plateau / gradual increase after day 15;

c. increase in respiration means more bacteria are present;

increase in gene ratio means the numbers of bacteria with C23O gene are increasing;

more bacteria with C23O gene breaks down more hydrocarbons;

after day 30 proportion of bacteria with C23O gene decreases so no longer effective/required;

e. halophiles

Examiners report

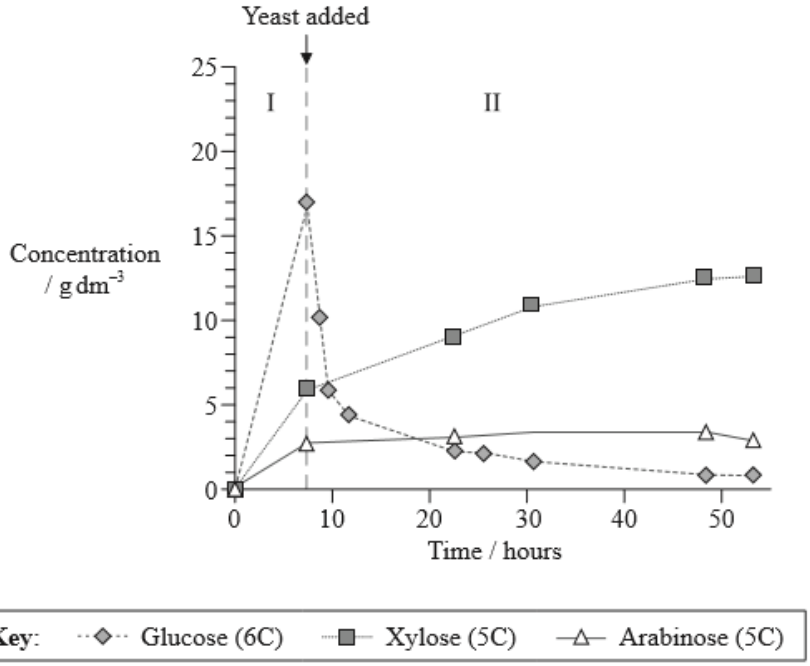
a . Many candidates gave a correct answer in (a), but a large number stated 44 a.u. instead of 30 a.u., indicating a confusion between variables and axes.

b. For (b), there were good answers, but many candidates did not relate to the number of days or were too vague in their description.

c. There were mixed answers for (c). The significance of the relationship between C23O gene ratio and hydrocarbon degradation was missed by many weaker candidates who also had difficulty in other questions, including (d) and (e).

e. The significance of the relationship between C23O gene ratio and hydrocarbon degradation was missed by many weaker candidates who also had difficulty in other questions, including (d) and (e).

Ethanol is an alternative energy source. Wheat straw can be converted into ethanol in two phases. Hydrolysis of complex polysaccharides in wheat straw (phase I) produces three monosaccharides (glucose, xylose and arabinose). Fermentation by yeast (*Saccharomyces cerevisiae*) then produces ethanol (phase II). The graph shows the changes in concentration of the three monosaccharides in both phases.



[Adapted from: Ronald H.W. Maas, Robert R. Bakker, Arjen R. Boersma, Iemke Bisschops, Jan R. Pels, Ed de Jong, Ruud A. Weusthuis and Hans Reith (2008) 'Pilot-scale conversion of lime-treated wheat straw into bioethanol: quality assessment of bioethanol and valorization of side streams by anaerobic digestion and combustion'. *Biotechnology for Biofuels*, 1, p. 14, Figure 1 (A).

a. State the maximum concentration of glucose reached during the two phases, giving the units. [1]

b. Distinguish between the changes in concentration of xylose and arabinose in phase II. [2]

c. Explain the changes in concentration of glucose and xylose during phase II. [3]

d. Suggest an advantage of the use of wheat straw as a source of energy. [1]

Markscheme

a. 17 g dm^{-3} (*accept answers in the range of 16.5 g dm^{-3} and 17.5 g dm^{-3}*)

b. concentration of xylose continues to increase while arabinose stays (approximately) constant;

concentration of xylose is always greater (than arabinose);

xylose concentration (appears to) stabilize at 50 hours while arabinose decreases slightly;

Do not accept answers stating numerical values alone.

c. glucose decreases/is used up due to fermentation/anaerobic respiration (by yeast);

xylose increases due to (continued) hydrolysis/production;

xylose not fermented by yeast / apparently yeast enzymes do not ferment 5-carbon sugars;

Do not accept xylose increasing due to breakdown of glucose

d. widely available / relatively inexpensive / waste product of food production / non-competitive with food applications / sustainable / renewable

Examiners report

a. Data showed the concentration of three monosaccharides issued from the hydrolysis of wheat straw and after the addition of yeast over time. The vast majority of candidates could read correctly the highest concentration of glucose and give the units for part (a).

b. Data showed the concentration of three monosaccharides issued from the hydrolysis of wheat straw and after the addition of yeast over time. The vast majority of candidates could read correctly the highest concentration of glucose and give the units for part (a) and distinguish between the concentrations of xylose and arabinose in part (b) although with few candidates obtaining two marks.

c. Part (c) was more challenging for them; a certain number stated incorrectly that the increase in xylose was the result of glucose fermentation, probably only looking at the data and failing to relate to their basic knowledge about fermentation.

d. They were not very successful either for part (d), a large number stating vaguely that wheat straw was a good source of energy without looking into the availability and cost of it.

Distinguish between the cell walls of Gram-positive and Gram-negative bacteria using the table below.

