SL Paper 3

Golden rice is a genetically modified variety of rice (*Oryza sativa*). The golden colour comes from beta-carotene, a precursor of vitamin A, in the edible parts of rice. The modification was achieved by the addition of two beta-carotene biosynthesis genes, one from a flower (*Narcissus pseudonarcissus*) and the other from a soil bacterium (*Erwinia uredovora*).

a. Using this information, outline the reason for Golden rice being considered a transgenic organism. [1]

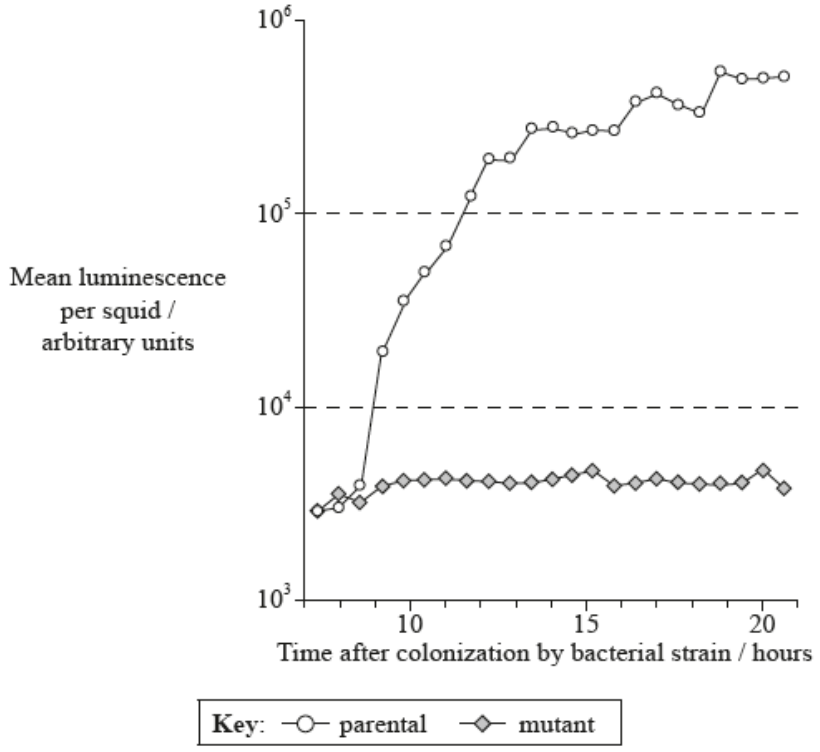
b. Outline the bioinformatics method used to identify the target gene in the plant. [1]

Discuss the environmental risks of the cultivation of genetically modified crops.

Explain how microorganisms can be used in response to pollution incidents such as an oil spill.

Outline the use of viral vectors in gene therapy.

The bacterium *Vibrio fischeri* produces an enzyme called luciferase. This bacterium often colonizes the squid (*Euprymna scolopes*). A mutant strain of *V. fischeri* was obtained that was unable to produce luciferase. The graph shows the mean luminescence per squid after being colonized by the parental and mutant bacterial strains.



[Source: K. L. Visick and M. J. McFall-Ngai (2000) '*Vibrio fischeri lux* Genes Play an Important Role in Colonization and Development of the Host Light Organ.' *Journal of Bacteriology*, **182**, pp. 4578–4586. Fig. 2. Reproduced with permission from American Society for Microbiology.]

a. State the mean luminescence per squid 11.5 hours after colonization by parental *V. fischeri*. [1]

b. Between 8.5 and 10.5 hours after colonization with the parental bacterial strain, luminescence increases by a factor of approximately 10. [1]

Estimate the factor by which luminescence increases between 8.5 and 17 hours after colonization with the parental bacterial strain.

c. Using the data in the graph, distinguish between luminescence in squid colonized by the parental and mutant bacterial strains. [2]

d. Bioluminescence only happens when *V. fischeri* becomes part of a population with high density, for example when bacteria colonize the light organs of squid. Evaluate whether the data supports this hypothesis. [2]

Gene therapy is a new technology which can be used to treat hereditary diseases.

Outline **two** risks of gene therapy.

State **two** fuels that can be produced from biomass using microbes.

State **two** roles of microbes in ecosystems.

b. Explain how Gram staining is used in microbiology. [3]

c. Discuss the possible consequences of gene therapy. [2]

a. Gene therapy may offer cures for inherited diseases and, perhaps, improve quality of life. Distinguish between somatic and germ line therapy. [2]

b. Discuss risks of gene therapy. [3]

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

Identify **one** risk associated with gene therapy.

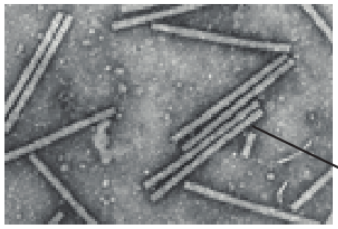
Researchers are studying several ways to treat cancer using gene therapy.

Discuss the risks of gene therapy.

Describe the use of viral vectors in gene therapy.

Discuss the risks of gene therapy.

Tobacco mosaic virus (TMV) was used as a vector in the development of a new process for Hepatitis B vaccine production.



[Source: Scholthof, K-B.G. 2000. Tobacco mosaic virus. The Plant Health Instructor. DOI: 10.1094/PHI-I-2000-1010-01. Updated 2005. © 2018 The American Phytopathological Society. All rights reserved.]

a. State the role of a vector in biotechnology. [1]

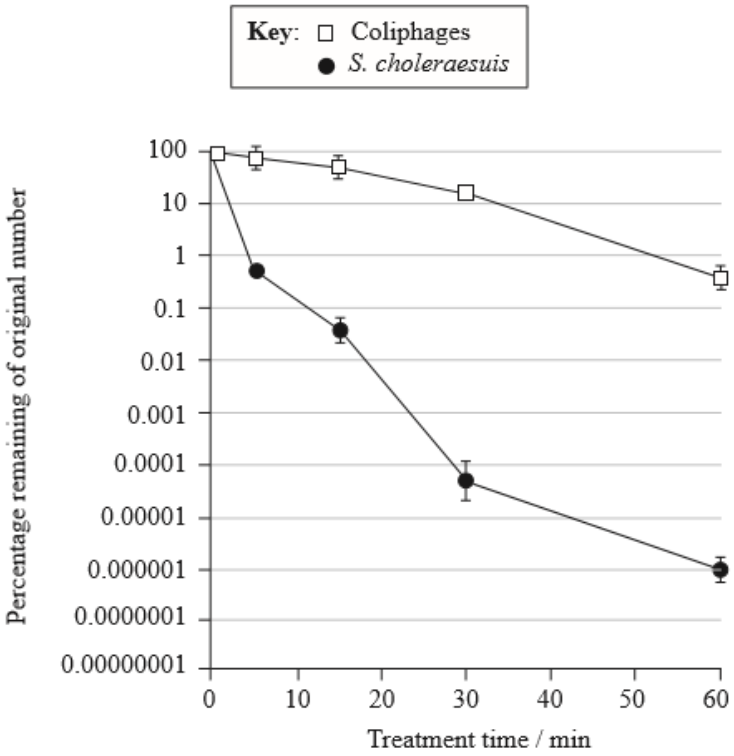
b. Explain how the Hepatitis B vaccine is produced using TMV. [3]

c. State the importance of marker genes in genetic modification. [1]

Outline the role of saprotrophic bacteria in the treatment of sewage.

The sludge produced in sewage treatment plants contains pathogenic microorganisms. In a study, sludge was heated to 80°C in order to kill the pathogens and the effectiveness of this treatment was compared using viruses (coliphages) and bacteria (*Salmonella choleraesuis*) which were added as indicators. The level of activity of either of these two indicators shows whether pathogenic microorganisms may have survived in the sewage sludge.

