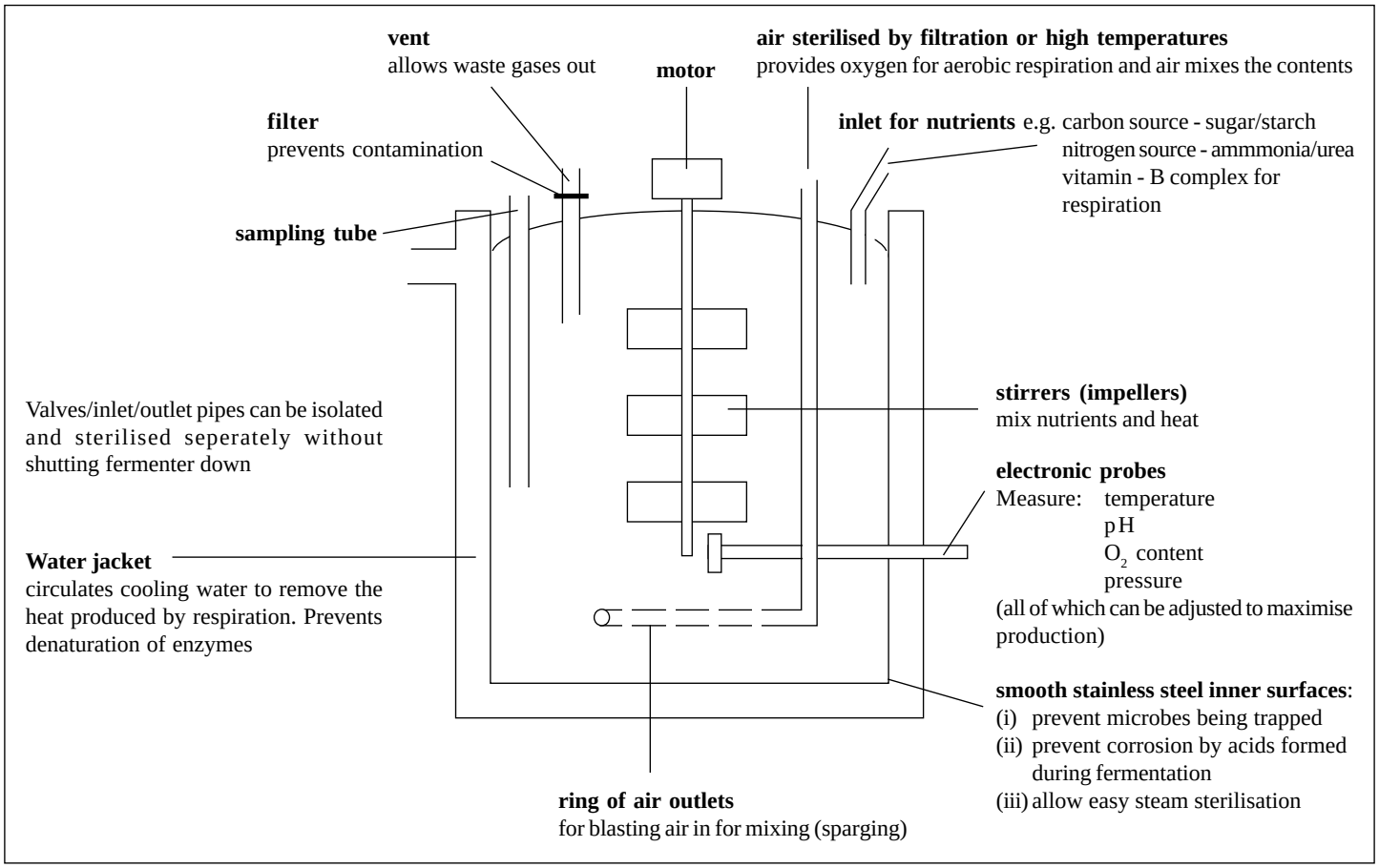
Bio Factsheet

Number 33

Fermentation made simple

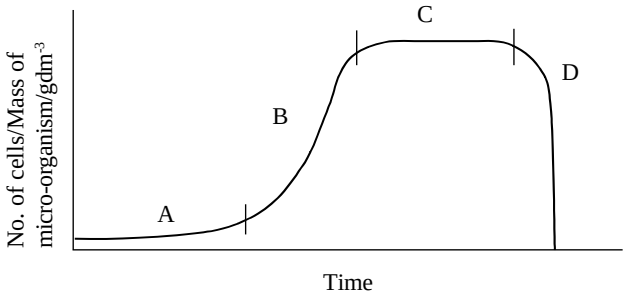
Fermentation involves the culture of cells in aerobic and anaerobic conditions. Industrial fermentation usually uses the large-scale culture of micro-organisms to produce useful products. In simple terms, fermenters are big tanks in which conditions can be carefully controlled. The key features of an industrial aerobic fermenter are shown in Fig 1.