Bacteria	Peptidoglycan content
Gram-positive	
Gram-negative	

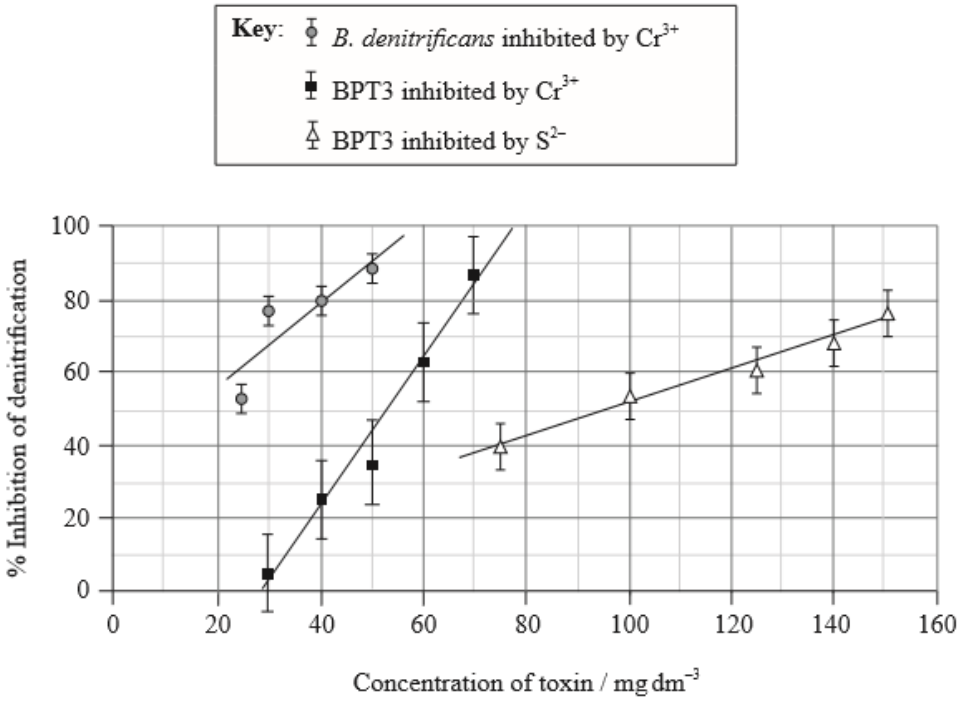
Markscheme

Bacteria	Peptidoglycan content
Gram-positive	thick wall / high content / 95% ;
Gram-negative	thin wall / low content / 5% ;

Examiners report

Of those answering this option most answered that Gram-positive bacteria have thicker walls that Gram-negative, thus suggesting that Gram-positive bacteria have more peptidoglycan.

Waste water from industrial processes contains a range of toxic substances that are harmful to the environment. These toxins include sulphide (S^{2-}) and metal ions such as chromium (Cr^{3+}). Microorganisms such as *Brachymonas denitrificans* that carry out denitrification of waste water, may be inhibited by these toxins. The effects of different concentrations of toxins on the rates of denitrification by *B. denitrificans* and a group of denitrifying bacteria named BPT3 are shown in the graph below.



[Source: With kind permission from Springer Science+Business Media: *World Journal of Biotechnology and Microbiology*, Identification of Efficient Denitrifying Bacteria from Tannery Wastewaters in Ethiopia and a Study of the Effects of Chromium III and Sulphide on Their Denitrification Rate, 20, 2004, 405–11, S. Leta]

a. Predict the Cr^{3+} concentration that would cause 50% inhibition in BPT3. [1]

b. Waste water from some industrial processes contains high levels of Cr^{3+}. State, with a reason, which of the bacteria investigated should be used to treat this water. [1]

c. Compare the effect of Cr^{3+} and S^{2-} on the inhibition of BPT3. [2]

d. Raw sewage contains high level of nitrates. Explain the importance of denitrification of raw sewage by bacteria such as *B. denitrificans* and BPT3 before it is released into rivers. [3]

Markscheme

a. 53 mg dm^{-3}

Accept answers in the range 51 to 54 (units required).

b. BPT3 because it is less inhibited by Cr^{3+}

c. a. Cr^{3+} more toxic than S^{2-} (between concentrations of about 67/70 and 80 $mgdm^{-3}$);

b. Cr^{3+} toxicity increases more rapidly with increasing concentration than S^{2-};

c. Cr^{3+} requires lower concentration to cause same percentage inhibition (between 40 and 70%) as S^{2-} / vice versa;

d. below (about) 30 ($mgdm^{-3}$) Cr^{3+} not toxic whereas S^{2-} effect not known/may be toxic / Cr^{3+} cannot be compared with S^{2-} below concentration of 70/67 ($mgdm^{-3}$);

d. a. denitrifying bacteria convert nitrate to nitrogen/N_2;

b. nitrate causes eutrophication/algal blooms in rivers / removing nitrate reduces the risk of eutrophication/prevents algal blooms;

c. high levels of nitrate/algal blooms lead to anoxic conditions / increase BOD / reduces oxygen levels;

d. nitrate is toxic for humans (in high concentrations);

e. removing nitrate reduces the risk of nitrate entering drinking water;

Examiners report

a. This was the least popular of the HL options but it was encouraging to note some schools studying it with some good standards seen.

Almost all candidates read the graph correctly for the 1 mark.

b. This was the least popular of the HL options but it was encouraging to note some schools studying it with some good standards seen.

Many candidates correctly identified BPT3 as the bacteria that should be used to treat the water but some could not give a reason so missed the mark.

c. This was the least popular of the HL options but it was encouraging to note some schools studying it with some good standards seen.

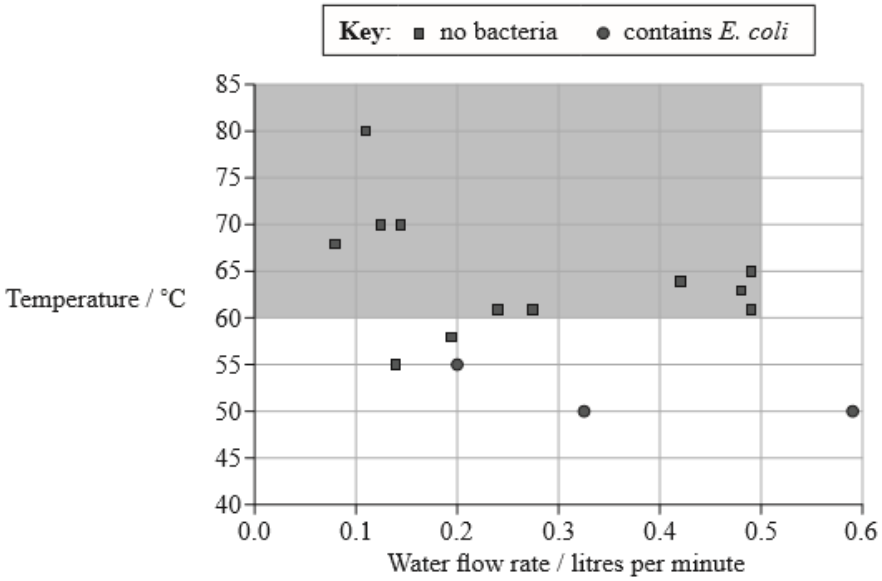
The better candidates were able to correctly compare the effects of sulphide and chromium ions on the inhibition of BPT3. Weaker candidates gave very confused and unclear answers.