The resistance of the indicators to heat treatment was studied and their level of activity is shown in the following graph.



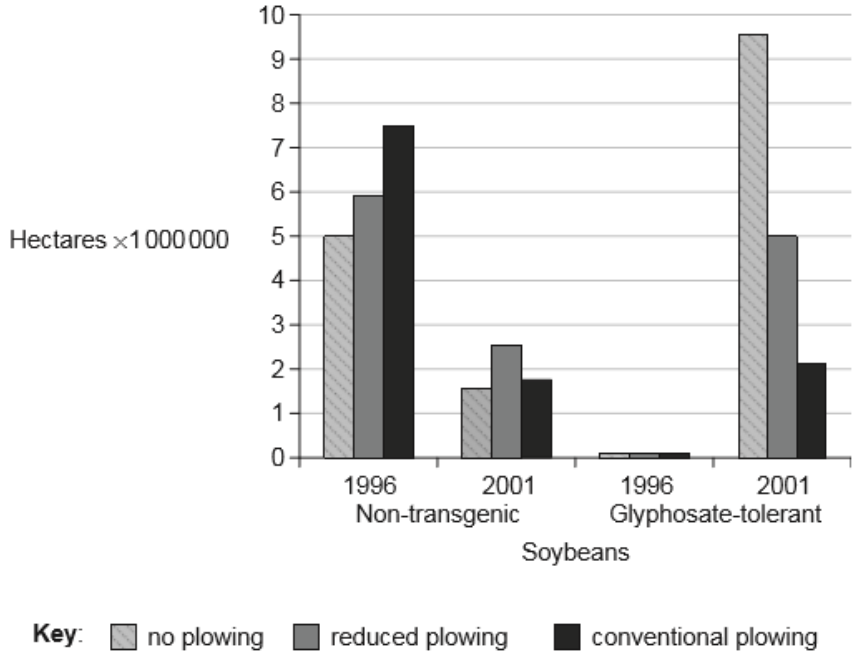
[Source: adapted from L Mocé-Llivina, *et al.*, (2003), *Applied and Environmental Microbiology*, **69** (3), pages 1452–1456]

State which indicator was more resistant to the heat treatment.

Explain the consequences of releasing raw sewage into rivers and the involvement of microorganisms in this process.

Explain, with reference to **one** example, how a polluted ecosystem can be restored through bioremediation.

a. Before planting their crops, farmers have traditionally plowed their land to suppress weed growth. Unfortunately, plowing causes the loss of valuable topsoil. Modern farming is shifting toward the use of chemical weed killers such as glyphosate in combination with genetically modified glyphosate-tolerant (GT) crops. The graph shows the area of plowed land in the USA for soybeans in 1996 and 2001. During that period GT soybean planting increased from a few percent to about 70 %. [2]



[Source: adapted from A. Cerdeira and S. Duke (2006) *Journal of Environmental Quality*, 35, pages 1633–1658. Reprinted by Permission, ASA, CSSA, SSSA]

Evaluate the hypothesis that increased planting of glyphosate-tolerant crops has resulted in the reduction of plowing.

b. Explain the role of bioinformatics in the determination of the function of an unknown target gene. [2]

c. Outline what is meant by open reading frame (ORF). [1]

d. Genetic engineers sometimes use physical methods to transform cells. Describe the method of biolistics. [2]

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b. Outline the role of saprotrophic bacteria in the treatment of sewage. [2]

c. Explain the formation of methane from biomass. [3]

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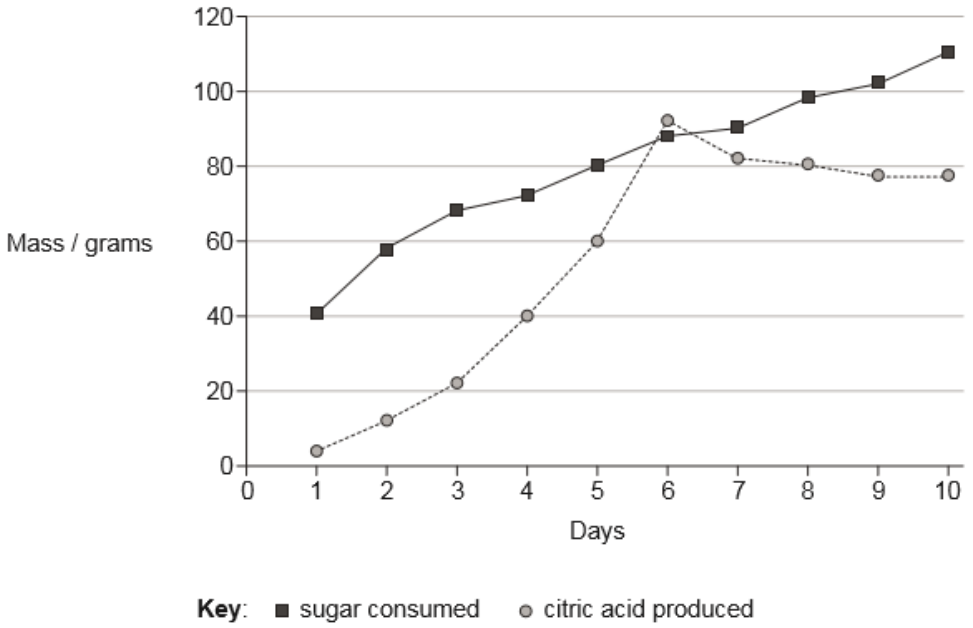
a. State the role of *Rhizobium*, *Nitrobacter* and *Azotobacter* in the nitrogen cycle. [3]

Rhizobium: .....
Nitrobacter: .....
Azotobacter: .....

b. Explain the production of methane from biomass. [4]

Sugar solution in a fermenter was inoculated with a culture of fungus, incubated at 30°C and left for 10 days to produce citric acid. The mass of sugar consumed and the mass of citric acid produced was measured daily.



[Source: adapted from Ali, S.; ul-Haq, I.; Qadeer, M.; Iqbal, J. (2002), Production of citric acid by Aspergillus niger using cane molasses in a stirred fermentor. *Electronic Journal of Biotechnology*, Vol. 5, No. 3]

a. State a suitable fungus for the production of citric acid in the fermenter. [1]

b. Suggest a reason that fermentation is most successful at 30°C. [1]

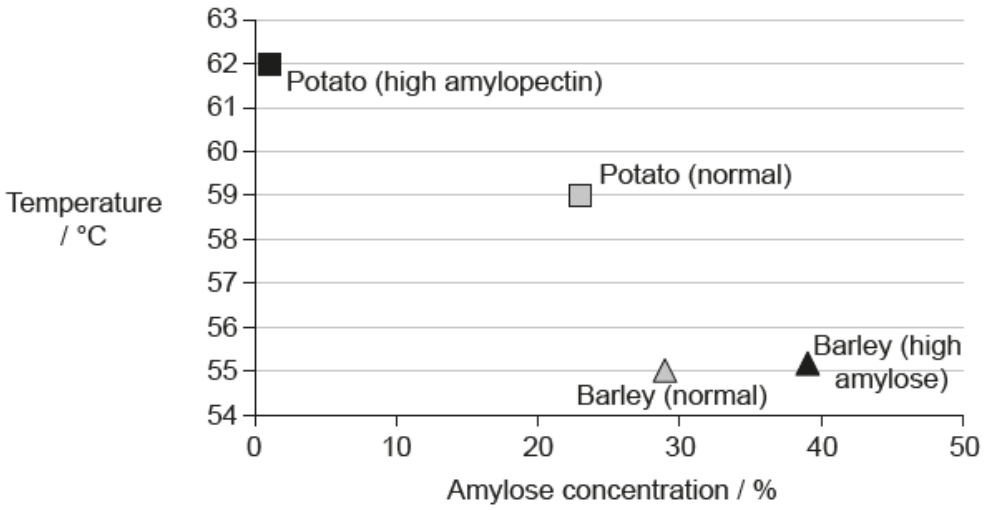
c. Suggest reasons for the changes in mass of sugar and citric acid after day 6. [2]

d. State **two** uses of the citric acid produced. [2]

1. . . . . .

