BIOWELSH WORKSHEET FOR WJEC GCE A LEVEL BIOLOGY
UNIT 3 – ENERGY, HOMEOSTASIS AND THE ENVIRONMENT
TOPIC 3.4 MICROBIOLOGY: WORKSHEET 1 (BW-A2-3.4-W1) MARK SCHEME

GENERAL INSTRUCTIONS

Recording of marks

Mark in red ink. One tick equals one mark, except in the extended response question, where a level of response scheme is used.

Write each question total in the box at the end of the question, and copy the totals onto the grid on the front cover of the worksheet. Add them to give the worksheet total out of 159.

Marking rules

Look at all of the work. Where the scheme says working is needed, the working must be seen. Mark crossed-out work if it has not been replaced. Give credit for correct, relevant answers that are not listed in the scheme.

Extended response question

Read the whole answer first. Decide which level descriptor fits the answer best, judging the overall quality. Then choose the mark within the level: the top mark when the answer matches both the content and the communication statements well, the middle mark when most of the content is there and the communication statement is partly met, and the bottom mark when only the content statements are matched.

Marking abbreviations

cao	=	correct answer only
ecf	=	error carried forward
bod	=	benefit of doubt
ORA	=	or reverse argument
owtte	=	or words to that effect

Notation: / separates alternatives; { } groups alternatives within a point; words in ( ) are not needed for the mark; underlined words are essential; (1) is one mark.

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
1	(a)			Spherical / cocci (1) In (grape-like) clusters / staphylococci (1) Reject round / circular	2			2		
	(b)			C rod shaped / bacilli (1) B curved rods / comma shaped / vibrios (1) Reject cylinders	1	1		2		
	(c)			780 nm (2) Accept 700 to 850 nm / 0.70 to 0.85 µm Award 1 mark for (measured diameter of P ÷ measured length of scale bar) × 500		2		2	2	
	(d)			The cells stay attached to each other after dividing (1) They divide in several / different planes (so they form clusters rather than chains) (1)		2		2		
	(e)			*B. subtilis* stains purple and *E. coli* stains pink / red (1) *B. subtilis* has a thick peptidoglycan layer that keeps the crystal violet; *E. coli* is Gram-negative with a thin layer (and outer membrane) (1)		1	1	2		
	(f)			Electron microscopes use (a beam of) electrons, not light, so images are black and white / have no colour (1)	1			1		
				Question 1 total	4	6	1	11	2	0

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
2	(a)			J outer membrane / lipopolysaccharide (layer) (1) K peptidoglycan / murein (1) Reject 'cell wall' alone for K; reject cell membrane for J	2			2		
	(b)	(i)		Lysozyme hydrolyses the peptidoglycan so the cell wall is weakened (1) Water enters by osmosis and the cells burst / lyse, so there are fewer whole cells (1)		2		2		
		(ii)		62.5 (%) (2) Award 1 mark for (0.80 − 0.30) ÷ 0.80 (× 100)		2		2	2	
		(iii)		*E. coli* has wall **W** / is Gram-negative with an outer (lipopolysaccharide) membrane (1) The outer membrane stops lysozyme reaching the (thin) peptidoglycan layer (1)	1	1		2		
		(iv)		Any two (×1) from: • EDTA damages only part of the outer membrane / not every cell is affected (1) • So lysozyme reaches the peptidoglycan of only some cells / reaches it more slowly (1) • The damaged outer membrane still partly covers the peptidoglycan / lysozyme has to pass through it first (1)			2	2		
	(c)			Each suspension in buffer with no lysozyme / with boiled (denatured) lysozyme (1) Accept *E. coli* with EDTA but no lysozyme			1	1		1
				Question 2 total	3	5	3	11	2	1

