HL Paper 3

Discuss biopharming.

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

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]

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]

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]

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]

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]

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

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]

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

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

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

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]

Describe the consequences of releasing raw sewage into rivers.

Explain the use of bacteria in the bioremediation of water.

Explain how methane can be generated from biomass.

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

Outline how bacteria can be classified by Gram staining.

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

Explain how bacteria are used in bioremediation of soil.

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

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]

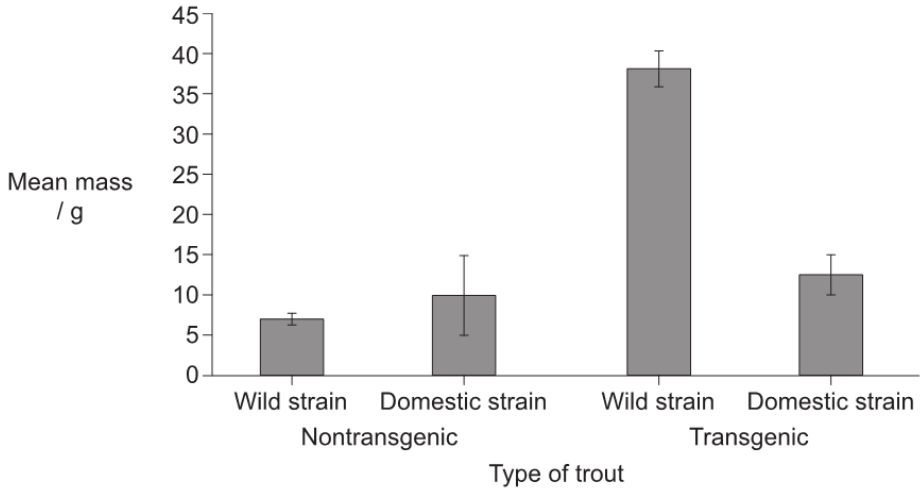
b. State how similar nucleotide sequences can be identified. [1]

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]

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

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]

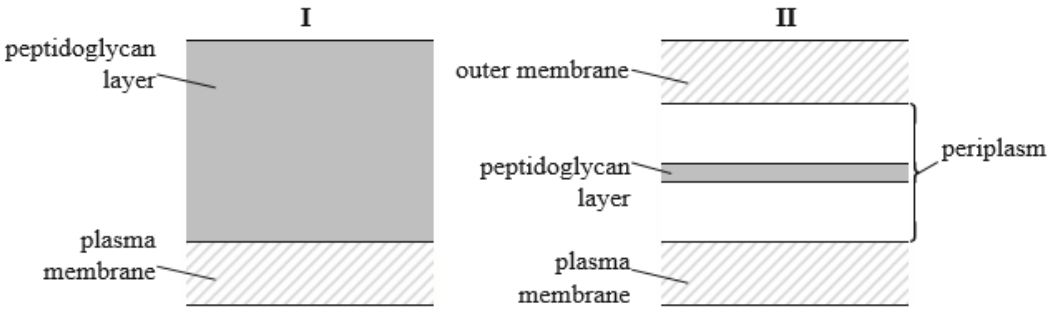
b. Suggest a reason for the growth differences between the nontransgenic trout and transgenic trout. [1]

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]

---

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]

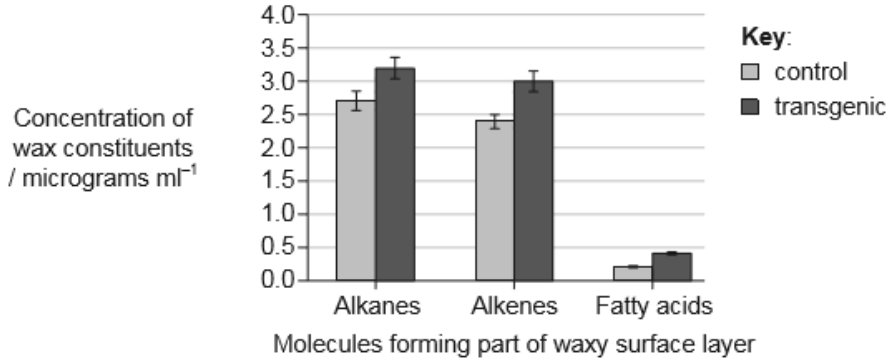


c. Microorganisms play many roles in ecosystems. List **two** of these roles. [2]

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

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

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).]