2. . . . . .

Starch from different sources contains differing proportions of amylose and amylopectin. Potatoes (*Solanum tuberosum*) have been genetically modified to produce high-amylopectin starch (Amflora potatoes). Heat induces starch to form a gel in excess water. The graph shows gel formation temperature at different amylose concentrations.



[Source: adapted from H Fredriksson et al. (1998) *Carbohydrate Polymers* 35, pages119–134, with permission from Elsevier]

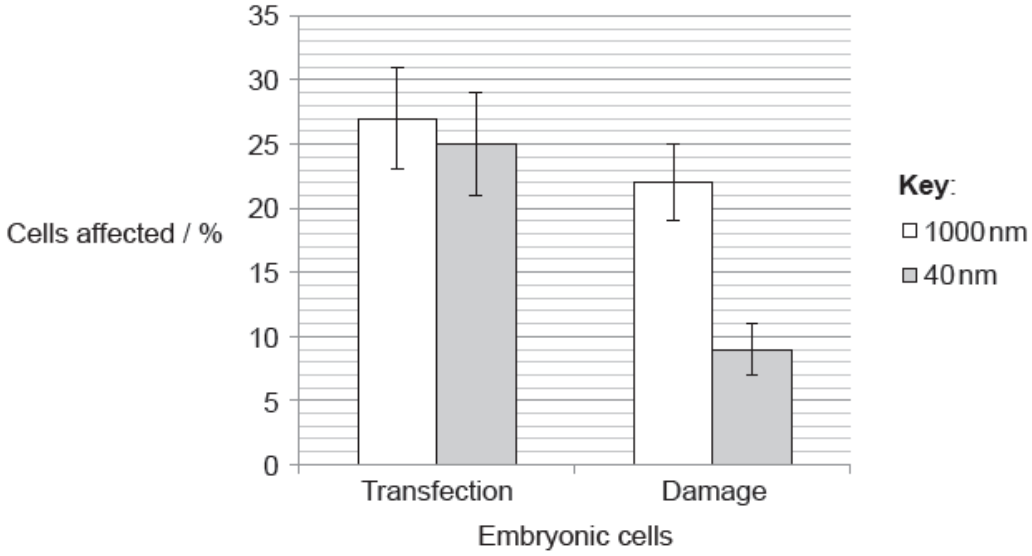
a. Discuss the hypothesis that the temperature at which starches form a gel depends on the degree of cross-linking of amylopectin. [2]

b. State **one** advantage of potatoes with a high amylopectin content. [1]

c. The Amflora potato was approved for industrial applications in the European Union (EU) in 2010 and was withdrawn in January 2012 due to opposition. Discuss reasons for people supporting or opposing the introduction of the Amflora potato in the EU. [3]

Usually the size of particles used in biolistics with plant cells is 1000 nm. Researchers tested the effect of using smaller sized particles (40 nm) in the biolistic treatment of animal cells.

The degree of transfection by DNA and the damage to embryonic kidney cells was assessed using particles of the two different sizes. The amount of DNA attached to each particle, whether large or small, was the same.



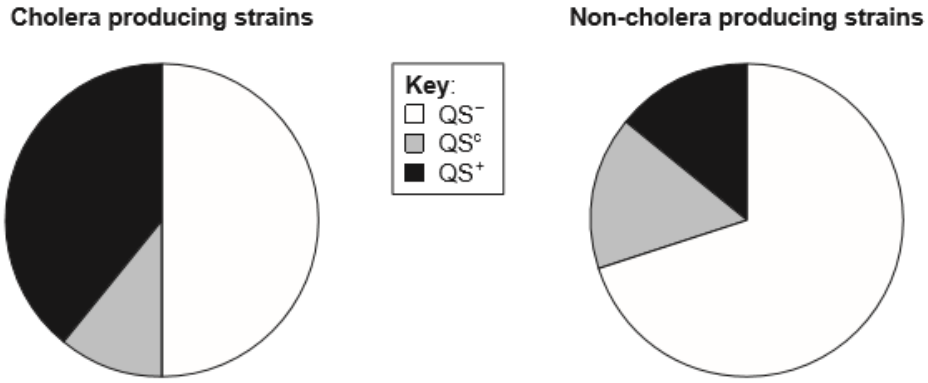
[Source: John A O'Brien and Sarah C. R. Lummis (2011) 'Nano-biolistics: a method of biolistic transfection of cells and tissues using a gene gun with novel nanometer-sized projectiles.' *BMC Biotechnology*, 11: p. 66.]

a. Describe the effect of the different sized particles on the treatment of these animal cells. [2]

b. State **one** other physical method used to introduce DNA into plants. [1]

Vibrio cholerae live in aquatic environments and cause cholera. Some *V. cholerae* form aggregates that show characteristics not seen in individual bacteria. The bacteria in these aggregates monitor the population densities by quorum sensing. They produce quorum sensing proteins (QS+). Some *V. cholerae* strains do not produce quorum sensing proteins (QS–) and some only produce quorum sensing proteins in low amounts (QSc).

V. cholerae strains isolated in China were examined. The pie charts show the percentage of different quorum-sensing systems in strains that contain cholera toxin genes and in strains that do not contain cholera toxin genes.



[Source: © International Baccalaureate Organization 2015]

a. State the percentage of cholera producing strains that do not produce quorum sensing proteins (QS^-). [1]

b. Determine the approximate percentage of non-cholera producing strains that produce quorum sensing proteins in low amounts (QS^c). [1]

c. Compare the percentage of strains that do not produce quorum sensing proteins (QS^-) in strains with and without the cholera toxin genes. [2]

d. Deduce, using the data, whether the genes for quorum sensing and for toxicity of cholera evolved together. [1]

e. *Vibrio cholerae* is Gram-negative. Describe the structure of the cell wall of this bacterium. [2]

The sludge produced in sewage treatment plants contains pathogenic microorganisms. In a study, sludge was heated to 80°C in order to kill the pathogens and the effectiveness of this treatment was compared using viruses (coliphages) and bacteria (*Salmonella choleraesuis*) which were added as indicators. The level of activity of either of these two indicators shows whether pathogenic microorganisms may have survived in the sewage sludge.