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
3	(a)			Kills the bacteria (so the slide is safe to handle) (1) Sticks the cells to the slide so they are not washed off during staining and rinsing (1)	2			2		2
	(b)			Steps 2 and 3: both purple (1) Step 4: *B. subtilis* purple; *P. fluorescens* colourless (1) Step 5: *B. subtilis* purple; *P. fluorescens* pink / red (1) Accept violet for purple	1	2		3		
	(c)			Any two (×1) from: • *P. fluorescens* has a thin peptidoglycan layer that cannot hold the crystal violet (and iodine) (1) • Alcohol dissolves lipid in its outer (lipopolysaccharide) membrane so the dye is washed out (1) • *B. subtilis* has a thick peptidoglycan layer that holds the crystal violet (1) Reject 'safranin stains Gram-positive bacteria'	2			2		
	(d)			Alcohol left too long washed the crystal violet out of the thick peptidoglycan as well / over-decolourised (1) So *B. subtilis* took up the safranin and gave a false Gram-negative result (1)			2	2		2
	(e)			So cells lie in a single layer / are separate, so the shape, arrangement and colour of individual cells can be seen / all cells are stained and decolourised evenly (1)			1	1		1
	(f)			Bacteria are very small (about 1 μm), so the highest magnification and resolution of the light microscope are needed (1)		1		1		1
				Question 3 total	5	3	3	11	0	6

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
4	(a)			1 mark for each correct row: X: Gram-positive; spherical / coccus; clusters / groups (1) Y: Gram-negative; rod / bacillus (1) *S. agalactiae*: Gram-positive; spherical / coccus; chains (1) Reject round / circular		3		3		
	(b)			Thick peptidoglycan layer holds the crystal violet (and iodine) (1) No outer (lipopolysaccharide) membrane, so the alcohol does not wash the dye out (1) Reject 'stained by safranin'	2			2		
	(c)			*S. agalactiae* is Gram-positive so its peptidoglycan is exposed / penicillin reaches it, so the wall weakens and the cell bursts (1) **Y** is Gram-negative; its outer (lipopolysaccharide) membrane stops penicillin reaching the peptidoglycan (1)		2		2		
	(d)			Treatment can start straight away; the infection could become serious / fatal while waiting (1) The Gram reaction and shape suggest which antibiotic is likely to work (for example one effective against Gram-positive cocci) (1)			2	2		
	(e)			Many different species have the same Gram reaction, shape and arrangement (1)			1	1		
				Question 4 total	2	5	3	10	0	0

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
5	(a)			A and it contains no agar (1) Both needed		1		1		
	(b)			C (1) Its exact chemical composition is known; the others contain peptone / yeast extract, whose exact composition is not known (1) Second mark dependent on C	1	1		2		
	(c)			Source of nitrogen (1) To make amino acids / proteins / nucleotides / nucleic acids / ATP (1)	1	1		2		
	(d)			Agar is not digested / hydrolysed by (most) bacteria, but many bacteria secrete proteases that digest gelatine (1) Agar stays solid at incubation temperatures / gelatine melts at about 30 to 35 °C (1)	1		1	2		
	(e)			The high concentration of sodium chloride gives the medium a very low / very negative water potential (1) Water leaves most bacteria by osmosis so they cannot grow, but *S. aureus* tolerates / survives it (1)		1	1	2		
	(f)			On a solid medium each bacterium stays in one place and grows into a separate colony (1) The colonies can be counted; in a liquid the cells spread throughout / no separate colonies form (1)		2		2		
				Question 5 total	3	6	2	11	0	0

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
6	(a)			Many bacteria were put on the agar at **R**, so the colonies grew into each other / merged (1) The loop carried fewer bacteria as the streaks continued, so at **S** single cells were far enough apart to form separate colonies (1)		2		2		
	(b)			Each colony grows from a single cell (by binary fission), so all its cells are the same species / genetically identical (1)	1			1		
	(c)			Heated in a (blue) Bunsen flame until it glows red hot (1) A hot loop would kill the bacteria being transferred (1)	1	1		2		2
	(d)			To stop the culture being contaminated by unwanted microorganisms from the air, surfaces or the student (1) To stop people and the environment being contaminated by the cultured microorganisms (which could be pathogens) (1)	2			2		2
	(e)			Not sealed: lets oxygen in so anaerobic conditions do not develop, which could allow the growth of anaerobic pathogens (1) Upside down: condensation does not drip onto the agar and spread / merge the colonies (1) 25 °C: below body temperature, so human pathogens are less likely to grow (than at 37 °C) (1)	1	2		3		3
	(f)			Sterilised in an autoclave (unopened) before disposal (1) Accept 121 °C			1	1		1
				Question 6 total	5	5	1	11	0	8