Fig 1. Industrial aerobic fermenter



The aim of the process is to put the micro-organism - usually bacteria or fungi - into a sterile, carefully controlled environment (a steel tank) and supply it with the exact nutrients and conditions it needs to grow optimally. Usually, but not always this involves aerobic conditions. Under optimal conditions the growth of a micro-organism such as bacteria or yeast is shown in Fig 2.

Fig 2. Growth curve of a micro-organism



A **(lag phase)** Growth is slow, organisms are synthesising enzymes.

B **(log or exponential phase)** Growth is faster than at any other time. No limiting factors.

C **(deceleration phase)** Nutrients and/or oxygen may be running out/ becoming a limiting factor. Toxic waste products may also be accumulating, slowing growth rates.

D **(decline phase)** Death rate is greater than birth rate, severely limiting the population growth or cell growth.

Remember, the usual reason that we grow these millions of bacterial or fungal cells is to get a useful product. That product may be a substance which is excreted by the micro-organism into the liquid surrounding it or it may be trapped inside their cells. Equally importantly, the desired product may be either a primary metabolite or a secondary metabolite.

Primary metabolite
Released as a result of metabolic processes which are essential for the life of the micro-organism e.g. ethanol from Saccharomyces cerevisiae. Thus, primary metabolites are produced throughout the growth of the micro-organism, especially through the exponential phase.

Secondary metabolite
A substance which is not essential for the life of the micro-organisms and which is not produced as a result of the growth process e.g. penicillin. Secondary metabolites are produced **after** the exponential growth phase has stopped. This is important because it means that secondary metabolites such as penicillin cannot be produced in continuous fermenters – which deliberately maintain the micro-organism in the exponential growth stage.

These differences allow us to identify primary or secondary metabolites from graphs. Fig 3 shows typical growth and product curves for a micro-organism and its primary metabolites.

Fig 3. Growth and product curves for a micro-organism and its primary metabolite

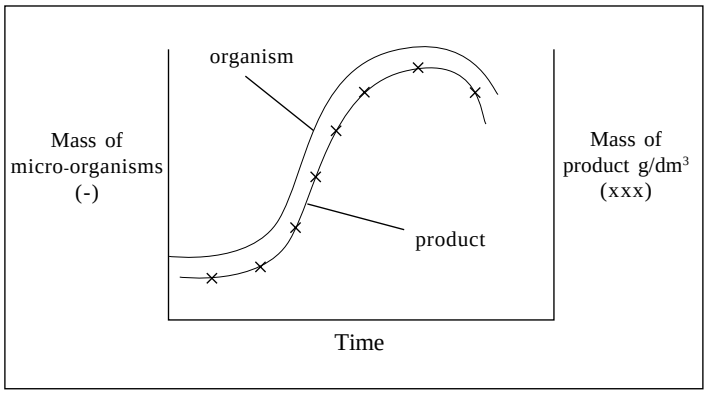
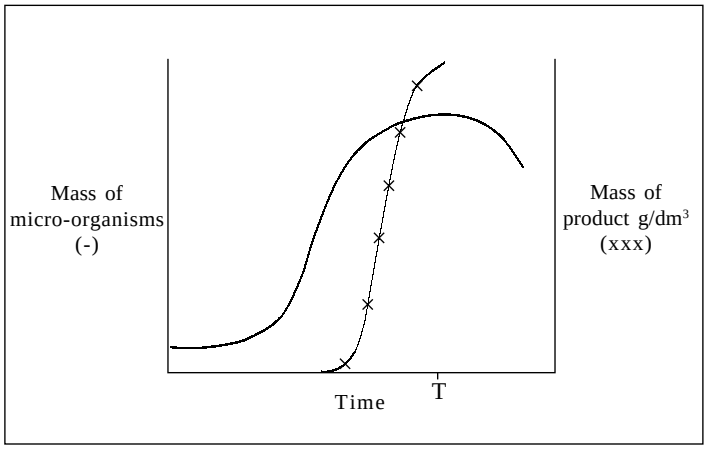


Fig 4 shows the typical growth and product curves for a micro-organism and its secondary metabolites.

Fig 4. Growth and product curves for a micro-organism and its secondary metabolite



Note: Production of secondary metabolite starts when exponential growth stops and growth of cells starts to slow. Adding a lot of extra nutrients at time T (see graph) will simply increase the growth of the micro-organism but not formation of the product. However, by adding a small amount of extra nutrients at this time, the amount of product formed can be increased.