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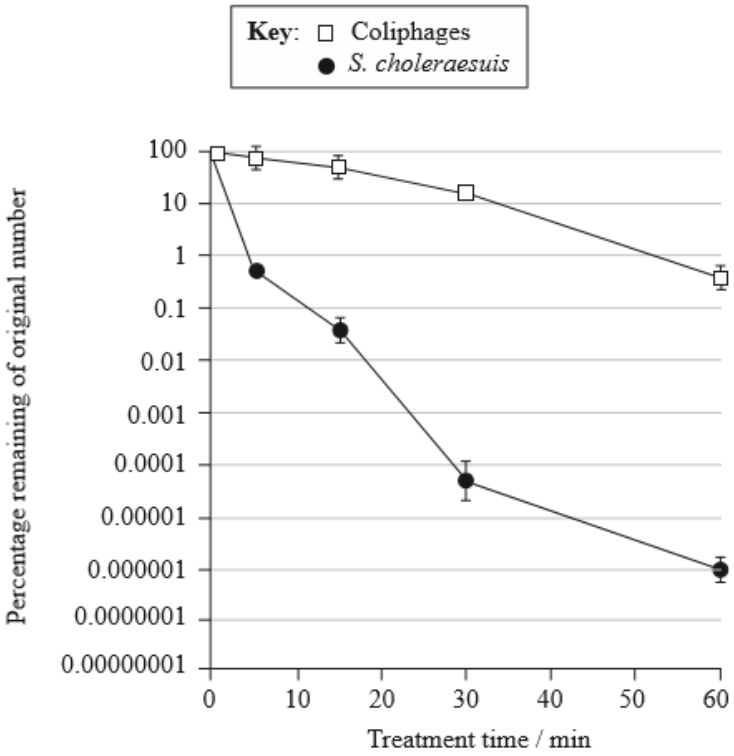


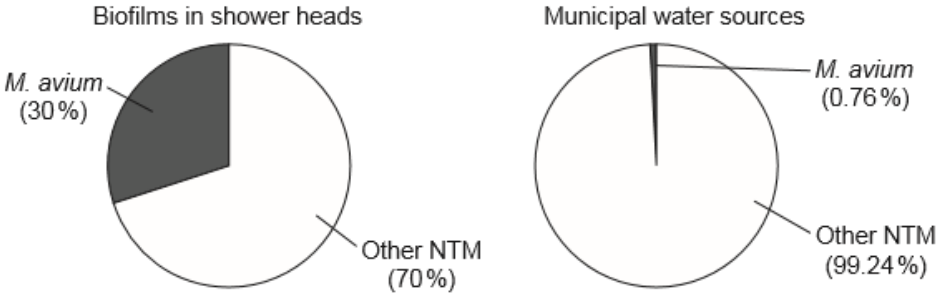
[Source: adapted from L Mocé-Llivina, *et al.*, (2003), *Applied and Environmental Microbiology*, **69** (3), pages 1452–1456]

b. Compare the effect of the 80°C heat treatment on coliphages and *S. choleraesuis*. [2]

c. Discuss whether the heat treatment should be continued beyond 60 minutes if this technique were to be used in sewage treatment plants. [2]

d. In many areas, sewage is discharged directly into the environment. State **two** potential environmental consequences of releasing sewage into rivers. [2]

Many people around the world wash themselves under a warm shower. This personal hygiene may expose individuals to harmful microorganisms such as *Mycobacterium avium* through inhalation of water droplets from the shower head and direct water contact. Samples taken from biofilms inside shower heads and municipal water sources were analysed. Proportions of other non-tuberculous mycobacteria (NTM) were also analysed. The results are shown in the pie charts.



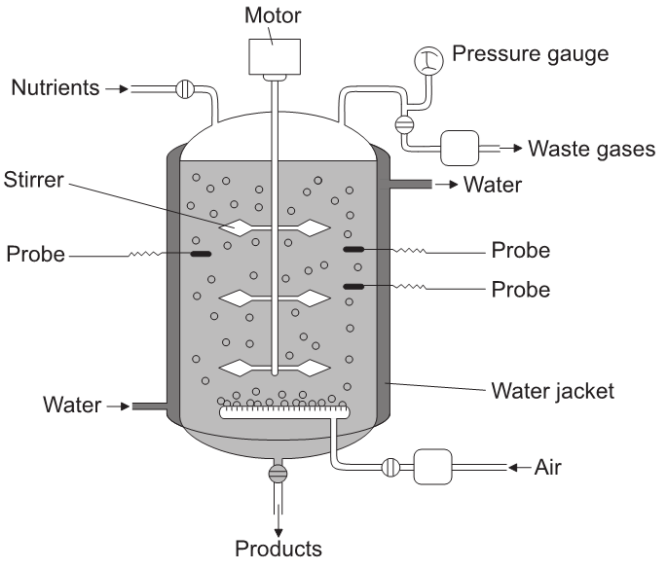
[Source: L. M. Feazel *et al.* (2009) 'Opportunistic pathogens enriched in showerhead biofilms.' *PNAS*, 106 (38), pages 16393–16399, Figure 3 (pie charts B & C).]

a. List **two** properties of biofilms. [2]

b. Distinguish between the data for shower head biofilms and municipal water sources. [1]

c. Suggest reasons for biofilms developing inside shower heads. [3]

The diagram shows a simplified fermenter used in the production of penicillin.



[Source: Valero, F, del Rio, JL, Poch, M and Sola, C (John Wiley and Sons, 1992). Studies on Lipase Production by Candida rugosa Using On-line Enzymatic Analysis. Annals of the New York Academy of Sciences, 665, pp. 334–344.
doi: 10.1111/j.1749-6632.1992.tb42596.x]

a. State **two** conditions in the fermenter that would be monitored by the probes. [1]

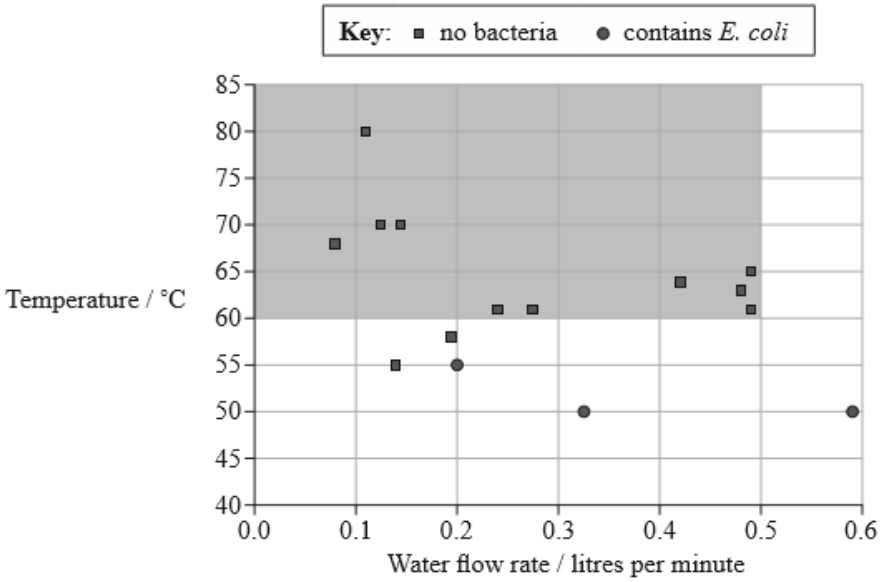
b. Suggest a reason that the fermenter is surrounded by a water jacket. [1]

c. Identify the waste gas produced. [1]

d. Explain the process of penicillin production in the fermenter. [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 treatment to eliminate microbes from water.

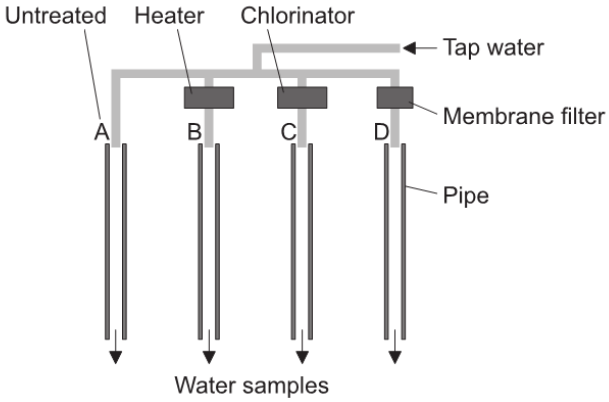
Water samples from 15 different locations containing high levels of the bacterium E. coli were passed through the purifier at different flow rates and temperatures to test its effect on contaminated water. The shaded area of the graph below represents the recommended temperature and flow rate for using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