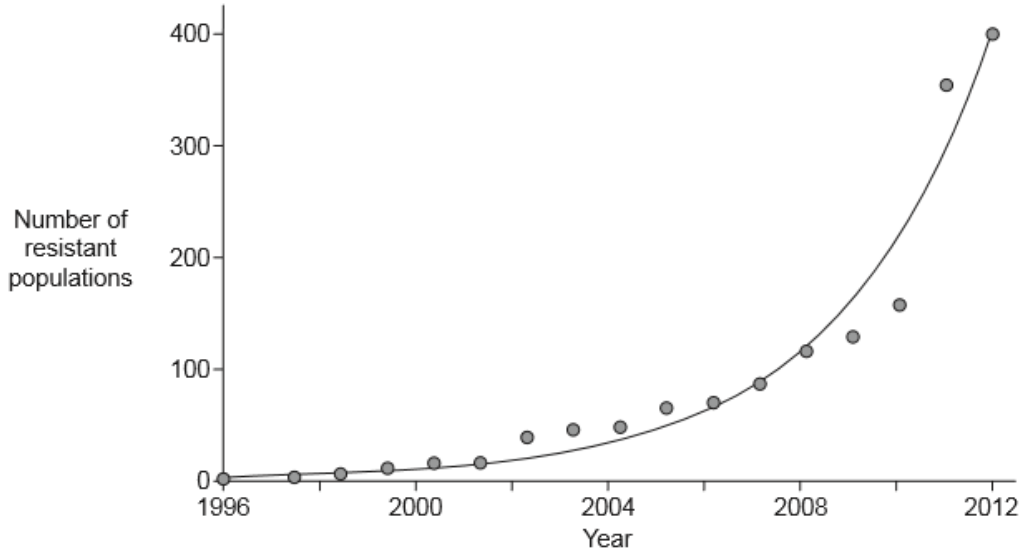
a. Outline the use of kanamycin in the selection of transgenic cotyledons. [2]

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]

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

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]

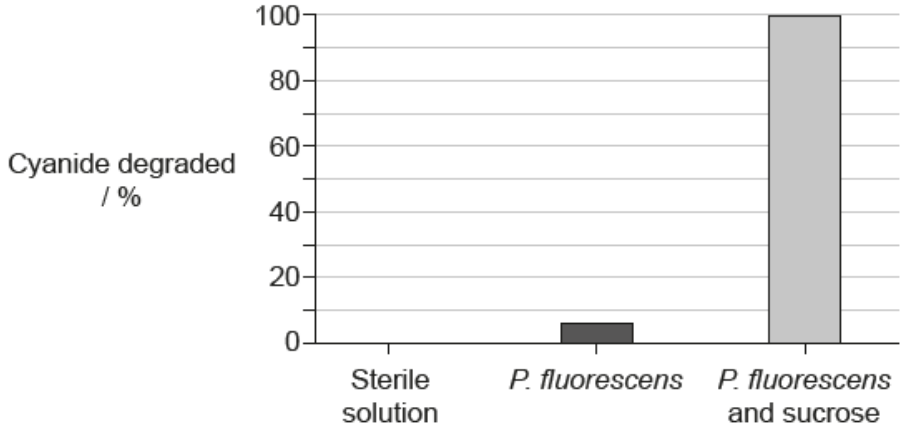
c. Explain the role of the *Agrobacterium tumefaciens* Ti plasmid in genetic modification. [3]

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.

$$\text{cyanide} + \text{oxygen} + \text{organic carbon source} \rightarrow \text{carbon dioxide} + \text{ammonia} + \text{nitrates}$$

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.



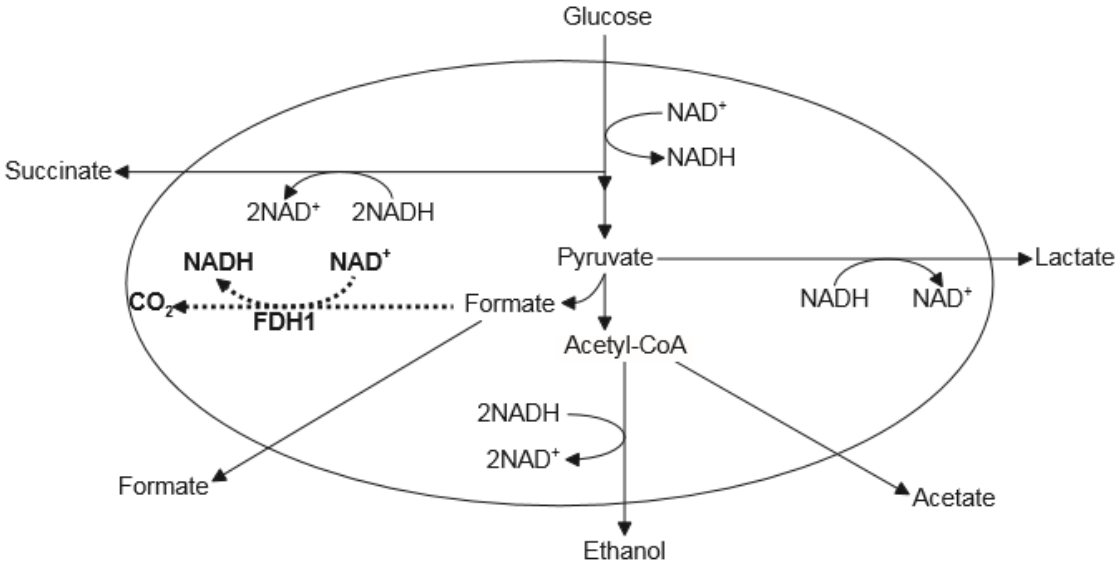
[Source: adapted from C White and J Markweise, (1994) *Journal of Soil Contamination*, 3, pages 271–283. http://www.informaworld.com]

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]

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.]