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
7	(a)			Obligate aerobe A (1) Obligate anaerobe B (1) Facultative anaerobe C (1)		3		3		
	(b)			The bacteria can respire anaerobically, so they grow at all depths (1) Aerobic respiration releases more ATP from each glucose, so they grow faster / more near the surface where there is oxygen (1)		2		2		
	(c)			They need oxygen for (aerobic) respiration / as the final electron acceptor, and oxygen is only present near the surface (1)	1			1		
	(d)			It does not use oxygen, so it grows at the same rate at all depths (1) Oxygen is not toxic to it, so it also grows near the surface / colonies are spread evenly (1)		1	1	2		
	(e)			Incubate the plates in an anaerobic jar / container where the oxygen has been removed / replaced by nitrogen or carbon dioxide (1)			1	1		1
	(f)			There is no oxygen / conditions are anaerobic inside a sealed can (and some cells / spores survived the heating) (1)			1	1		
				Question 7 total	1	6	3	10	0	1

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
8	(a)			40 °C (1) Accept 38 to 41 °C		1		1		
	(b)			Enzyme and substrate molecules have more kinetic energy (1) More (successful) collisions / more enzyme–substrate complexes form, so metabolic reactions are faster (1)	2			2		
	(c)			Hydrogen / ionic bonds in the enzymes break, so the enzymes are denatured (1) The active site changes shape so substrates no longer fit / fewer enzyme–substrate complexes form (1)	2			2		
	(d)			24 (minutes) (2) Award 1 mark for 60 ÷ 2.5 / 1 ÷ 2.5 = 0.4 hours Allow ecf from a misread growth rate		2		2	2	
	(e)			*P. fluorescens* still grows at 4 °C, although slowly (about 0.1 generations per hour) (1) Over ten days there are many generations / about 24 doublings, so the number of bacteria becomes very large (1) Accept *E. coli* cannot grow at 4 °C as part of the answer			2	2		
	(f)			It is very heat resistant, so if these spores are killed all other microorganisms will have been killed too (1) At 60 °C only *G. stearothermophilus* can grow / contaminants such as *E. coli* cannot grow, so any growth shows the autoclave failed (1)			2	2		
				Question 8 total	4	3	4	11	2	0

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
9	(a)			There are so many bacteria that the colonies would overlap / merge (1) Separate colonies are needed so that they can be counted (1)	2			2		1
	(b)	(i)		10^{-5} and at 10^{-4} the colonies merge / are too many to count accurately (1) At 10^{-6} there are fewer than 30 colonies, too few to give a reliable estimate (1) No marks unless 10^{-5} is chosen			2	2		2
		(ii)		1.24×10^8 / 1.2×10^8 (2) Award 1 mark for mean of 124 used with $\times 10^5$ (1.24×10^7) / correct answer not in standard form (124 000 000)		2		2	2	
		(iii)		$1.24 \times 10^8 \times 65 = 8.1 \times 10^9$ (per bottle) (1) This is more than 6.5×10^9, so it supports the claim (1) Allow ecf from (ii)		1	1	2	1	
	(c)			A chain of several cells grows into a single colony (1) So the number calculated is an underestimate (1)		1	1	2		
	(d)			An old pipette would carry bacteria from the more concentrated tube, so the dilutions / count would be too high / inaccurate (1)		1		1		1
				Question 9 total	2	5	4	11	3	4

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
10	(a)			P lag and Q log / exponential (1) R stationary and S death / decline (1)	2			2		
	(b)			The bacteria are adjusting to the new medium and are not yet dividing (1) They are synthesising enzymes / taking up water and nutrients (1)	2			2		
	(c)	(i)		5.0×10^7 (1) Accept 4.0×10^7 to 6.3×10^7 (from a reading of 7.6 to 7.8)		1		1	1	
		(ii)		30 (minutes) (2) Award 1 mark for n = 12 / 11.96 generations Accept 30.1		2		2	2	
	(d)			Nutrients are used up / toxic waste products build up (1) The rate of cell division equals the rate of cell death (1)		2		2		
	(e)			Turbidity gives a total count, which includes dead cells as well as living cells (1) Dead cells still make the broth cloudy, so the readings stay high / level (1)			2	2		
				Question 10 total	4	5	2	11	3	0