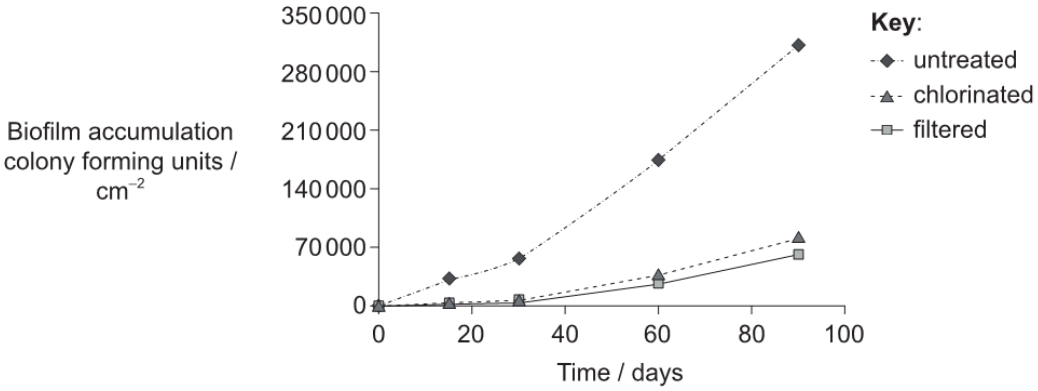
[Source: S. K. Gupta et al. (2008) *American Journal of Tropical Medicine and Hygiene*, 78, pages 979–984]

Evaluate the chulli purifier as a method of controlling microbial growth.

Researchers in Korea set up an experiment to measure how accumulation of biofilm changes in water pipes under different conditions.



The graph shows the accumulation of biofilm in steel pipes when the water was untreated, treated with chlorine and filtered through a membrane.



[Source: adapted from Yoonjin Lee, (2013), Journal of Environmental Research Public Health 2013, 10 (9), pages 4143 – 4160]

a. State the effect chlorination has on the accumulation of biofilm in the pipe. [1]

b. Suggest why membrane filtration may be more suitable than chlorination in purifying the water. [1]

c. Identify which **two** pipes would be required to study the effect of heat on biofilm accumulation. [1]

d. Explain how quorum sensing benefits the bacteria within the steel pipes. [2]

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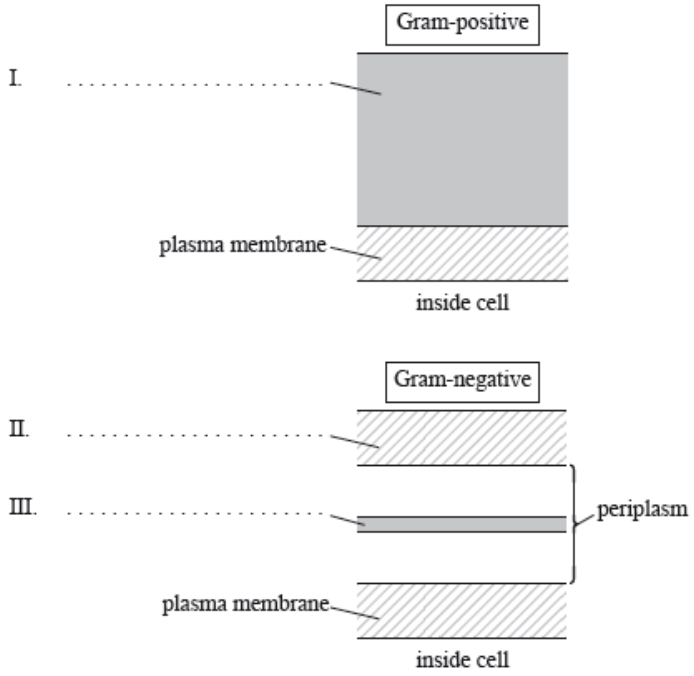
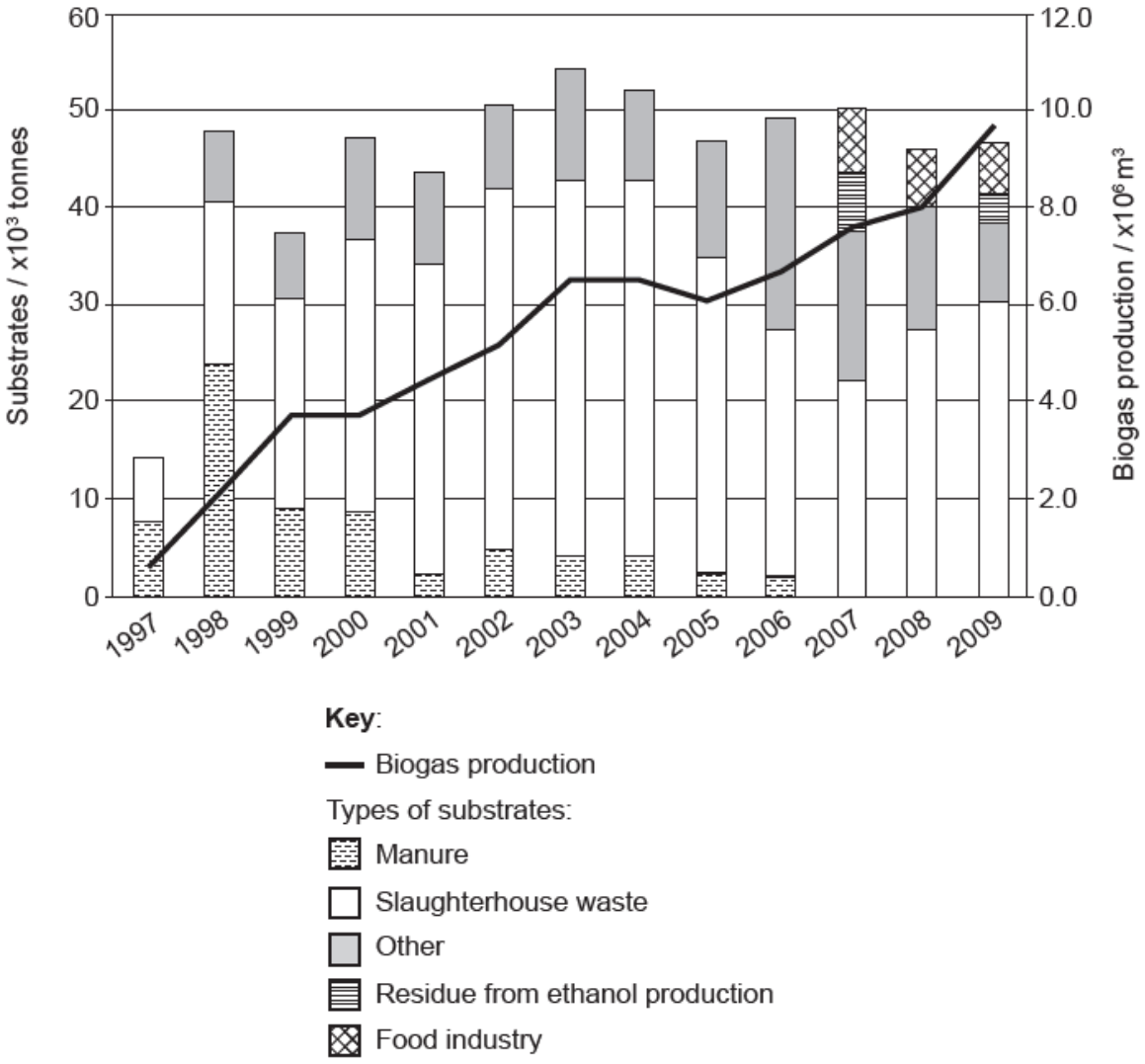
a. Distinguish between Archaea and Eukarya. [2]

b. Label the parts of the cell walls in Gram-positive Eubacteria and Gram-negative Eubacteria shown below. [3]



The graph shows the development of biogas production and substrate utilization at Svensk Biogas (Sweden) from 1997 to 2009.



