RECOGNISING ACHIEVEMENT

2805/04 Microbiology and Biotechnology

June 2003

Mark Scheme

ADVICE TO EXAMINERS ON THE ANNOTATION OF SCRIPTS

1. Please ensure that you use the **final** version of the Mark Scheme.
   You are advised to destroy all draft versions.

2. Please mark all post-standardisation scripts in red ink. A tick (✓) should be used for each answer judged worthy of a mark. Ticks should be placed as close as possible to the point in the answer where the mark has been awarded. The number of ticks should be the same as the number of marks awarded. If two (or more) responses are required for one mark, use only one tick. Half marks (½) should never be used.

3. The following annotations may be used when marking. No comments should be written on scripts unless they relate directly to the mark scheme. Remember that scripts may be returned to Centres.

   x = incorrect response (errors may also be underlined)
   ^ = omission mark
   bod = benefit of the doubt (where professional judgement has been used)
   ecf = error carried forward (in consequential marking)
   con = contradiction (in cases where candidates contradict themselves in the same response)
   sf = error in the number of significant figures

4. The marks awarded for each part question should be indicated in the margin provided on the right hand side of the page. The mark total for each question should be ringed at the end of the question, on the right hand side. These totals should be added up to give the final total on the front of the paper.

5. In cases where candidates are required to give a specific number of answers, (e.g. 'give three reasons'), mark the first answer(s) given up to the total number required. Strike through the remainder. In specific cases where this rule cannot be applied, the exact procedure to be used is given in the mark scheme.

6. Correct answers to calculations should gain full credit even if no working is shown, unless otherwise indicated in the mark scheme. (An instruction on the paper to 'Show your working' is to help candidates, who may then gain partial credit even if their final answer is not correct.)

7. Strike through all blank spaces and/or pages in order to give a clear indication that the whole of the script has been considered.

8. An element of professional judgement is required in the marking of any written paper, and candidates may not use the exact words that appear in the mark scheme. If the science is correct and answers the question, then the mark(s) should normally be credited. If you are in doubt about the validity of any answer, contact your Team Leader/Principal Examiner for guidance.

Abbreviations, annotations and conventions used in the Mark Scheme	/	=	alternative and acceptable answers for the same marking point
	;	=	separates marking points
	NOT	=	answers which are not worthy of credit
	R	=	reject
	( )	=	words which are not essential to gain credit
	___	=	(underlining) key words which **must** be used to gain credit
	ecf	=	error carried forward
	AW	=	alternative wording
	A	=	accept
	ora	=	or reverse argument

Question **Expected Answers** **Marks**

1 **(a)** glucose in blood small enough to diffuse through (selective) membrane;
shape of glucose fits recognition, molecule / layer;
(glucose combines with) immobilised enzyme / AW;
glucose oxidase;
glucose, reacts with oxygen / is oxidised;
product formed / e.g. hydrogen peroxide / gluconic acid / gluconate;
less oxygen present;
mention of platinum oxygen electrode;
transducer creates electrical, current / signal;
(current) related to, concentration of product / more glucose;
calibration using known concentration of glucose; **max 5**

(b) (other molecules) do not pass through membrane / membrane selectively permeable;
(enzyme / glucose oxidase) specific to glucose; **2**

(c) quantitative;
sensitive (to low concentration);
specific to glucose / not affected by other reducing sugars;
'immediate' result / portable / read values continuously / reusable / cheaper;

treat ref to accuracy / easy to set up as neutral **max 2**

[Total: 9]