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
11	(a)			The total count includes dead cells as well as living cells (1) Only living cells form colonies in a viable count, and many cells had died by 16 hours (1)	2			2		
	(b)			20 (%) (2) Award 1 mark for 2.5 ÷ 12.5 (× 100)		2		2	2	
	(c)			Any two (×1) from: • Lactic acid builds up so the pH falls (1) • Enzymes are denatured (by the low pH) so cells die (1) • Lactose / nutrients are used up (1)			2	2		
	(d)			The result is available at once, rather than after the plates have been incubated for a day or more (1)		1		1		1
	(e)			At 12 hours (1) The viable count is highest / 10.1×10^8 cells cm^{-3}, before many cells die (1)			2	2		
	(f)			Other microorganisms would compete with *L. lactis* / produce unwanted products that spoil the cheese (1) Contaminants could include pathogens that multiply in the milk and cause food poisoning (1)		2		2		
				Question 11 total	2	5	4	11	2	1

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
12	(a)			So the only difference is the storage time / both start with the same bacteria (same source, same pasteurisation) (1)			1	1		1
	(b)			To kill microorganisms on the spreader so no contaminants are added (1) A hot spreader would kill bacteria from the milk, giving too few colonies / an underestimate (1)	1	1		2		2
	(c)			A plate spread with 0.1 cm^3 of the sterile distilled water used for the dilutions (1) It should grow no colonies, showing that the water and technique added no bacteria / all colonies came from the milk (1)			2	2		2
	(d)	(i)		12 (stale milk, plate 3) (1) Spreader not cooled so bacteria were killed / less than 0.1 cm^3 transferred / sample not mixed before it was taken (1)			2	2		2
		(ii)		2430 (2) Accept 2400 to 2440 Award 1 mark for fresh 3.77×10^4 and stale 9.15×10^7 per cm^3 / mean colony counts 37.7 and 91.5		2		2	2	2
	(e)			Keep the lids taped on and do not open the plates, as pathogens may have grown from the milk / autoclave the plates before disposal / wash hands (1)			1	1		1
				Question 12 total	1	3	6	10	2	10

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
13	(a)	(i)		Day 2: 7.1×10^6 (1) $\log_{10}$ values: 6.85 (day 2) and 8.65 (day 4) (1) Allow ecf for day 2 log from an incorrect number		2		2	2	
		(ii)		The numbers cover a very large range (from 10^4 to 10^9), so log values let them all fit on one scale (1)		1		1	1	
	(b)			Axes the correct way round, labelled with quantity and unit: x = age of milk / days; y = $\log_{10}$ number of bacteria per cm^3 (1) Suitable linear scales using more than half of the grid; y axis need not start at 0 (1) All points plotted correctly ± half a small square (1) Points joined with ruled straight lines (1) Allow ecf from (a)(i)		2	2	4	4	4
	(c)			The increase slows down / levels off (log value from 8.65 to 8.99) (1) Stationary phase: nutrients / lactose are used up or lactic acid builds up (1)		1	1	2		
	(d)			Pasteurisation kills most (pathogenic) bacteria but does not sterilise the milk / some heat-resistant bacteria survive (1)	1			1		
	(e)			The colonies merged / overlapped, so separate colonies could not be counted accurately (it would give an underestimate) (1)	1			1		1
				Question 13 total	2	6	3	11	7	5

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
14	(a)			The pores (0.45 μm) are smaller than the bacteria (about 1 to 2 μm) (1)	1			1		
	(b)			0.1 cm^3 would contain very few / no bacteria, so too few colonies for a reliable count (1) Filtration collects the bacteria from a large volume onto one membrane, giving enough colonies to count (1)			2	2		1
	(c)	(i)		L 355 (1) M 192 (1)		2		2	2	
		(ii)		K and M (1) Allow ecf from (i)		1		1	1	
	(d)			Gut bacteria such as *E. coli* (from warm-blooded animals) can grow at 44 °C (1) Most bacteria that live naturally in seawater cannot grow at this temperature / their enzymes are denatured (1)		1	1	2		
	(e)			A colony may grow from a clump / chain of several cells, not always from one cell (1)			1	1		
	(f)			Bacteria left from one sample would be counted in the next, giving too high a count (1)	1			1		1
				Question 14 total	2	4	4	10	3	2

