GROWTH KINETICS IN CONTINUOUS CULTURE

Exponential growth in batch culture may be prolonged by the addition of fresh medium, provided that the medium is designed to be substrate-limiting. If the vessel is designed with an overflow mechanism, such that the added medium displaced an equal volume of spent medium, then continuous culture of cells can be achieved. A steady state will be achieved if the medium is fed continuously at a suitable rate, i.e., formation of new biomass by the culture is balanced by the loss of biomass from the vessel. The flow of medium is related to the volume of the vessel by the dilution rate (D) as follows:

$$D = F/V$$

Where F is the flow rate (l/h) and V is the volume (l).

The net change in cell concentration over time may be expressed as:

$$dx/dt = \text{growth} - \text{output or } dx/dt = \mu x - Dx$$

Under steady state conditions, the cell concentration remains constant, therefore

$$dx/dt = 0 \text{ and } \mu x = dx \text{ and } \mu = D$$

A continuous culture may be operated at dilution rates below the maximum specific growth rate and so within certain limits, the dilution rate may be used to control the growth rate of the culture. Cell growth in such a continuous culture is controlled by the availability of the growth limiting substrate and the system is referred to as a chemostat. The mechanism underlying the controlling effect of the dilution rate is expressed in the Monod equation:

$$\mu = \mu m s/(Ks + s)$$

AT steady state,

$$\mu = D$$

Therefore, $D = \mu m\, \cancel{S}/(Ks + \cancel{S})$

where ~~S~~ is the steady state concentration of substrate in the chemostat.

Rearranging the equation:

$$\bar{s} = K_sD/(\mu m - D)$$

which predicts that the substrate concentration is determined by the dilution rate. This occurs by the growth of the cells depleting the substrate to a concentration that supports the growth rate equal to the dilution rate. If the substrate becomes depleted below the level that supports the growth rate dictated by the dilution rate, the following would occur:

(i) The growth rate of the cells will be less than the dilution rate and they will be washed out of the vessel at a rate greater than they are being produced resulting in a decrease in biomass concentration.

(ii) The substrate concentration in the vessel will rise because fewer cells are left in the vessel to consume it.

(iii) The increased substrate concentration in the vessel will result in the cells growing at a rate greater than the dilution rate and biomass concentration will increase.

(iv) The steady state will be re-established.

Therefore a chemostat is a nutrient-limited, self-balancing culture system which may be maintained in a steady state over a wide range of sub-maximum specific rates. The cell concentration in a chemostat is defined by:

$$\bar{x} = Y(S_R - \bar{s})$$

Where $\bar{x}$ is the steady state cell concentration. By combining equation of steady state substrate and biomass concentrations:

$$\bar{x} = Y[S_R - \{K_sD/(\mu_m - D)\}]$$

Therefore biomass concentration at steady state is defined by operational variables SR and D. If SR is increased, $\bar{x}$ increases but $\bar{s}$ remains the same. If D is increased, μ will increase (μ = D), $\bar{s}$ at the new steady state would have increased to support the elevated growth rate and less substrate will be available to be converted to biomass resulting in a lower $\bar{x}$

An alternative type of continuous culture to a chemostat is a turbidostat. Here the concentration of the cells in the vessel is kept constant by controlling the flow of medium such that the turbidity of the culture is kept within certain narrow limits. To achieve this, biomass is monitored using a photoelectric cell, signals are sent to a pump controlling medium flow into the vessel. If the biomass exceeds a set point, the pump is switched on and if the biomass falls below the set point it is switched off. Other biomass measurement systems include CO2 concentration or pH - biostat. However, the chemostat is the more commonly used system as it has the advantage over the biostat of not requiring complex control systems to maintain steady state.

Bioreactors (Fermenters): Function, Designs and types

Bioreactors (Fermenters): Function, Designs and types!

A bioreactor is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Bioreactors arc extensively used for food processing, fermentation, waste treatment, etc.

On the basis of the agent used, bioreactors are grouped into the following two broad classes: (i) those based on living cells and, (ii) those employing enzymes. But in terms of process requirements, they are of the following types: (i) aerobic, (ii) anaerobic, (iii) solid state, and (iv) immobilized cell bioreactors.