d. This was the least popular of the HL options but it was encouraging to note some schools studying it with some good standards seen.

Many were able to get two marks for why denitrification of raw sewage before release into rivers was important with many mentioning eutrophication, algal blooms and reduced oxygen levels as problems of high nitrate levels.

In 2003, the Integrated Approach to Community Development (IACD) organization introduced the chulli water purifier to homes in Bangladesh that had not previously had access to safe drinking water. It was designed to be made cheaply from local materials. The purifier uses sand filtration to remove organic particles and heat pasteurization to eliminate microbes from water.

Water samples from 15 different locations containing high levels of the bacterium *E. coli* were passed through the purifier at different flow rates and temperatures to test its effect on contaminated water. The shaded area of the graph below represents the recommended temperature and flow rate for using the purifier.



[Source: S. K. Gupta et al. (2008) *American Journal of Tropical Medicine and Hygiene*, 78, pages 979–984]

a. State the highest temperature at which bacteria were found in water that had passed through the chulli purifier. [1]

b. Calculate the maximum volume of safe drinking water that could be produced by the chulli purifier in one hour. [1]

c. Discuss whether 80°C is the best temperature to operate the chulli purifier. [2]

d. The results suggest that there may be a relationship between the water flow rate and the minimum temperature needed to eliminate microbes. [1]

State this relationship.

e. Evaluate pasteurization as a method of controlling microbial growth. [2]

Markscheme

a. 55°C (*units required*)

b. 30 litres (*accept answers in the range of 28.8 to 30.0 litres*)

Working not required.

c. a. good because it kills/is free of bacteria;

b. no bacteria between 80° and 60° even at higher flow rates;

c. not good as it would use too much energy/be expensive to heat water;

d. the slower the flow rate, the lower the temperature.

e. *implications:*

a. kills bacteria/most pathogens by heating food/liquids to specific/high/ 60° – 72° temperature;

b. (usually pasteurization temperature) does not alter the taste/quality/chemical structure;

limitations:
c. may not kill heat-resistant/all bacteria;
d. requires immediate cooling to prevent (further) microbial growth;

Examiners report

a. Most had 55° and 30 (or 29.4) litres for (a) and (b).

b. Most had 55° and 30 (or 29.4) litres for (a) and (b).

c. Many confused between the independent and dependent variables and/or were distracted by the only data point at 80°; they stated a reversed relationship in (d) (e.g. "the higher the flow rate, the lower the temperature"); their answer to the previous question (c) was influenced by this, but some managed to get the two marks there anyways.

d. Many confused between the independent and dependent variables and/or were distracted by the only data point at 80°; they stated a reversed relationship in (d) (e.g. "the higher the flow rate, the lower the temperature").

e. For (e), some read the question as relating to the graph and the chulli purifier, others read it as a theoretical question (F.6.4); among the latter, some confused pasteurization and sterilization; the markscheme allowed for both perspectives and most candidates gained some marks.

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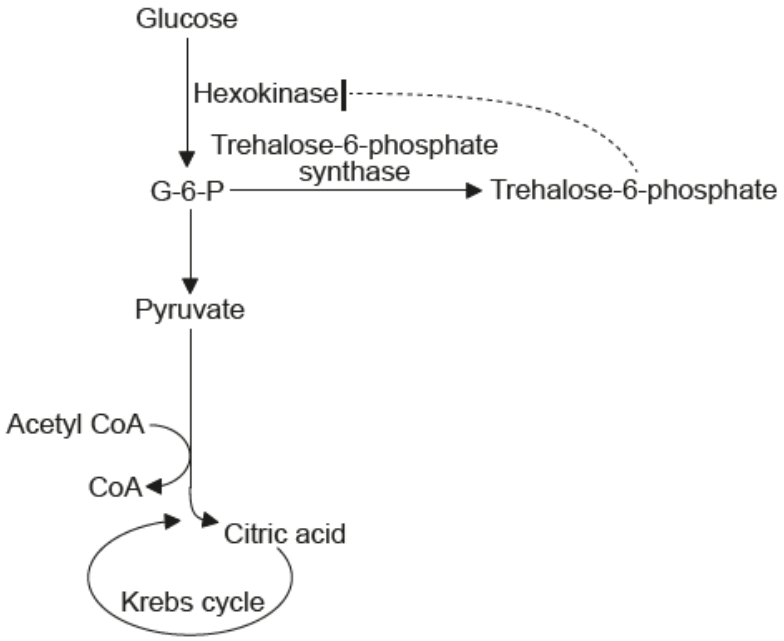
c. Distinguish between batch fermentation and continuous fermentation. [2]

d. *Aspergillus niger* is used to produce citric acid by continuous fermentation. Glucose is converted to pyruvate by glycolysis. Trehalose-6-phosphate normally inhibits hexokinase, an important enzyme in the glycolysis pathway. [2]



Suggest how pathway engineering could be used to address this factor which reduces yields of citric acid.