Question	Expected Answers	Marks
2 (a)	alginate beads / **A** any other correct method;	1
(b)	enzyme is protein; biuret test / sodium hydroxide and dilute copper sulphate; lilac / purple colour, if contaminated; *or* add starch (and incubate); iodine test / add iodine solution; if it remains brown then enzyme present / if it goes blue black enzyme is not present / AW;	3
(c)	Benedict's test / reducing sugar test / glucose electrode; **A** Clinistix compare intensity of colour / use colorimeter / record data / plot graph; until no significant change / when graph levels off;	max 2
(d)	enzyme not lost / can be retrieved or recovered; therefore reusable; cost effective, qualified; product not contaminated; therefore not costly to purify / no need to purify; matrix protects the enzyme; therefore it is more stable; *credit one of the second points below to explain importance of its stability* enzyme stable at <u>higher</u> temperatures; therefore reaction temperature can be higher / higher yield; enzyme stable at extremes of pH; e.g. use in gut / washing powder; larger surface area (of enzymes); more rapid reaction / more accessible to substrate; *advantage and explanation – mark as pairs*	max 4
		[Total: 10]

Question	Expected Answers	Marks
3 (a)	difficult to maintain temperature / needs cooling / water jacket; **R** refs to the 'time' it takes to heat up / cool down maintaining sterility / prevent entry of contaminants; hard to clean; mixing / stirring, needed; oxygen needs to be distributed; ref to, quality control / flavour; downstream processing on large scale; AVP; e.g. justified use of equipment - high pressure / venting gas	**max 3**

(b) *max 4 for differences*
not necessary to state both sides of the argument for credit if the opposite viewpoint is implied

batch

1 'closed fermenter' / AW;
2 (microorganism and) nutrients added initially;
3 reaction continues for a defined time / continues until reaction stops;
4 product separated from the rest of mixture;

continuous

1 'open fermenter' / AW;
2 nutrients added at steady rate;
3 reaction continues, indefinitely / all of the time;
4 products removed continuously;

max 4 for advantages / disadvantages
ora for all points

advantage of batch

5 easy to control environmental factors / example / simple equipment;
6 vessels usable for different processes;
7 complete conversion to products possible;
8 used to produce, secondary metabolites / e.g. penicillin;
9 if contaminated only one batch lost;

disadvantage of batch

10 vessel needs to be sterilised at the end of each batch;
11 smaller amounts produced; **A** yield is smaller
12 product not always available;

advantage of continuous

13 microorganisms maintained in exponential growth;
14 higher productivity;
15 smaller vessels used;

disadvantage of continuous

16 product contaminated with, unused raw materials / cells;
17 blocking of inlets due to, foaming / clumping; **A** cost of antifoaming agent
18 flavour / alcohol content, reduced; **max 7**

QWC – legible text with accurate spelling, punctuation and grammar; 1

(c) **(i)** tolerance to higher alcohol, content / concentration;
tolerance to higher temperature;
more rapid fermentation / faster fermentation, at lower temperatures;
produces a higher concentration of enzymes / more active enzymes;
smaller cell size / increase surface area;
ability to metabolise, cheaper substrates / waste products; **max 2**

(ii) identify suitable (phenotypic) characteristic;
identify gene;
isolate gene / cut out gene;
restriction enzyme and function;
ligase enzyme and function;
use of, vector / plasmid / virus;
identify cells containing gene;
culture cells;
AVP; e.g. any further detail **max 5**

[Total: 18]

Question			Expected Answers	Marks
4	(a)	(i)	cell number not increasing / cells not dividing / cells not reproducing; **R** cell replication lag phase; cells making, enzymes / organelles; **A** DNA replication **R** 'getting used to the environment'	**2**
		(ii)	includes, dead cells / dust / other microorganisms / cell fragments;	**1**
		(iii)	curve flattens off / curve falls toward x axis;	**1**
	(b)		known volume of culture; dilution series; detail / 1 cm^3 culture and 9 cm^3 distilled water, repeated; **R** refs to 10^{-1}, 10^{-2} etc with out detail on preparation of these dilutions plated / described; defined volume of dilution added to plate; use aseptic technique / described; incubated; count colonies; **R** cells select plate with, sensible number of colonies to count / 30-300 colonies; each colony represents a single individual; multiply by dilution factor; calculate number in a known unit volume; replicates;	**max 6**
	(c)	(i)	*primary* chemicals produced as part of normal growth / result of respiration; **R** *'not produced when plenty of nutrients'* *secondary* chemicals produced when, short of nutrients / under stress / not growing;	**2**
		(ii)	antibiotic / named antibiotic e.g. penicillin;	**1**
	(d)	(i)	**X** marked on flat section of graph;	**1**
		(ii)	limit / reduce / remove / do not replace, nutrients; provide lactose (instead of glucose); **A** 'do not 'feed' the culture'	**max 1**
				[Total: 15]