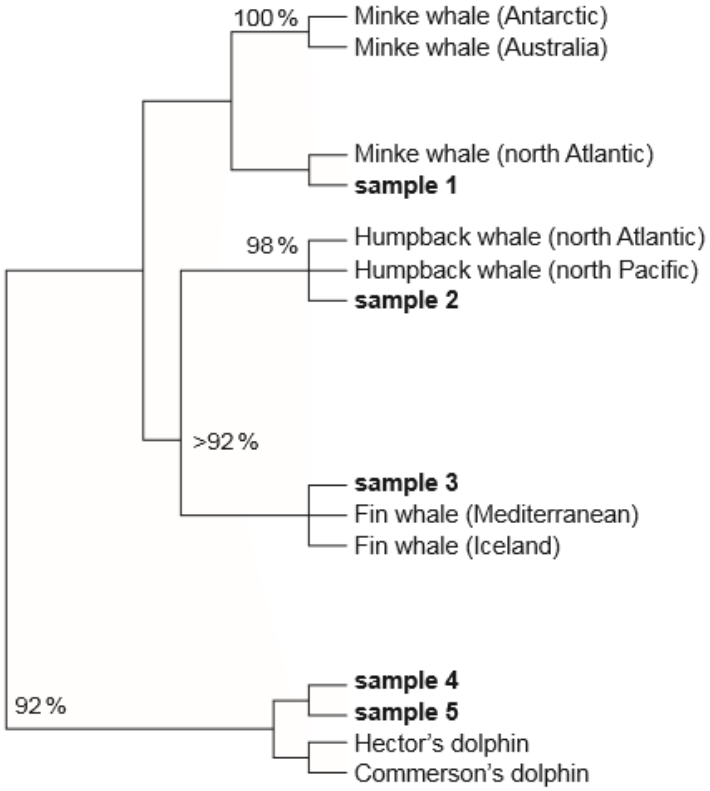
a. Using the diagram, suggest a reason for high concentrations of NADH favouring the production of succinate. [1]

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]

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.



[Source: Adapted from C. S. Baker and S. R. Palumbi (1994), *Science*, 256 (5178), pages 1538–1539. (http://www.soest.hawaii.edu/oceanography/courses_html/OCN331/Baker%26Palumbi.pdf). Reprinted with permission from AAAS. Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal or classroom purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher.]

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

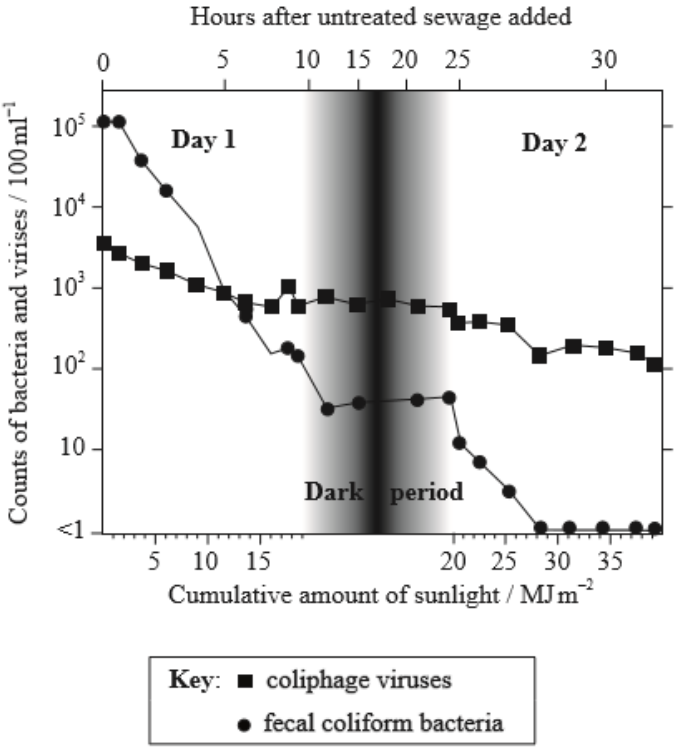
b. Using the data in the cladogram, state the reason for sale of Sample 4 meat being illegal in Japan. [1]

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]

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]