Markscheme

c.

factor	*batch*	*continuous*
a. introduction of nutrients	at the beginning	all the time ✓
b. collection of products	all products at the end/*OWTTE*	small quantities throughout/*OWTTE* ✓
c. cleaning and sterilization	between batches	after a long time/*OWTTE* ✓
d. contamination	ruins only one batch	ruins the whole production ✓

d. a. «genetically modify to» incorporate gene for low/blockage of TPS activity into *A. niger*

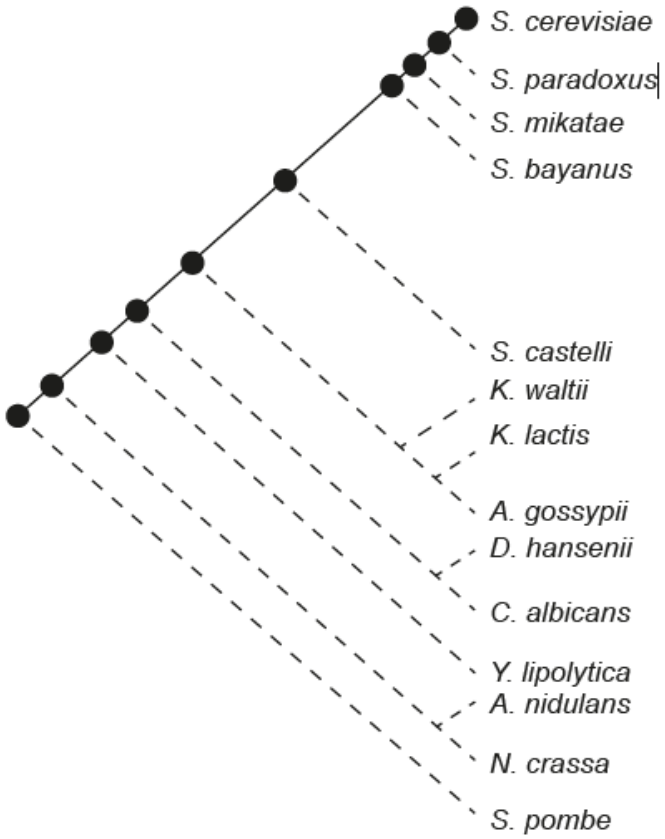
b. «genetically modify to» incorporate gene that breaks down trehalose-6-phosphate

c. selectively breed *A. niger* cultures for low/no TPS activity

Examiners report

c. [N/A]
d. [N/A]

The cladogram is based on a comparison of open reading frames in DNA taken from fungi. It is an example of how open reading frames can be used in phylogenetic studies.



[Source: Reprinted by permission from Macmillan Publishers Ltd: *Nature*, Vol. 487, Anne-Ruxandra Carvunis *et al*. Proto-genes and de novo gene birth, pp. 370–374, copyright (2012), http://www.nature.com/]

a. Outline how open reading frames are identified in DNA. [2]

b. Explain what the branching off points represent in the cladogram of these fungi. [1]

c. There are several methods of introducing DNA into a cell in the laboratory. Outline the introduction of recombinant DNA in plant cell protoplasts. [2]

Markscheme

a. a. identify a start codon and stop codon

b. identify base sequences for a gene/that could code for a polypeptide

c. possible correlation with existing open reading frames in databases

b. a. represent common ancestors shared by the organisms that emanate from the point

b. indicates time since divergence

c. indicates number of differences in DNA

c. a. plant cells made into protoplasts by removing their cell wall / use cellulase to produce protoplasts

b. physical methods such as electroporation /microinjection/biolistics

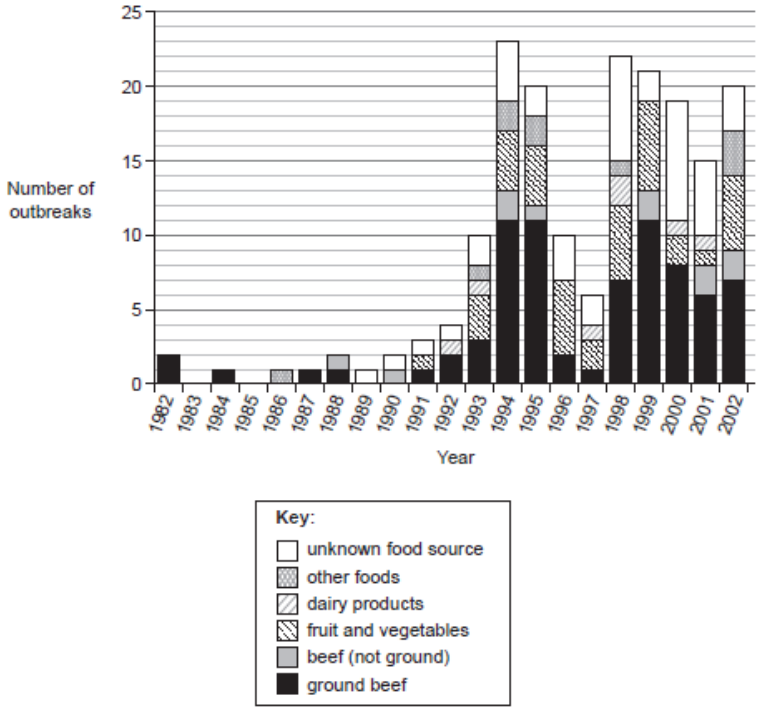
c. chemical methods such as liposomes/calcium chloride/polyethylene glycol «PEG»

d. vectors such as *Agrobacterium*/tobacco mosaic virus

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]

The bacterium *Escherichia coli* is responsible for over 70 000 cases of illness each year in the US. More than half of these cases are due to transmission of the bacteria in food, particularly from ground beef in undercooked burgers. Epidemiologists collected evidence from 183 outbreaks of food poisoning between the years 1982 and 2002 and identified the food responsible for the outbreak. They divided the foods into dairy products, fruit and vegetables, beef, ground beef (beef which has been minced) and other foods. In some cases they were unable to identify the food that had caused the outbreak. The results are displayed in the bar chart.