All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors. But, in general, theoretical explanation usually lags behind technical realization.

A bioreactor should provide for the following: (i) agitation (for mixing of cells and medium), (ii) aeration (aerobic fermenters; for O_2 supply), (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (iv) sterilization and maintenance of sterility, and (v) withdrawal of cells/medium (for continuous fermenters). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

The first truly large-scale aseptic anaerobic fermentation vessels were developed in the wake of the process developed (during the First World War, 1914-1918) by Weizmann and co-workers of U.K. to produce acetone by a deep liquid fermentation using Clostridium acetobutylicum.

For this, large cylindrical vessels of mild steel that permitted sterilization with steam under pressure were constructed, and piping, joints and valves were specially designed to maintain aseptic conditions, which were the major problem; mixing was achieved by the large volumes of gas produced during fermentation.

The large-scale aerobic fermentation vessels were first used in Central Europe during 1930s for the production of compressed yeast; these fermenters had large cylindrical tanks in which air was introduced at the base via a network of perforated pipes.

In later modifications, mechanical impellers were used to improve mixing of broth and dispersal of air bubbles. Fermenter design was considerably improved during 1940s to accommodate the requirements of strict aseptic conditions, and good agitation and aeration for penicillin production from submerged cultures; for this, steel fermenters with working volumes of 54,000 1 were built in U.S.A. In 1944, Cooper and co-workers (and several others) reported the findings from studies on agitation in baffled stirred tank fermenters, which had a major influence on the design of later fermenters.

Basic Functions of a Fermenter:

1. It should provide a controlled environment for optimum biomass/product yields.

2. It should permit aseptic fermentation for a number of days reliably and dependably, and meet the requirements of containment regulations. Containment involves prevention of escape of viable cells from a fermenter or downstream processing equipment into the environment.

3. It should provide adequate mixing and aeration for optimum growth and production, without damaging the microorganisms/cells. The above two points (items 2 and 3) are perhaps the most important of all.

4. The power consumption should be minimum.

5. It should provide easy and dependable temperature control.

6. Facility for sampling should be provided.

7. It should have a system for monitoring and regulating pH of the fermentation broth.

8. Evaporation losses should be as low as possible.

9. It should require a minimum of labour in maintenance, cleaning, operating and harvesting operations.

10. It should be suitable for a range of fermentation processes. But this range may often be restricted by the containment regulations.

11. It should have smooth internal surfaces, and joints should be welded wherever possible.

12. The pilot scale and production stage fermenters should have similar geometry to facilitate scale-up.

13. It should be contrasted using the cheapest materials that afford satisfactory results.

14. There should be adequate service provisions for individual plants.

Fermenter Design:

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On the basis of the agent used, bioreactors are grouped into the following two broad classes: (i) those based on living cells and, (ii) those employing enzymes. But in terms of process requirements, they are of the following types: (i) aerobic, (ii) anaerobic, (iii) solid state, and (iv) immobilized cell bioreactors.

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But, in general, theoretical explanation usually lags behind technical realization. A bioreactor should provide for the following: (i) agitation (for mixing of cells and medium), (ii) aeration (aerobic fermenters; for O_2 supply), (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (iv) sterilization and maintenance of sterility, and (v) withdrawal of cells/medium (for continuous fermenters). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

Agitation and Aeration:

In scaling up, both chemical (O_2, pH, medium constituents and removal of wastes) and physical (the configuration of bioreactor and power supplied to the reactor) factors have to be optimised for good results.

The medium must be suitably stirred to keep the cells in suspension and to make the culture homogeneous; it becomes increasingly difficult with the scaling up. Various types of stirrers range from simple magnetic stirrers, flat blade turbine impellers, to marine impellers, to those using pneumatic energy, e.g., airlift fermenter, and those using hydraulic energy, e.g., medium perfusion.

Improved mixing can be obtained by changing the design of stirrer paddle or by using multiple impellers. The objective of stirring is to achieve good mixing without causing damage to the cells. Vibro-mixer achieves stirring by vertical reciprocating motion of 0.1-3 mm at a frequency of 50 cycles/sec of a mixing disc fixed horizontally to the agitator shaft. These stirrers cause random mixing, less foaming and lower shear forces.