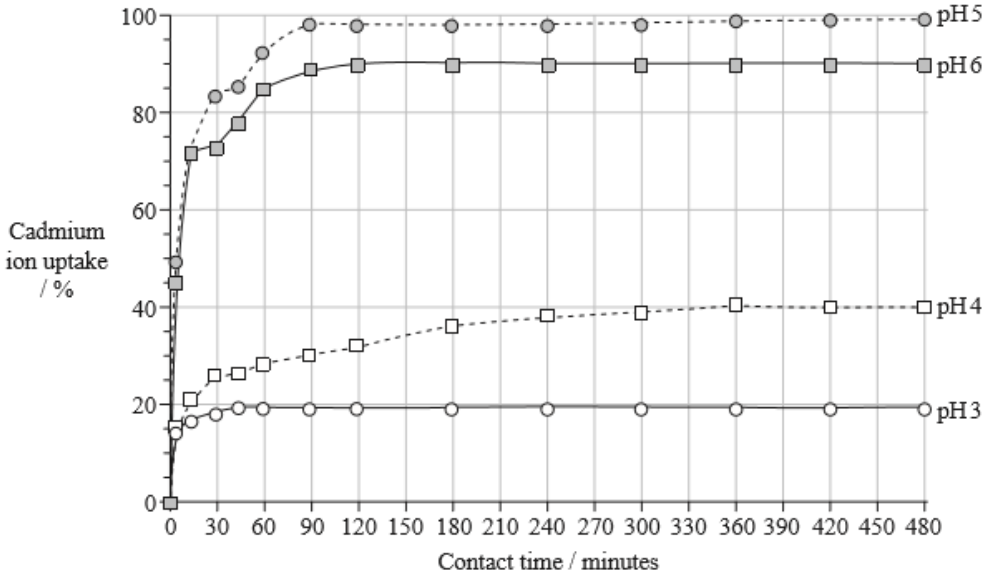
b (i) Deduce, using the data in the graph, the effect of sunlight on fecal coliform bacteria. [2]

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]

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]

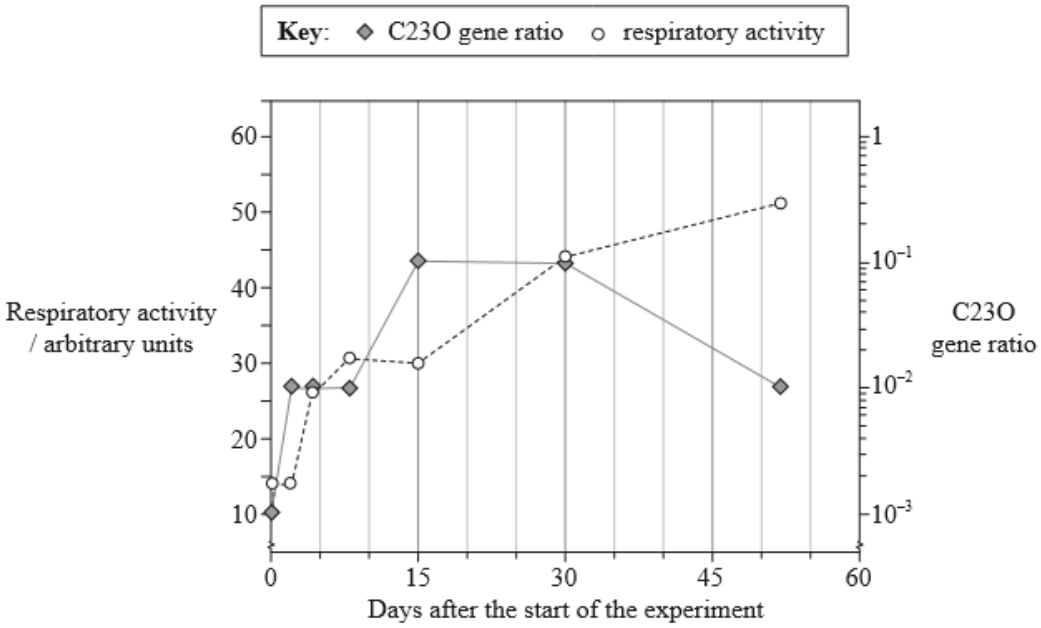
b. Calculate the difference in cadmium ion uptake between pH 4 and pH 5 at 60 minutes. [1]

.....%

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]

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.]