[Source: L Vallin, (2012), Svensk Biogas AB]

a.i. Biogas production in a fermenter requires a substrate. State another requirement for this process. [1]

a.ii. Suggest reasons based on the data in the graph for increases in biogas production at Svensk Biogas. [2]

b. Outline the principles of fermentation by continuous culture. [3]

Freshwater invertebrates were sampled by students at three sites along a river in central France. The animals were identified and counted. The diversity of each site can be compared using Simpson's reciprocal index.

Species	Number of animals in the sample		
	Site A	Site B	Site C
Baetis rhodani	0	30	7
Ecdyonurus dispar	1	0	9
Ephemerella ignita	4	0	0
Limnephilus lunatus	0	0	2
Brachycentrus subnubilus	2	1	0
Polycentropus flavomaculatus	0	1	0
Rhyacophila obliterata	1	0	0
Gammarus pulex	0	1	0
Asellus aquaticus	8	0	0
Simulium equinum	17	0	0
Dexia	0	5	0
Chironomus annularis	0	0	1
Hirudinea	0	4	2
Simpson's reciprocal index	**3.09**	**1.91**	

[Source: © International Baccalaureate Organization 2017]

Simpson's reciprocal index is given by the following formula:

$$D = \frac{N(N-1)}{\sum n(n-1)}$$

a. Calculate the diversity of site C. Working should be shown. [2]

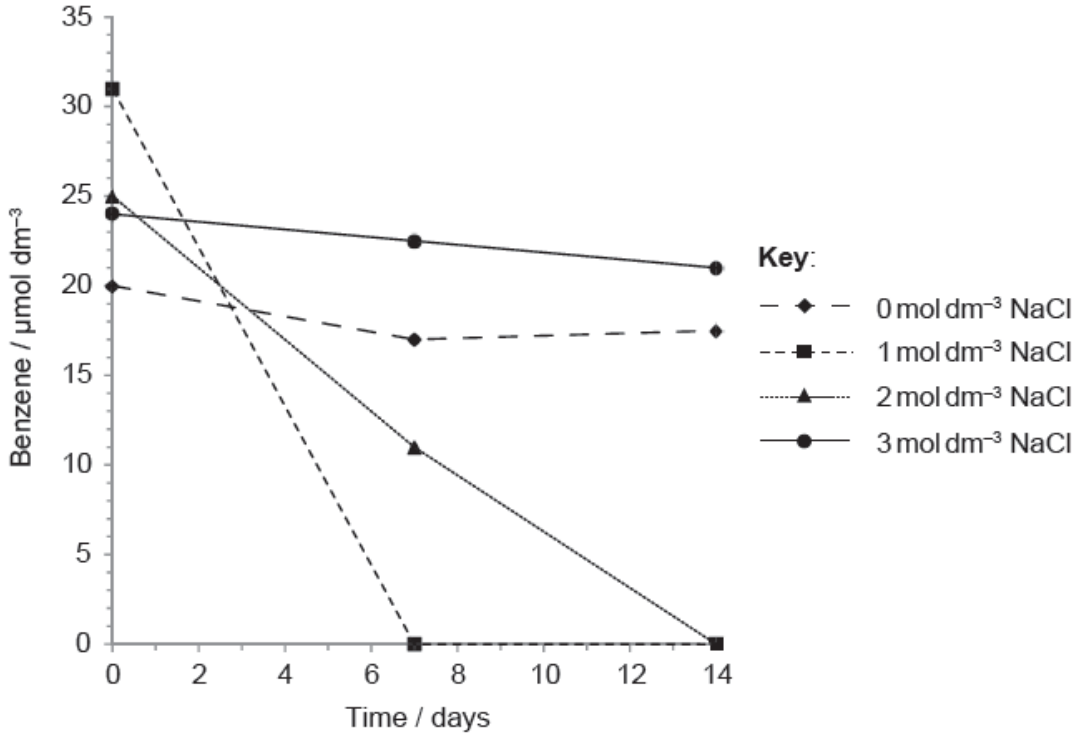
b. Site A has a higher Simpson's reciprocal index than Site B showing that its diversity is higher. [2]

Explain the reason that ecologists consider Site A to have a higher diversity than Site B, despite both sites having six different species present.

c. Discuss the advantages and disadvantages of in *situ* conservation methods. [4]

Benzene is a cancer-causing component of crude oil. Some halophilic bacteria degrade benzene. Using a culture of bacteria obtained from an oil fi eld in the US, degradation of benzene was studied by microbiologists.

The microbiologists cultured the bacteria at different concentrations of sodium chloride (NaCl) and measured the amount of benzene left at different times.

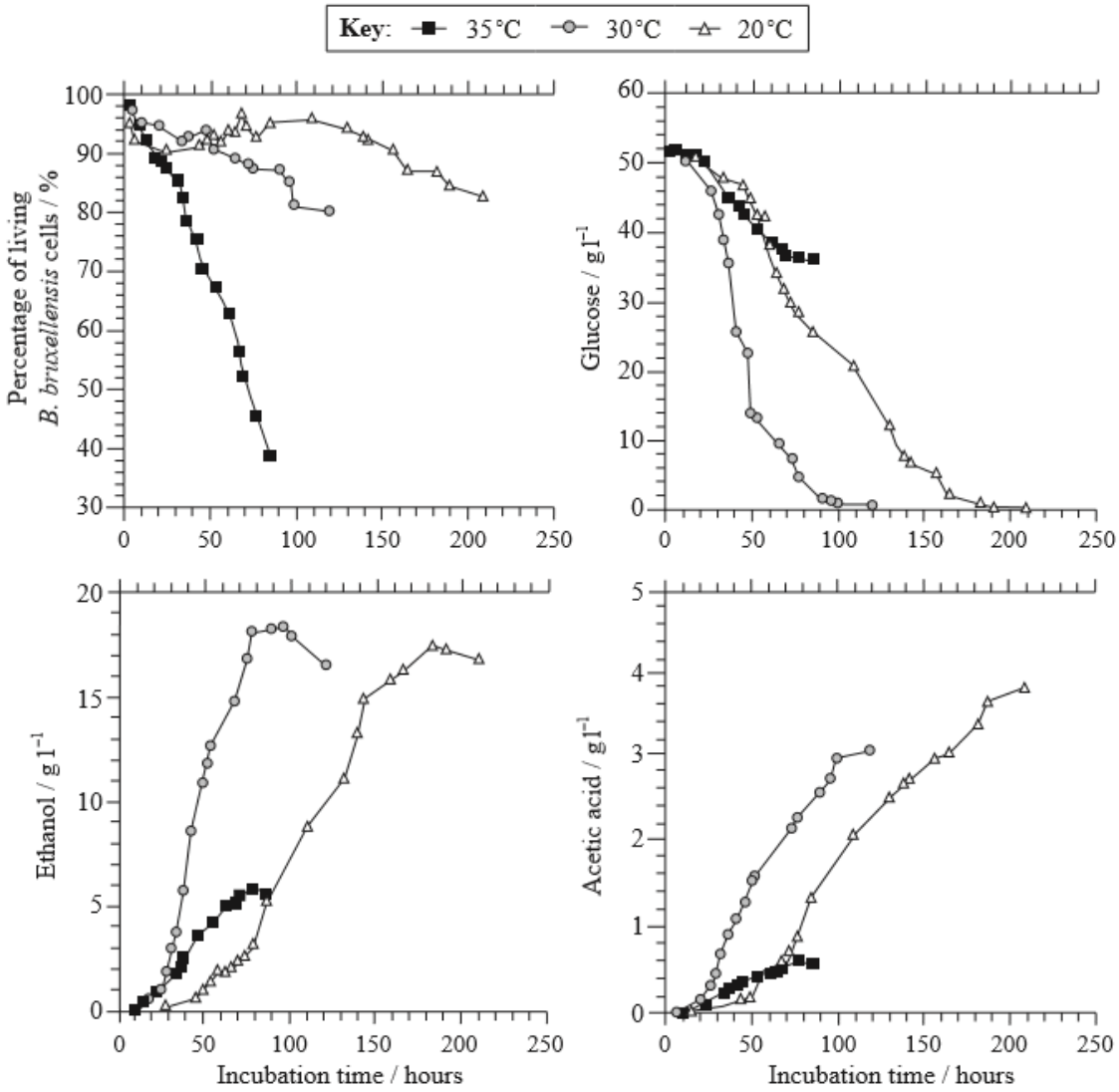


[Source: C A Nicholson and B Z Fathepure, (2004), *Applied and Environmental Microbiology*, pages 1222–1225]

a. Determine the optimum concentration of sodium chloride for benzene degradation. [1]

b. State the genus of halophilic bacteria in the soil that could be degrading the benzene. [1]