Question	Expected Answers	Marks
5 (a) (i)	**A** – nucleus and **B** – rough ER;	1
(ii)	mitochondria; tonoplast / membrane around vacuole; smooth endoplasmic reticulum; vesicles / lysosomes; Golgi; **A** – rough ER if not given above	max 2
(iii)	measurement from drawing with units; **A** within range 41 – 42 mm divided by 3420; *ecf* 42 / 3420 = 0.01228 **A *figure given for measurement*** converted to μm / 12 μm ; **R** if incorrect conversion of decimal point correct answer (12 μm) = max 3 *if initial measurement inaccurate allow max 2 for correct method*	3

(b)

	*microorganism **S***	*microorganism **T***
type of microorganism	bacterium / prokaryote;	virus / phage;
name of structure…	plasmid;	capsid / protein core; **R** capsomeres
another feature…	capsule / mesosome / circular DNA / pili;	genetic material / nucleic acid / DNA / RNA;

6

(c) **1** some stain red / pink, some stain purple / colour ref (if not qualified elsewhere)
2 stain with crystal violet;
3 and, iodine solution / grams iodine / Lugol iodine;
4 clear / rinse with, alcohol / ethanol / acetone alcohol;
5 flood with, safranin / carbol fuchsin;

6 Gram positive cell wall, mostly polysaccharide / peptidoglycan / murein;
7 this retains crystal violet / AW (when rinsed with ethanol);
8 Gram positive stain purple;

9 Gram negative cell wall, more lipid / phospholipid / lipoprotein / lipopolysaccharide;
10 which dissolves in ethanol;
11 crystal violet washes off;
12 Gram negative stain red / pink;

13 AVP; technique e.g. fix by flaming **max 7**

QWC – clear well organised using specialist terms; **1**

[Total: 20]

Question			Expected Answers	Marks
6	(a)	(i)	sample from, broth / agar, with, loop / spreader / AW; streak on agar / use selective medium; incubate; (*where it occurs in the answer*) sample from one colony; aseptic technique / e.g. ; use heat / flame / alcohol / handle in transfer chambers / airflow hoods / negative pressure / use sterile equipment / AW	**max 3**
		(ii)	incubate at temperatures below 30 ^{o}C; **A** - not at 37 ^{o}C / not at body temperature tape to keep lid in place / seal Petri dish;	**2**
		(iii)	never observe live cultures without lid in place / do not open dishes / kill bacteria using alcohol before observing them;	**1**
		(iv)	put unopened culture dishes in heat resistant plastic / autoclave / biohazard bags; autoclave / irradiate / microwave; detail / e.g. 120 ^{o}C / 100+ kPa for stated time (20 - 30 mins); disinfect / use bleach (virkon / hypochlorite), on other equipment; incinerate;	**max 2**
	(b)		containment of microorganisms / easily released into environment; antibiotic resistance gene(s) are used as markers; (pathogenic) microorganisms could develop resistance to antibiotics; development of new pathogens / e.g. MRSA; **R** 'superbug' unqualified competition between transgenic organisms and existing species; AVP;	**max 3**
	(c)		transmission of genes in pollen; genetically modified crop becomes a 'weed'; closely related wild plants become resistant to herbicide / superweed idea; pesticide production in competitors; emergence of resistant insects; reduces populations of competitors (plants); impact on organic farming;	**max 3**

(d) improved resistance to, pests / disease;
resistance to herbicides;
crops more resistant to extremes of temperature;
crops more resistant to drought;
crops salt tolerant;
faster maturation; **A** faster growth rate
addition of nitrogen fixing, allele / gene / enzyme;
AVP; **max 4**

[Total: 18]