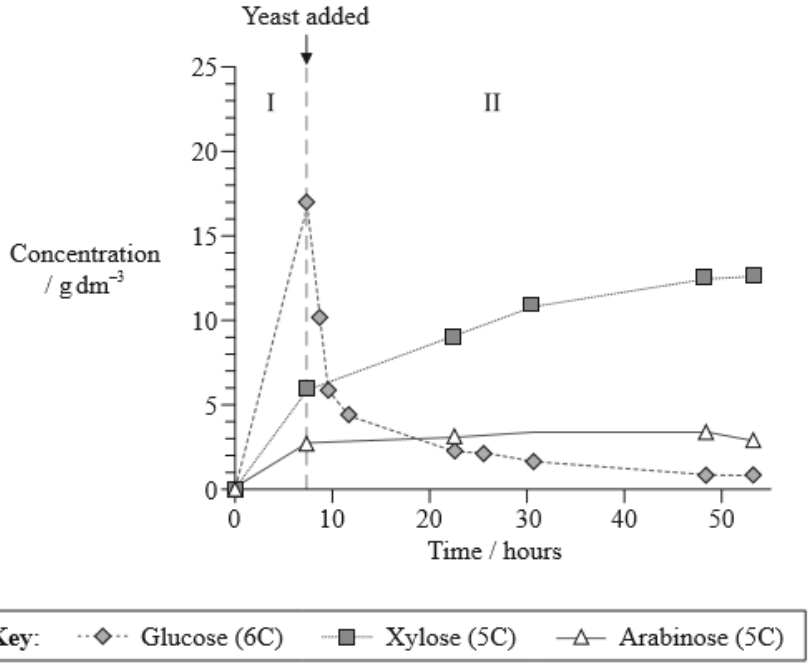
a . State the respiratory activity when the C23O gene ratio first reached its highest level. [1]

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]

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]

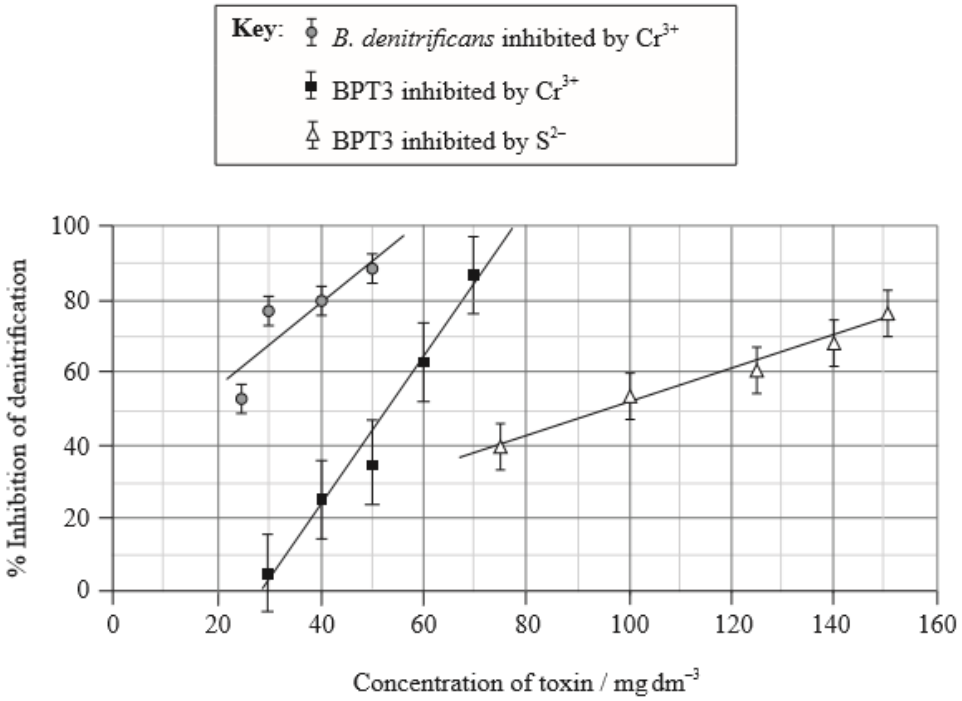
c. Explain the changes in concentration of glucose and xylose during phase II. [3]

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

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

Bacteria	Peptidoglycan content
Gram-positive	
Gram-negative	

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]

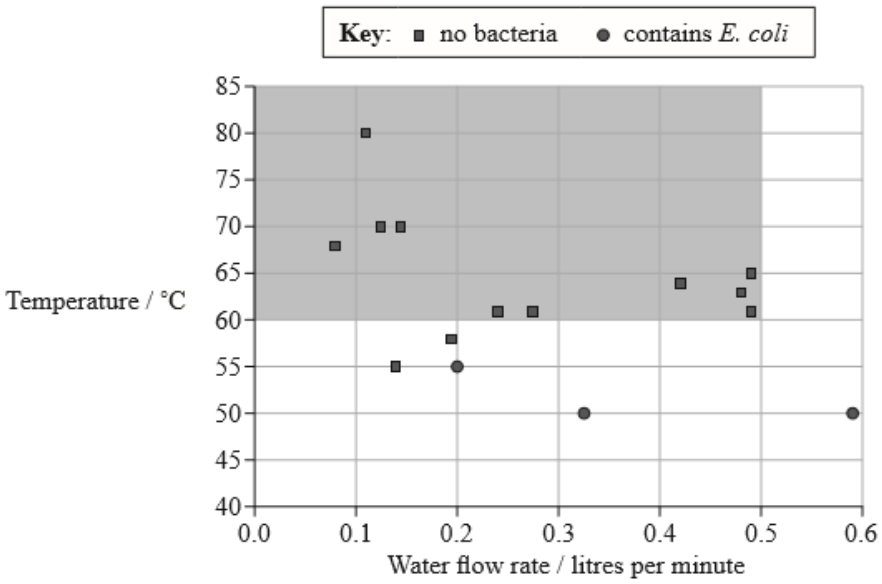
b. Waste water from some industrial processes contains high levels of Cr^{3+}. State, with a reason, which of the bacteria investigated should be used to treat this water. [1]

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]

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.