The yeast *Brettanomyces bruxellensis* is a contaminant of wine which when present produces acetic acid, the main component of vinegar. The presence of acetic acid can lead to economic losses as it alters the taste of the wine and inhibits the growth of *Saccharomyces cerevisiae*, thus decreasing the ethanol production. Scientists investigated the effect of changing the temperature in fermentation tanks containing only *Brettanomyces bruxellensis* and a growth medium containing glucose in order to understand the dynamics of this contaminant.



[Source: Cédric Brandam, Claudia Castro-Martínez, Marie-Line Délia, Felipe Ramón-Portugal, Pierre Strehaiano (2008) "Effect of temperature on Brettanomyces bruxellensis: metabolic and kinetic aspects", *Canadian Journal of Microbiology*, vol 54 (1), pp. 11–18 © Canadian Science Publishing or its licensors.]

a. State the concentration of glucose at 20°C after 110 hours of incubation, giving the units. [1]

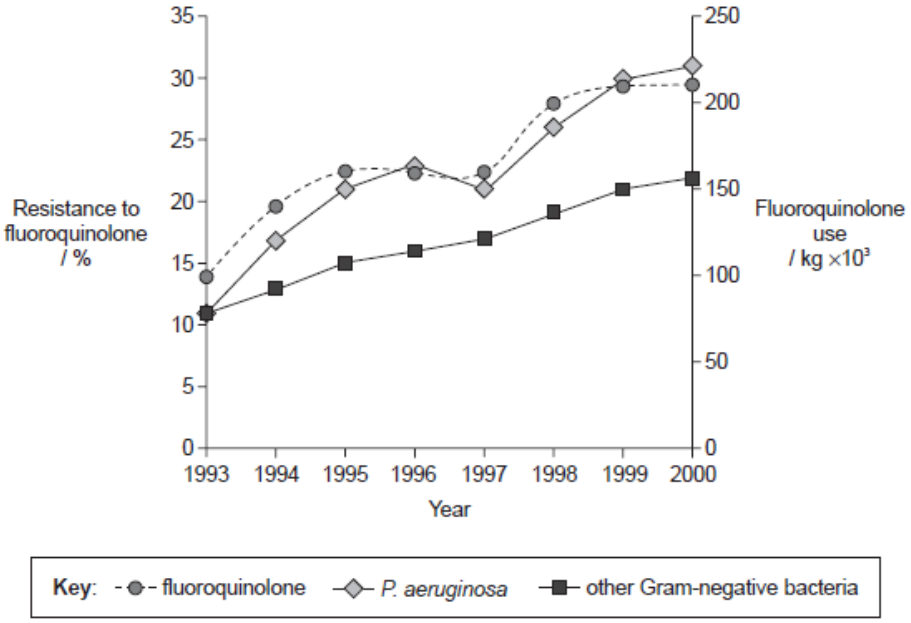
b. State the effect of increasing temperature from 20°C to 30 °C on the rate of production of ethanol. [1]

c (i) Deduce one reason why there were no more rises in ethanol concentration after 120 hours at 30°. [1]

c (ii) Deduce one reason why the concentration of ethanol and acetic acid at 35°C does not rise after 80 hours despite the fact that the concentration of glucose is still high. [1]

d. Discuss the idea of producing wine using a lower temperature range to avoid economic losses due to contamination by yeasts other than *S. cerevisiae*. [3]

Data on microbial resistance to the fluoroquinolone family of antibiotics was collected in US hospitals. The graph shows the relationship between *Pseudomonas aeruginosa*, other Gram-negative bacteria and the use of fluoroquinolone from 1993 to 2000.



[Source: adapted from M Neuhauser, *et al.*, (2003), *Journal of the American Medical Association*, **289** (7), pages 885–888]

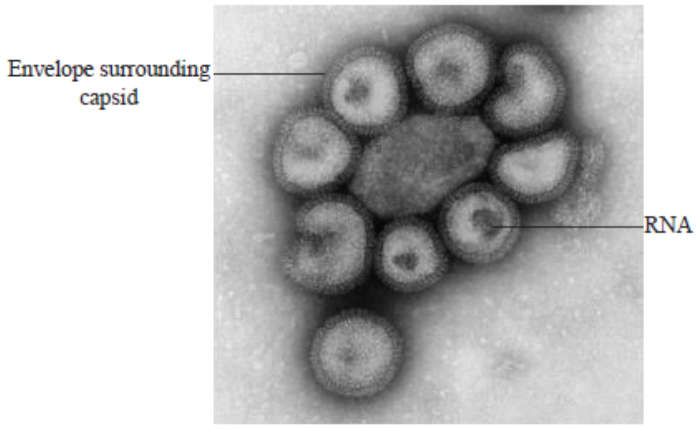
a. State the percentage of *P. aeruginosa* that were resistant to fluoroquinolone in 1996. [1]

b. Compare the trends in fluoroquinolone use and resistance to fluoroquinolone in other Gram-negative bacteria between 1993 and 2000. [2]

c. Predict the results if data from the same hospitals were collected for *P. aeruginosa* resistance in 2001. [1]

d. Discuss the implications of the data in the graph for the health of patients. [3]

The electron micrograph below shows a pathogen.

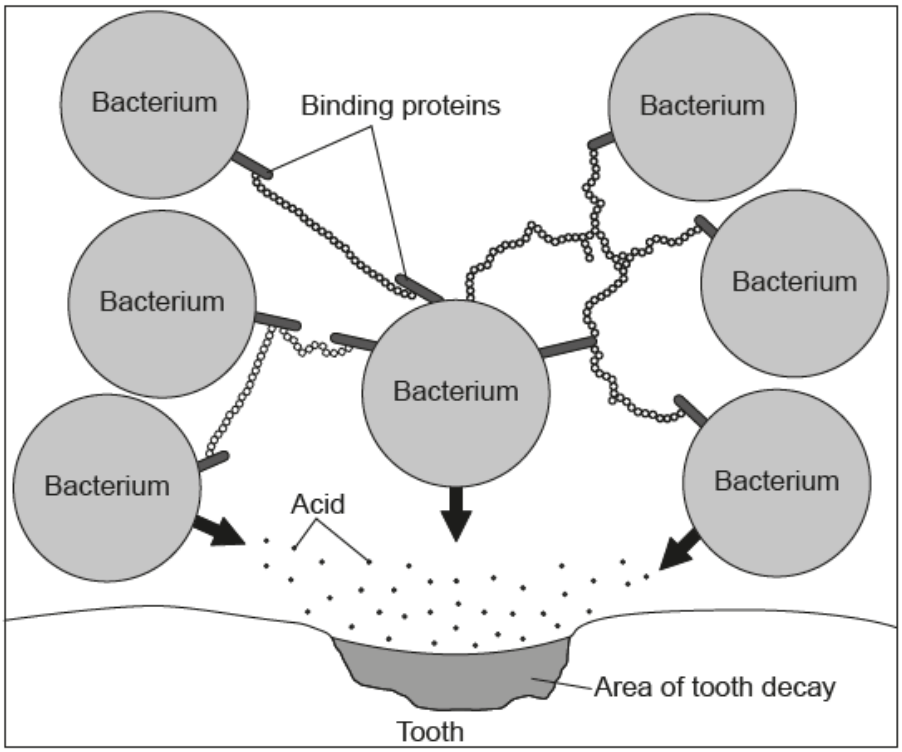


[Source: Professor Frederick A Murphy (University of Texas Medical Branch). Reprinted with permission.]