***Exam Hint** - Identifying whether a product is a primary or secondary metabolite is a favourite exam question.*

***Case Study:* The Production of Penicillin and Mycroprotein**

	Penicillin	Mycroprotein
Organism:	Penicillium chrysogenum/ P. notatum	Fusarium
Product:	Penicillin	Mycroprotein
Type of Product:	Antibiotic	Meat alternative
Primary / Secondary Metabolite:	Secondary metabolite – no role in cellular processes of Penicillin but may inhibit competition from other micro-organisms occupying same niche	Primary metabolite - Protein synthesis is essential for all life
Nutrient source:	Corn steep liquor – C, Yeast extract – N, Lactose – C	Starch, molasses or glucose syrup – C, Ammonia – N, B vitamins, metal sulphates e.g. copper sulphate
Fermenter conditions:	Batch or Batch Fed, aerobic, 27°C, pH 6.5. Penicillin is secreted into the surrounding liquid by the fungus	Continuous culture, aerobic
Downstream processing:	The mixture is filtered. Penicillin extracted using butylacetate solvent. Addition of pottasium causes Penicillin to precipitate. Precipitate is washed, filtered and dried	Cells are separated, filtered, washed and then killed by steam treatment. Cells filtered to remove RNA – consequence of high rate of protein synthesis

Advantages of using micro-organisms

- very rapid growth rates
- utilise waste products as substrates e.g. agricultural wastes
- can be grown continuously and on a large scale so there are less shut-downs and less re-sterilisations. This has economic advantages
- high protein content
- high yields from small factories
- can be genetically manipulated
- usually produce less toxic or non-toxic waste products
- because living organisms are used, temperatures used are lower than in chemical production which is therefore cheaper

Carbon Conversion Efficiency (CCE)

$$CCE = \frac{\text{Mass of carbon incorporated in biomass}}{\text{Mass of carbon supplied}} \times 100$$

May be low because:
1. some C will be lost as CO_2 via respiration
2. some C may be excreted in carbon containing waste products

Batch fermentation v Continuous fermentation

There are three types of fermentation processes:

1. **Batch -** The micro-organism and substance are cultures, fermentation occurs, and the product which forms is removed. The fermenter is then emptied, cleaned and the process begins again.
2. **Batch fed -** Where the substance may be cooled during the fermentation process.
3. **Continuous -** Substances are added and products removed continuously, without shutting the fermenter down.

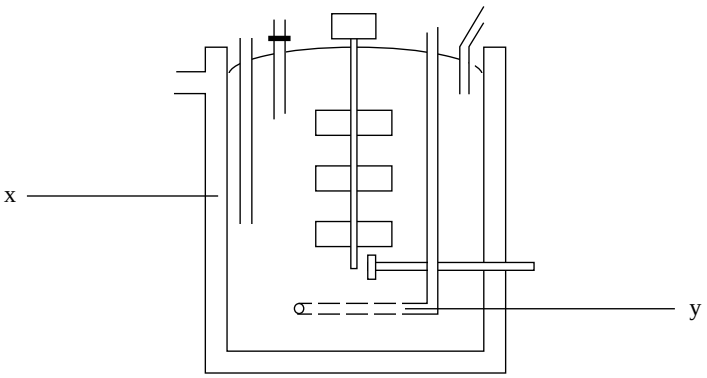
This is a very popular exam question! (Table 1).

Table 1. Batch and Continuous fermentation compared

Batch culture	Continuous culture
Nutrients added only at start • Product removed when fermentation stops • Growth rates and product formation are slower because limiting factors e.g. substrate levels/build up of toxins • slower growth rates = larger vessels are used • Easy to set up and maintain • If contamination occurs only one batch is wasted • Less efficient/more time wasted shutting down, removing product and starting up again • Product quality can vary between batches	Nutrients added continuously • Product continuously removed - organisms held in exponential growth phase giving higher productivity so can be on a smaller scale • Can be very difficult to maintain conditions so that exponential phase is maintained. Foaming, clumping and blocked inlets pose problems • Contamination can affect a huge volume of product/organism • Continuous, therefore more efficient use of time • Product quality more consistent

Practice Questions

1. The diagram shows an industrial fermenter



(a) Explain the precise function of structures x and y. (2 marks)

(b) Suggest how

(i) the fermenter is sterilised between fermentations. (1 mark)

(ii) correct pressure is maintained. (1 mark)

(c) Suggest explanations for the following observations.

(i) In mycoprotein production the slurry which is removed from the fermenter is rich in RNA deposits (2 marks)

(ii) In certain fermentation processes urea $CO(NH_2)_2$ is added as a substrate. (1 mark)

(d) State two advantages of

(i) using batch fermentation over continuous fermentation (2 marks)

(ii) using micro-organisms in industrial processes (2 marks)

Answers

Semicolons indicate marking points

1. (a) x Remove heat/cool fermenter;
prevent enzyme denaturation;

y Aeration/mixing;
increases aerobic respiration;

(b) (i) Steam/disinfectant;
(ii) Vents release gases;

(c) (i) RNA is component of ribosomes;
site of protein production;
(ii) Source of nitrogen;

(d) (i) easier to set up/maintain;
contamination affects only one batch;
(ii) high yields from small factories;
can be genetically manipulated;
very rapid growth rates;
utilise waste products as substrates;
usually produce less toxic or non-toxic waste products;
living organisms are used, therefore temperatures used are lower than in chemical production which is cheaper;
can be grown continuously and on a large scale so there are less shut-downs and less re-sterilisations = economic advantages;

Acknowledgements;
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