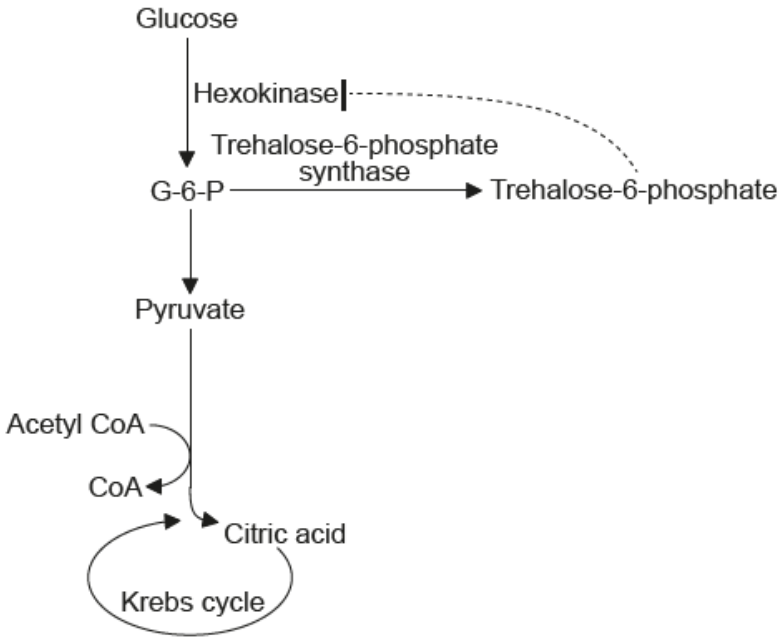
e. Evaluate pasteurization as a method of controlling microbial growth. [2]

---

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

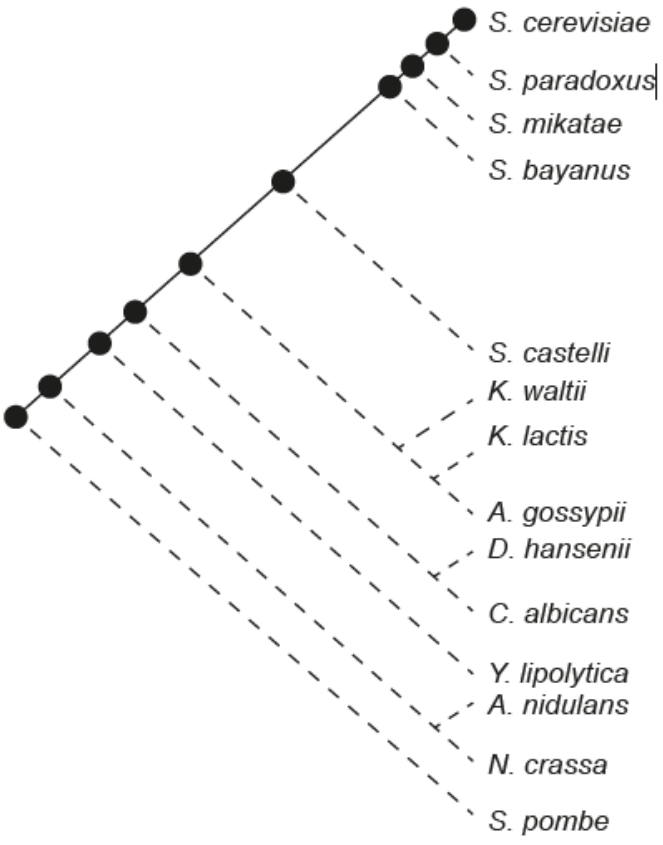
d. *Aspergillus niger* is used to produce citric acid by continuous fermentation. Glucose is converted to pyruvate by glycolysis. Trehalose-6- [2]

phosphate normally inhibits hexokinase, an important enzyme in the glycolysis pathway.



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

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.