a. Identify the type of pathogen shown in the electron micrograph, giving reasons for your answer. [2]

b. Outline the use of viral vectors in gene therapy. [3]

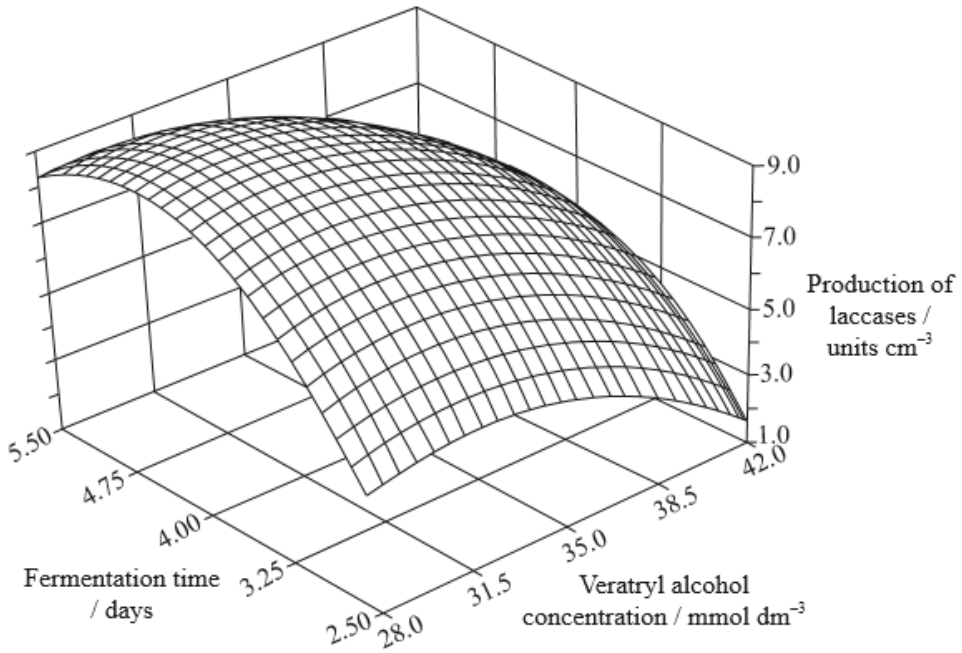
The diagram shows a biofilm that has formed on a tooth.



[Source: © International Baccalaureate Organization 2017]

Using the diagram, explain the concept of emergent properties of biofilms.

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.



Reprinted from *Process Biochemistry*, Volume 35/Issue 10. Ana Flora D. Vasconcelos, Aneli M. Barbosa and Maria Inês Rezende. "Optimization of laccase production by Botryosphaeria sp. in the presence of veratryl alcohol by the response-surface method", Pages 1131-1138, Copyright (2000), with permission from Elsevier

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. Deduce from the graph the optimal conditions for maximizing the biotechnological production of laccases. [2]

One method of inserting new genes into plants is by gene gun.

[Source: adapted from www.genomicon.com]

a. Outline how a gene gun inserts genes into plants. [2]

b. Marker genes are often inserted together with the new gene. State the function of the marker genes. [1]

c. Outline the characteristics of an open reading frame. [2]

d. Explain, using an example, how gene transfer to a plant could help increase crop yield. [3]

The photograph shows apparatus used to culture microorganisms in order to produce a metabolite.

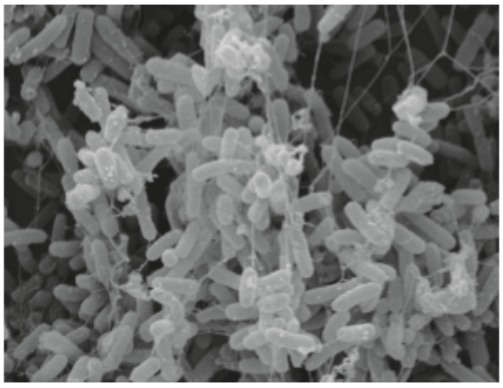
[Source: adapted from www.medicalexpo.com]

a. State the general term for the reaction, involving microorganisms, that takes place in the apparatus shown. [1]

b. Other than temperature and pH, state **one** variable that should be monitored during continuous culture in the apparatus shown. [1]

c. State the binomial name of an organism used in continuous culturing to produce citric acid used as a preservative. [1]

Cooperative aggregates of microorganisms can form biofilms. The micrograph shows a biofilm of *Escherichia coli*.

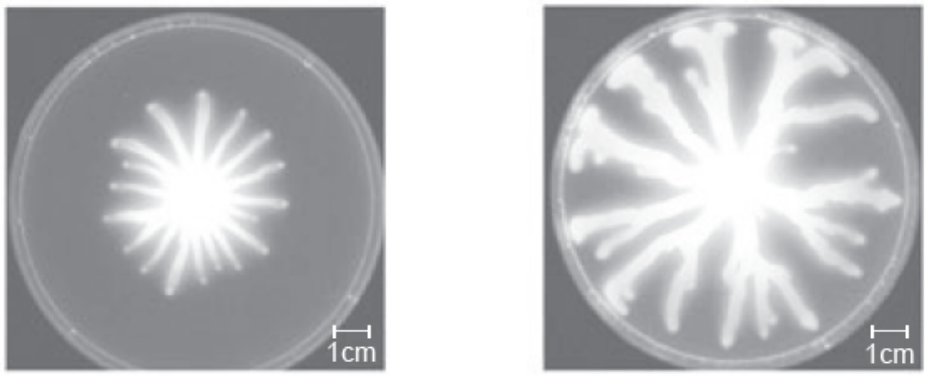


[Source: Brigit Pruess for North Dakota State University]

a. Outline the emergent properties of biofilms. [2]

b. Explain **two** ways in which bacteria of the genus *Pseudomonas* can be used for bioremediation. [4]

Korean microbiologists tested the effect of ginger root (*Zingiber officinale*) extracts on biofilm formation by the bacterium *Pseudomonas aeruginosa*. Formation of a biofilm prevents the bacteria from spreading. These bacteria were cultured on plates of agar and the results after 24 hours of growth are shown in the photographs below.



Control 1% ginger root extract

[Source: Han-Shin Kim and Hee-Deung Park (2013) Ginger Extract Inhibits Biofilm Formation by Pseudomonas aeruginosa PA14. *PLOS ONE*, September, 8(9). https://doi.org/10.1371/journal.pone.0076106.]

a. Evaluate the effect of 1 % ginger root extract on biofilm formation. [3]

b. Outline the importance of avoiding biofilm formation in pipes carrying drinking water. [2]

The picture shows workers cleaning up a polluted stretch of coastline in Alaska after oil was leaked from a tanker.

[Source: https://commons.wikimedia.org/wiki/File:OilCleanupAfterValdezSpill.jpg]
(https://commons.wikimedia.org/wiki/File:OilCleanupAfterValdezSpill.jpg])

Explain how oil pollution can be treated by bioremediation.