[Source: adapted from JM Rangel, *et al.*, (2005), *Centres for disease control and prevention*, **11** (4), and www.nc.cdc.gov]

a. State the number of years during the study when contaminated dairy products caused food poisoning. [1]

b(i). Compare the outbreaks of food poisoning in 1989 and 1994. [2]

b(ii). Suggest **two** reasons for these changes. [2]

c. Explain how pasteurization may have prevented food poisoning by dairy products. [2]

Markscheme

a. 6 (years) *(units not required)*

b(i). a. total number of outbreaks of food poisoning (much) greater in 1994/ changed from 1 to 23;

b. more unknown outbreaks in 1994 than in 1989;

c. food poisoning in 1994 due to ground beef/beef/fruit and vegetables/other sources which did not occur in 1989;

d. greatest increase in food poisoning due to ground beef;

e. no food poisoning due to dairy products in either year / increase in food poisoning from other sources from 1989 to 1994;

b(ii) a. increase in range of foods available;

b. increase in fast food outlets (short time of cooking) / change in preparation methods / *OWTTE*;

c. increase in technological advances to analyse outbreaks / more awareness (of occurrence of contaminations) / better data collection / *OWTTE*;

d. increase in bacterial resistance;

c. a. milk is quickly heated;

b. to high temperatures then rapidly cooled down;

c. this kills harmful bacteria;

Examiners report

a. Candidates handled the data without problems although some of the comparisons were weak. Some gave very vague reasons for the changes such as more food, more bacteria, etc., without any explanation of why these changes could have occurred in 5 years. The most common answers were related to food preparation and types of food available. Most had some knowledge about pasteurization, but few included sufficient detail as to timing.

b(i). Candidates handled the data without problems although some of the comparisons were weak. Some gave very vague reasons for the changes such as more food, more bacteria, etc., without any explanation of why these changes could have occurred in 5 years. The most common answers were related to food preparation and types of food available. Most had some knowledge about pasteurization, but few included sufficient detail as to timing.

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Lipid A is a phospholipid that makes up the external layer of the outer membranes of most Gram-negative bacteria. LpxC is an enzyme involved in the biosynthesis of lipid A. In this experiment, a lawn of the Gram-negative bacterium *Escherichia coli* was grown on a nutrient agar plate. Shortly after inoculation, before the lawn is formed, discs containing different test compounds were placed on top. The Petri dish shows the results after 24 hours incubation.

Key:
disc 1: LpxC inhibitor
disc 2: mutated LpxC inhibitor
disc 3: ampicillin
disc 4: control

[Source: © International Baccalaureate Organization 2016]

a. Outline the effect of disc 3 on the bacterial lawn. [2]

b. Outline the effect of mutating the LpxC inhibitor. [1]

c. Predict the results obtained with disc 1 in a Gram-positive bacterial lawn. [1]

Markscheme

a. a. the antibiotic/ampicillin diffuses out

b. killing bacteria/inhibiting growth of bacteria

c. zone of inhibition/clearing formed

b. lipid A production/synthesis is not inhibited so bacteria can grow

OR

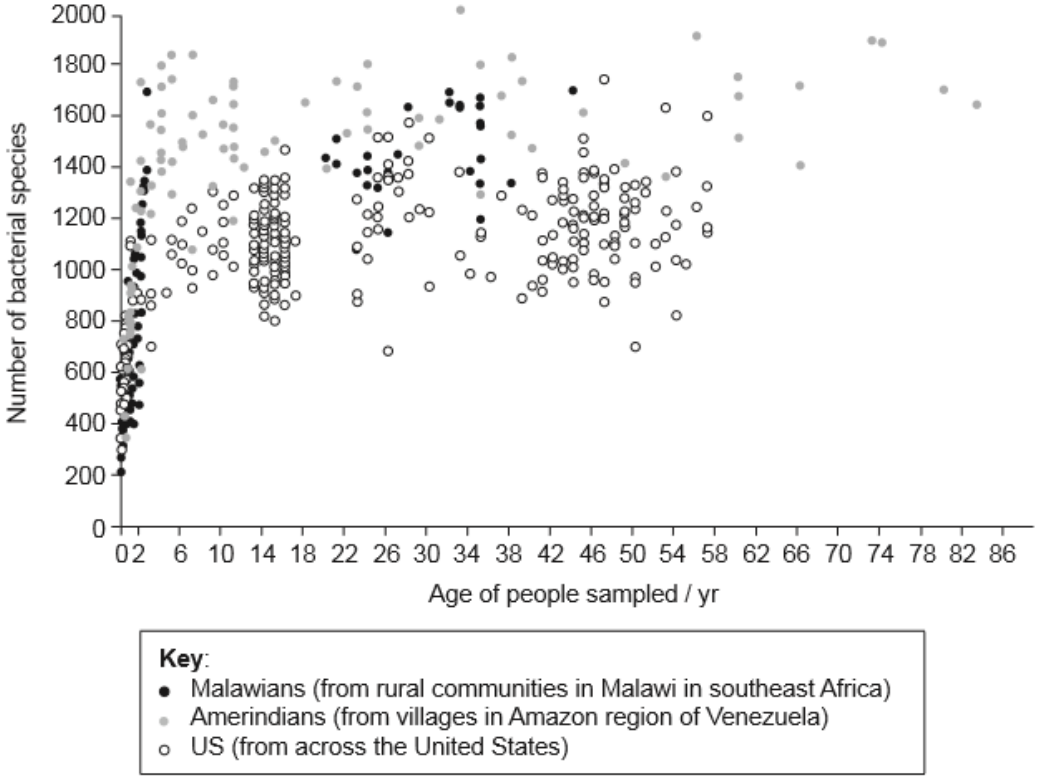
bacteria grow/are not affected since inhibitor function is lost

c. no inhibition of growth, since Gram-positive do not have lipid A in membrane/OWTTE

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]

Over a thousand bacterial species occupy the human gut. The gut bacteria show much larger genetic diversity than the host cells. Gut bacteria are vital to proper food digestion and vitamin synthesis. Fecal samples were collected from people in various locations so the genomes of their gut bacteria could be analysed. Bacteria with the same unique DNA sequences were identified as species. The graph shows the number of bacterial species in the digestive tract of people in three different parts of the world.



a. Identify the age and ethnic group of the individual with the highest diversity of gut bacterial species. [1]

b (i) Outline the trends in the number of bacterial species in the digestive tracts of Amerindians. [1]

b (ii) Distinguish between the trends seen in the three populations. [2]

c. Suggest **two** reasons for how the different environments of the three human populations affect the number of bacterial species in their digestive tracts after the age of four. [2]

d. A century ago, it was discovered that each person belonged to one of four blood types. Now some researchers are reporting that human gut ecosystems fall into three distinct types, each involving a great number of similar bacterial species. [1]

Suggest one medical application based on the knowledge that humans could be typed according to their gut ecosystem.