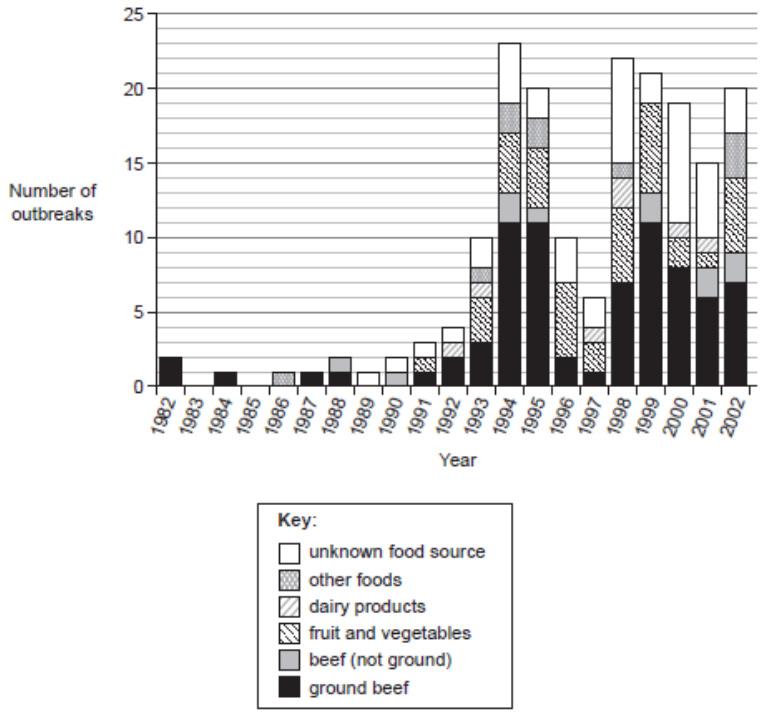
[Source: Reprinted by permission from Macmillan Publishers Ltd: *Nature*, Vol. 487, Anne-Ruxandra Carvunis *et al*. Proto-genes and de novo gene birth, pp. 370–374, copyright (2012), http://www.nature.com/]

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]

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]

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

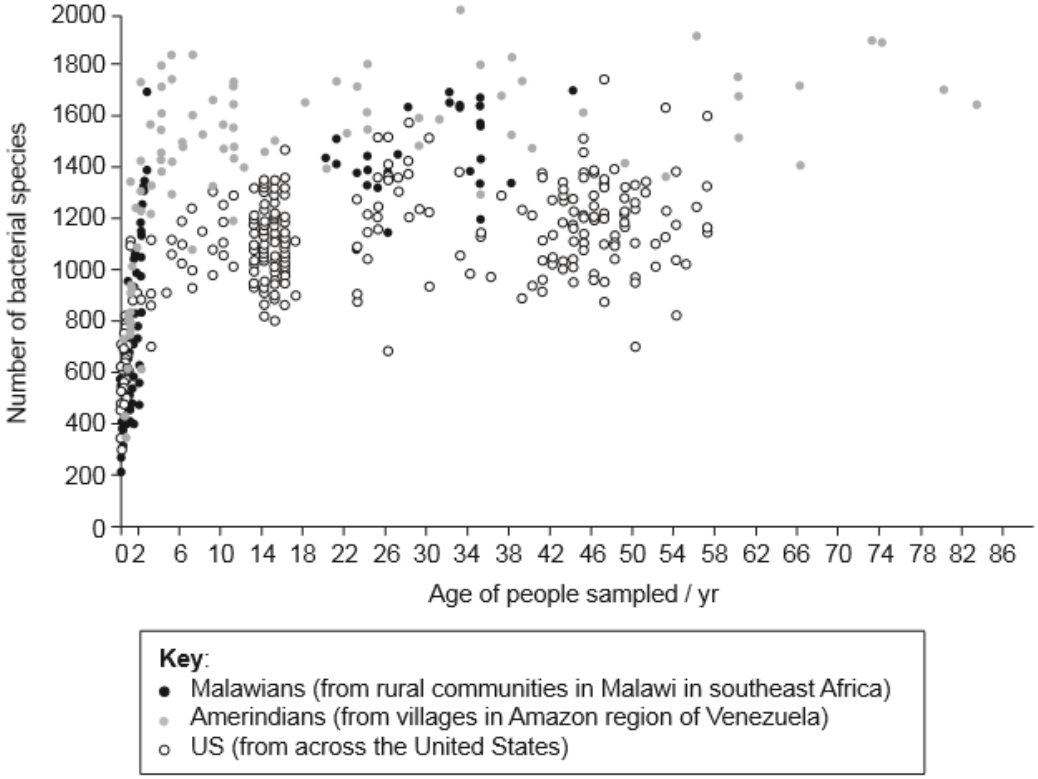
[Source: © International Baccalaureate Organization 2016]

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]

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]