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
15				**Indicative content** Credit correct use of log values converted to numbers.	3	3	3	9		
				A Gram stain A1 Thin smear on a slide, air dried and heat fixed A2 Crystal violet, then iodine, then alcohol (decolouriser), then safranin (counter-stain), rinsing between stages A3 Examined with an oil immersion lens at × 1000 A4 Gram-positive cells stay purple; rod-shaped cells are bacilli A5 Thick peptidoglycan layer holds the crystal violet (and iodine) when alcohol is added A6 No outer lipopolysaccharide membrane, so the dye is not washed out (and safranin does not show)						
				B Viable count with aseptic technique B1 Known mass of cheese (for example 1 g) mixed with 9 cm^3 of sterile diluent to give a 10^{-1} dilution B2 Serial dilution: 1 cm^3 into 9 cm^3 of sterile water / saline repeatedly, mixing each time B3 0.1 cm^3 of each dilution spread on agar plates with a sterile spreader B4 Incubated for a set time and temperature; colonies counted on plates with separate colonies (30 to 300) B5 Number per gram = mean colonies × 10 × dilution factor; assumes each colony grew from one cell B6 Aseptic technique: sterile pipettes and plates, flame necks of bottles, lift lid only slightly, work near a Bunsen flame, disinfect the bench B7 Plates taped and not opened after incubation (pathogen), autoclaved before disposal						

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
				C Using Graph 15.1 C1 Both start at log 1 / 10 bacteria per gram C2 At 20 °C the number passes the legal limit (log 2) in about 1.5 days and reaches about 4×10^7 per gram (log 7.6) / stationary phase C3 At 4 °C there is a lag and then slow growth; the limit is reached only after about 8.5 days C4 So cheese eaten within 7 days at 4 °C stays below 100 bacteria per gram C5 Low temperature slows enzyme activity / metabolism, so cells divide less often / longer generation time C6 Refrigeration slows but does not stop growth of *Listeria*, so a use-by date is still needed						
				7-9 marks Indicative content of this level is detailed information from all three lists. The candidate constructs an articulate, integrated account, correctly linking relevant points in a logical sequence. The answer fully addresses the question with no irrelevant inclusions or significant omissions. Scientific conventions and vocabulary are used appropriately and accurately.						
				4-6 marks Indicative content of this level is detailed information from two of the lists, or less detail from all three. The candidate constructs an account correctly linking some relevant points, showing some reasoning. The answer addresses the question with some omissions. Scientific conventions and vocabulary are usually used appropriately and accurately.						
				1-3 marks Indicative content of this level is some information from at least one of the lists, or a little information from more than one. The candidate makes some relevant points, showing limited reasoning. The answer addresses the question with significant omissions. Use of scientific conventions and vocabulary is limited.						

Question				Marking details	Marks available					
					AO1	AO2	AO3	Total	Maths	Prac
				0 marks The candidate does not make any attempt or give a relevant answer worthy of credit.						
				Question 15 total	3	3	3	9	0	0

UNIT 3: ENERGY, HOMEOSTASIS AND THE ENVIRONMENT, TOPIC 3.4

SUMMARY OF MARKS ALLOCATED TO ASSESSMENT OBJECTIVES

Question	AO1	AO2	AO3	TOTAL MARK	MATHS	PRAC
1	4	6	1	11	2	0
2	3	5	3	11	2	1
3	5	3	3	11	0	6
4	2	5	3	10	0	0
5	3	6	2	11	0	0
6	5	5	1	11	0	8
7	1	6	3	10	0	1
8	4	3	4	11	2	0
9	2	5	4	11	3	4
10	4	5	2	11	3	0
11	2	5	4	11	2	1
12	1	3	6	10	2	10
13	2	6	3	11	7	5
14	2	4	4	10	3	2
15	3	3	3	9	0	0
TOTAL	**43**	**70**	**46**	**159**	**26**	**38**

A Tutor Meets Tutee project

BioWelsh mark scheme BW-A2-3.4-W1 (MS). Not an official WJEC mark scheme.