Markscheme

a. 34 (years old) and Amerindian

Allow answers in the range 33–35.

b (i) a. rapid increase in diversity early in life/before age four;

b. (from age four into adulthood) bacterial diversity tends to level off/stay within same (broad) range of diversity/great variation;

b (ii) a. Amerindians reach highest plateau / Malawians and US reach a lower plateau than the Amerindians;

b. US reach lowest plateau / US reach a lower plateau than the Malawians and Amerindians;

c. a. US population use disinfectants/antiseptics / pasteurise/sterilise/irradiate food more than populations in Malawi or Amazon;

b. different diets support different populations of bacteria;

c. different soil/water/local animal bacteria;

d. different use of antibiotics;

e. contact with farm/wild animals by rural populations;

d. a. diets could be tailored to a particular gut ecosystem to maximize digestion / personal health/weight control;

b. antibiotics could be prescribed with minimal effect on gut bacteria/reduce diarrhoea;

c. fecal transplants; (*accept other reasonable answers*)

Examiners report

a. The majority of candidates were able to identify the point required from the graph.

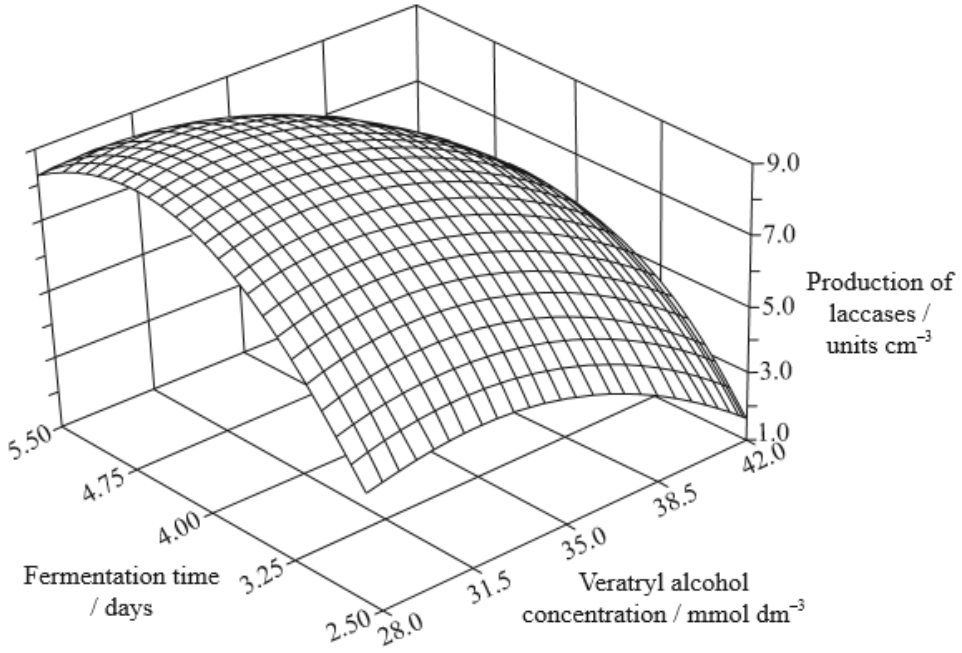
b (i) Candidates struggled to outline the trend for Amerindians in (i), perhaps due to the variation in the data. There was seldom reference to a plateau.

b (ii) Likewise, only the better candidates could clearly distinguish the trends in the 3 populations in (ii).

c. Usually only 1 of the 2 marks available was awarded, often for different food sources that would provide different bacteria. Many candidates did not mention environmental differences but individual habits.

d. A good number of candidates were able to make reasonable suggestions of how the knowledge of human gut flora could be applied despite this being a novel idea to most.

Fungi of the genus *Botryosphaeria* have been found to produce certain oxidizing enzymes, laccases, that are effective in treating contaminated water and soils. Studies were undertaken to test the effects of veratryl alcohol concentrations and fermentation time in order to optimize the industrial production of laccases. Statistical analysis of the data was used to develop the graph below.



a (i) Identify the amount of laccases produced when the veratryl alcohol concentration is at its highest level and the fermentation time is at its shortest. [1]

a (ii) Identify the amount of laccases produced when the veratryl alcohol concentration is at its lowest level and the fermentation time is at its longest. [1]

b. Analyse the overall effects of the veratryl alcohol concentration and fermentation time on the production of laccases. [3]

c. Suggest **two** other conditions that might affect the production of laccases. [2]

Markscheme

a (i) 1.6 (units) cm^{-3} (*accept answers in range of 1.5 units cm^{-3} and 1.7 units cm^{-3}*)

a (ii) 8.3 (units) cm^{-3} (*accept answers in range of 8.2 units cm^{-3} and 8.4 units cm^{-3}*)

b. as fermentation time increases laccase production rises then falls; (*accept converse*)

as veratryl alcohol concentration increases laccase production rises then falls; (*accept converse*)

optimum fermentation time is 4.75/5.0/5.25 days; (*accept converse*)

most laccase overall with low veratryl alcohol concentration and long fermentation; (*accept converse*)

optimum veratryl alcohol concentration is 33/34/35 mmol dm^{-3};

fermentation time has greater effect than veratryl alcohol concentration;

c. temperature;

pH;

oxygen concentration;

carbon dioxide concentration;

build up of waste/toxic products of metabolism;

amount of contamination;

light;

supply of raw materials/nutrients/sugar;

concentration of fungi;

presence of other fungi/bacteria;

Examiners report

a (i) This proved to be very challenging, but it was balanced by relatively straightforward questions later in this option. The three-dimensional graph was not studied carefully enough by some candidates. Answers to (a)(i) and (ii) were sometimes therefore incorrect.

a (ii) This proved to be very challenging, but it was balanced by relatively straightforward questions later in this option. The three-dimensional graph was not studied carefully enough by some candidates. Answers to (a)(i) and (ii) were sometimes therefore incorrect.

b. Part (b) was also answered poorly by many candidates. As they increase, both veratryl alcohol concentration and fermentation time cause an increase in the production of laccases, up to an optimum, beyond which there is a decrease. Few candidates described this clearly.

c. Part (c) was more successfully answered and in most cases two acceptable other conditions were given.