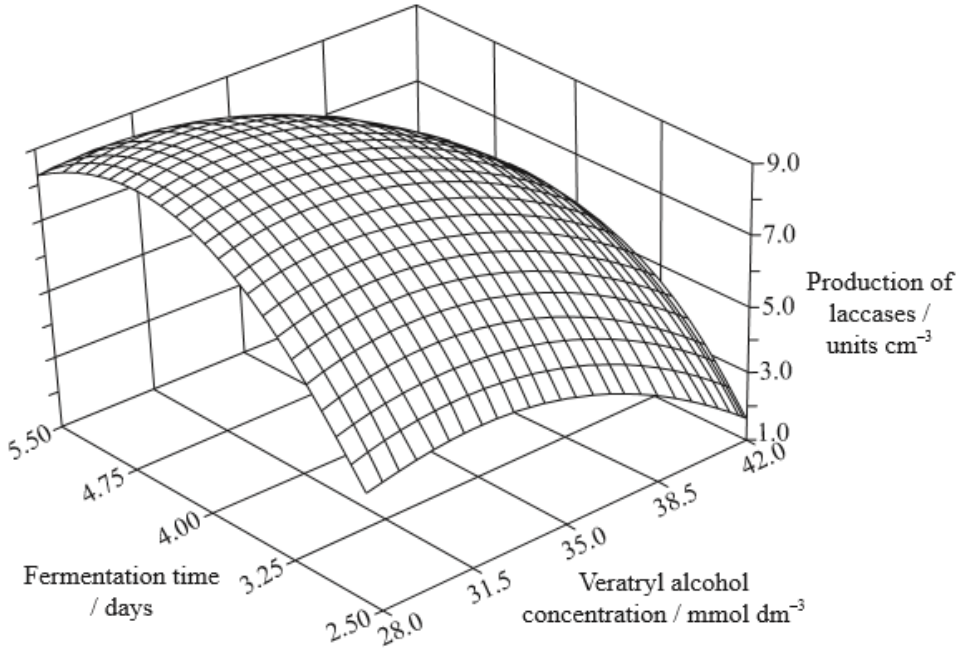
b (ii) Distinguish between the trends seen in the three populations. [2]

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.

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]

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

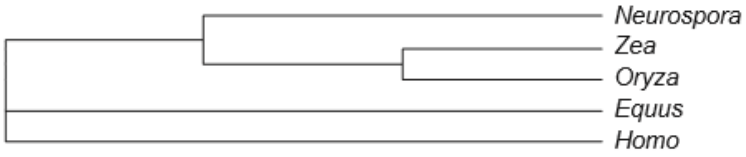
First position	Second position: U		Second position: C		Second position: A		Second position: G		Third position
U	UUU	Phe (F)	UCU	Ser (S)	UAU	Tyr (Y)	UGU	Cys (C)	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu (L)	UCA		UAA	**STOP**	UGA	**STOP**	A
	UUG		UCG		UAG		UGG	Trp (W)	G
C	CUU	Leu (L)	CCU	Pro (P)	CAU	His (H)	CGU	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]

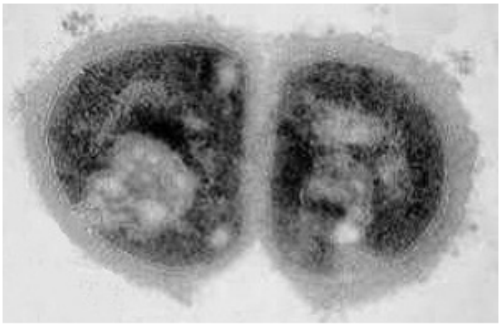
c. (i) Identify the longest amino acid sequence where there are no differences amongst the five genera. [2]

(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]

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]

---

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

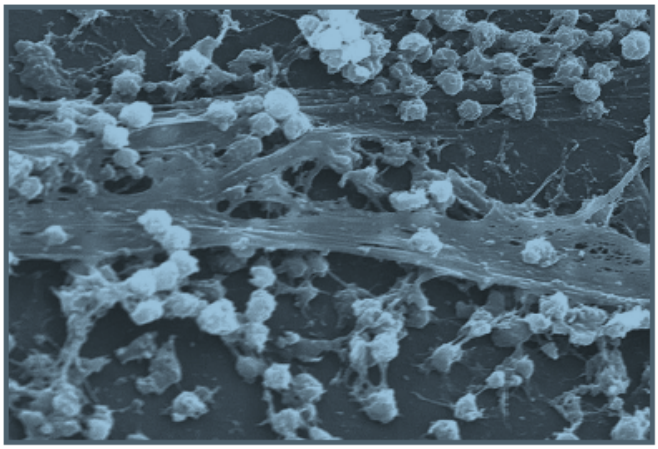
c. Outline the process of nitrogen fixation by a **named** free-living bacterium. [2]

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.

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



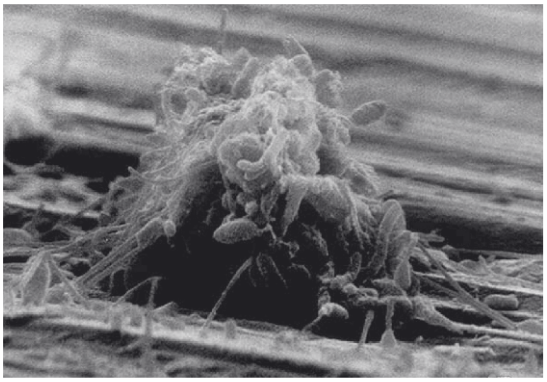
[Source: https://en.wikipedia.org/wiki/Biofilm#/media/File:Staphylococcus_aureus_biofilm_01.jpg]

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]

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]