The genetic code is the information encoded within the mRNA sequence that is translated into proteins by living cells. The codon table is shown.

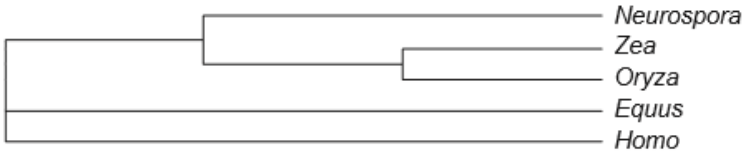
First position	Second position: U		Second position: C		Second position: A		Second position: G		Third position
U	UUU	Phe (F)	UCU	Ser (S)	UAU	Tyr (Y)	UGU	Cys (C)	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu (L)	UCA		UAA	**STOP**	UGA	**STOP**	A
	UUG		UCG		UAG		UGG	Trp (W)	G
C	CUU	Leu (L)	CCU	Pro (P)	CAU	His (H)	CGU	Arg (R)	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln (Q)	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile (I)	ACU	Thr (T)	AAU	Asn (N)	AGU	Ser (S)	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys (K)	AGA	Arg (R)	A
	AUG	**Met (M)**	ACG		AAG		AGG		G
G	GUU	Val (V)	GCU	Ala (A)	GAU	Asp (D)	GGU	Gly (G)	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu (E)	GGA		A
	GUG		GCG		GAG		GGG		G

The first part of the cytochrome c protein sequence alignment of mold fungus (*Neurospora*), horse (*Equus*), human (*Homo*), corn (*Zea*) and rice (*Oryza*) is shown using the amino acids as a one letter code.

```
Neurospora ----MGFSAGDSKKGANLFKTRCAQCHTLEEGGGNKIGPALHGLFGRKTGSVDGYAYTDA
Equus      --------MGDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFSYTDA
Homo       --------MGDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGYSYTAA
Zea        MASFSEAPPGNPKAGEKIFKTKCAQCHTVDKGAGHKQGPNLNGLFGRQSGTTAGYSYSAG
Oryza      MASFSEAPPGNPKAGEKIFKTKCAQCHTVDKGAGHKQGPNLNGLFGRQSGTTPGYSYSTA
```

[Source: © International Baccalaureate Organization 2016]

The alignment was used to obtain a cladogram of these organisms.



a. State the bioinformatics tool used to obtain the alignment. [1]

b. State the meaning of the dash (–) in the alignment. [1]

c. (i) Identify the longest amino acid sequence where there are no differences amongst the five genera. [2]

(ii) Suggest, with a reason, whether the DNA coding for the amino acid sequence identified in (c)(i) must be identical for the five genera.

d. Describe briefly how the cladogram was obtained. [2]

e. Determine which **two** genera are most closely related according to their cytochrome c protein sequence. [1]

Markscheme

a. BLASTp

Do not allow "BLAST" alone but accept BLASTx.

b. gap/no amino acid present «for cytochrome c in that organism in that position»

OR

protein is shorter

c. (i) GLFGR

Can be shown directly on the alignment.

(ii) no, because more than one codon can code for an amino acid/degeneration of the genetic code

d. ***ALTERNATIVE 1***

a. alignment used to quantify differences and similarities

b. algorithms for cladograms
OR
named algorithms

Named algorithms: least squares, neighbour-joining, parsimony, maximum likelihood or Bayesian inference.
Allow other verifiable algorithms.

c. selection of best model

ALTERNATIVE 2
d. comparing amino acid sequences between organisms

e. more similar sequences correspond to closer evolutionary links/OWTTE
OR
number of differences indicate number of mutations accumulated «over time»
OR
«if mutation rate is assumed to be constant», more mutations imply further evolutionary distance

f. length of lines/position of nodes established by the number of differences/mutations between organisms

Marks should be awarded for statements from only one alternative, not both.

e. Zea «corn» and Oryza «rice»

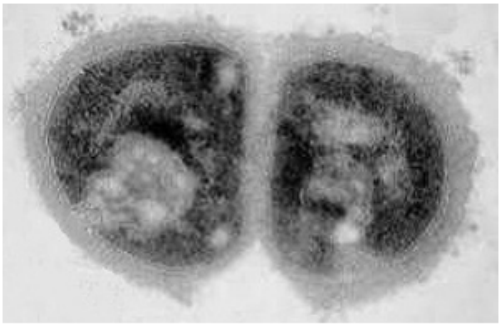
Both required.

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]
d. [N/A]
e. [N/A]

---

a (i)The electron micrograph below shows a thin section of the Gram-positive bacterium *Micrococcus lysodeikticus*. [2]



Compare the cell wall structure of this bacterium with one classified as Gram-negative.

b. Outline the role of saprotrophic bacteria in the treatment of sewage using reed bed systems. [2]

Markscheme

a (i) *similarities*:

both have walls made of murein net;

both have polysaccharide chains cross-linked by short peptide chains/ peptidoglycan;

differences:
Gram-positive have thicker/more rigid walls while Gram-negative walls are thinner;
Gram-positive walls contain other components/polysaccharides and proteins while Gram negative do not;
Gram-negative walls coated on outside with lipid-rich layer while Gram-positive are not;
*To award **[2 max]** responses need to address a similarity and a difference.*

b. bacteria cause decay by feeding on dead/decaying organic matter (in sewage);

nitrifying bacteria convert ammonia to nitrates /nitrites;

plant/reed roots absorb nitrates;

denitrifying bacteria convert nitrates to nitrogen;

Examiners report

a (i) Whilst knowledge of the differences in wall thickness was evident, further comparative detail was not.

b. Poorly answered, showing little appreciation of the different roles played in sewage treatment by bacteria. Many candidates answering in Spanish seemed to ignore this topic, although the question is nearly an exact statement in the guide.

---

a. State **one** example of a bacterium that forms aggregates. [1]

c. Outline the process of nitrogen fixation by a **named** free-living bacterium. [2]

d. The image shows part of a sewage treatment plant. [3]

[Source: http://purewatergazette.net]

Outline the role of bacteria in trickling filter bed treatment of sewage.

Markscheme

a. *Pseudomonas aeruginosa* / *Vibrio fischeri*

Accept other correct answers.

c. a. (atmospheric) nitrogen is converted to ammonia;

b. by *Azotobacter*;

Do not accept Rhizobium.

d. a. (saprotrophic) bacteria/biofilm fix on the surface of the rocks/material in the trickling filter;

b. bacteria decompose the sewage/organic matter as it runs over the filter bed;

c. bacteria break down organic matter aerobically;

d. the rocks increase the surface area for the decomposition of organic matter;

e. filter bed can treat high amounts of sewage quickly;

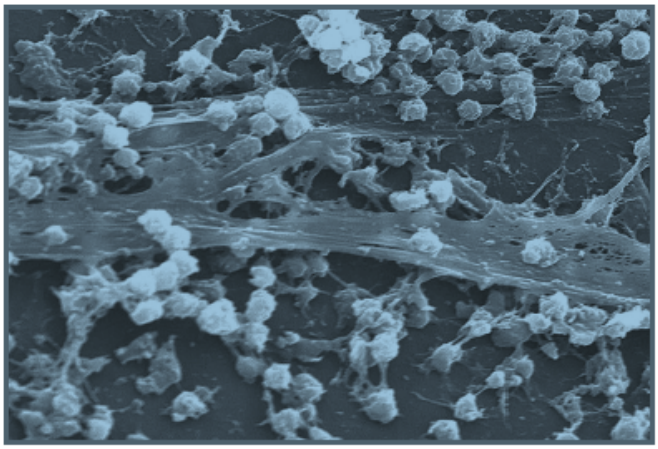
Examiners report

a. Many mentioned *Vibrio fischeri* or *Pseudomonas aeruginosa* as an example of bacteria forming aggregates, but some gave far too vague categories, such as methanogenic bacteria.

c. Most could state halophiles and *Azotobacter*, although there was some confusion with *Rhizobium*.

d. Many knew only that bacteria fix on the rocks, but did not give enough detail for more, showing poor understanding of the treatment of sewage.

The micrograph below shows an example of a biofilm including *Staphylococcus aureus*.



[Source: https://en.wikipedia.org/wiki/Biofilm#/media/File:Staphylococcus_aureus_biofilm_01.jpg]

a. Biofilms can be formed in many different environments. [1]

State **one** example of an environment where biofilms can be formed.

a.ii. Discuss the emergent properties of biofilms. [3]

Markscheme

a. cooling- or heating-water systems / rocks at the bottom of a river / teeth «of most animals» / prepared on sewage treatment plants / boat hulls / medical catheters

Accept other verified examples

a.ii. a. have «new» properties that are not present in the individual microorganisms

b. organisms form a matrix «EPS» / biofilms have a complex architecture

c. increased resistance to antibiotics/treatments
OR
bioluminescence

d. biofilms can be formed by different types of micro organisms that interact/cooperate

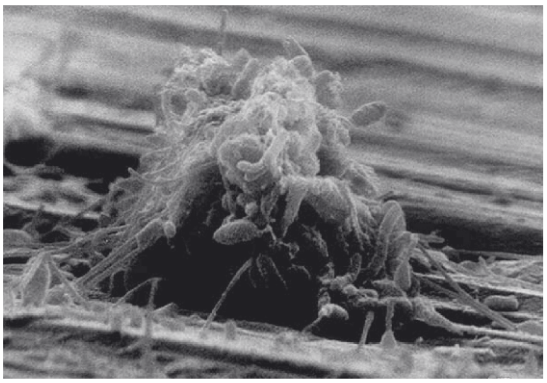
e. quorum sensing
OR
high population/cell density determines expression of genes

Examiners report

a. [N/A]

a.ii. [N/A]

The scanning electron micrograph shows a biofilm on a metal surface from an industrial water system.



[Source: Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms, Rodney M. Donlan, J. William Costerton, Clinical Microbiology Reviews, 2002, 15 (2), pp. 167–193. Reproduced with permission from American Society for Microbiology]

a. Outline the emergent properties of biofilms. [3]

b. State a positive application of biofilms. [1]

c. Suggest **two** problems that could be caused by the presence of biofilms in water systems. [2]

Markscheme

a. a. properties not present in individuals but present/develop only in the aggregate

OWTTE

b. develop structure/architecture/scaffolding

OR

develop an «extracellular» matrix/EPS

c. signaling/communication

d. migration/movement

e. resistant to antimicrobial agents

f. cooperates through quorum sensing

[Max 3 Marks]

b. a. sewage/waste water treatment/trickle filter beds

b. «bio»remediation of contaminated soil/water

c. metal extraction from ore deposits/microbial leaching

Accept other valid positive application

eg: decay/breakdown contaminants, such as petroleum

[Max 1 Mark]

c. a. contamination/pollution «of water system»

OR

«microbial growth of biofilm» causes disease through water systems

b. difficult to eliminate «from water systems»

c. fouling/clogging of water pipes

d. corrosion of water pipes

OWTTE

[Max 2 Marks]

Examiners report

a. [N/A]
b. [N/A]
c. [N/A]