It is important to supply sufficient O2 without damaging the cells. Mean O2 utilization rate by cells is about 6 mg $O_2/10^6$ cells/hour. But O2 is only sparingly soluble in culture medium; the oxygen transfer rate (OTR) from gas phase into medium is about 17 µg/cm/hr.

Therefore surface aeration can support about 50 x 10^6 cells in I 1 culture vessel. Efficient aeration is achieved by bubbling air through the medium (sparging), but this may damage animal cells due to the high surface energy of the bubble and on the cell membrane.

The damage can be reduced by using larger bubbles, lower gassing rates and by adding non-nutritional supplements like Pluronic F-68 (polyglycol) and sodium carboxymcthyl cellulose (these protect cells from damage due to shear forces and bubbles, respectively). Silicone tubing (highly gas permeable) can be arranged inside the culture vessel (2-5 cm tubing of 30 111 length for a 1000 1 culture) and air is passed though the tube; however it is inconvenient to use.

Aeration may be achieved by medium perfusion, in which medium is continuously taken from culture vessel, passed through an oxygenation chamber and returned to the culture. The cells are removed from the medium taken for perfusion so that the medium can be suitably altered, e.g., for pH control. Perfusion is used with glass bead and, more particularly, with micro-carrier systems.

Where considered safe and desirable, O_2 supply in the culture vessel can be enhanced from the normal 21% to a higher value and the air pressure can be increased by 1 atmosphere. This increases the O_2 solubility and diffusion rates in the medium, but there is a risk of O_2 toxicity.
The basic objective of aeration is to provide microorganisms growing in submerged cultures with adequate oxygen for their metabolic needs. Agitation, on the other hand, aims to ensure a homogeneous distribution of microorganisms and the nutrients in the broth.

The type of aeration- agitation system used in the fermenter is dictated by the characteristics of the fermentation process. For example, in processes based on low viscosity, low total solids broths, agitation may not be needed as aeration itself would create the necessary agitation.

Fine bubble aerators without mechanical agitation offer the advantage of lower equipment and power costs. Such fermentations are usually carried out in vessels having height/diameter ratio of 5 : 1, but a tall column of liquid would require a higher energy input for the compression of air used for aeration.

However, mechanical agitation is usually necessary for fermentation processes based on actinomycetes and fungi. The following components of the fermenter are required for aeration and agitation: (t) agitator (impeller), (ii) stirrer glands and bearings, (iii) baffles, and (iv) sparger (the aeration system).

1. Agitator (Impeller):

Agitators achieve the following objectives; (a) bulk fluid and gas-phase mixing, (b) air dispersion, (c) oxygen transfer, (d) heat transfer, (e) suspension of solid particles, and (f) maintenance of a uniform environment throughout the vessel.

These objectives are achieved by a suitable combination of the most appropriate agitator, air sparger and baffles, and the best positions for nutrient feeds, acid or alkali for pH control and antifoam addition. Agitators are of several different types, e.g., (i) disc turbines, (ii) vaned discs, (iii) open turbines of variable pitch and (iv) propellers.

Disc turbine consists of a disc with a series of rectangular vanes set in a vertical plane around its perpheri (Fig. 14.1 A). The vaned disc turbine has a series of rectangular vanes attached vertically to the underside of the disc (Fig. 14. 1B). In case of variable pitch open turbine, the vanes are attached directly to a boss on the agitator shaft (Fig. 14.1C).

The marine propeller is similar to variable pitch open turbine, except that it has blades in the place of vanes (Fig. 14.1D). In case of disc and vaned disc turbines, the air bubbles from the sparger first-hit the underside of disc before being broken into smaller bubbles and dispersed by the vanes.

But in the case of the latter two types of agitators, air bubbles contact the vanes/blades directly and arc broken up and dispersed by them. These basic agitation devices have been variously modified. For example, the variable pitch open turbine scheme has been modified to develop four modern agitator types, viz., Scaba 6SRGT, Prochem Maxflo T, Lightning A315 and the Ekato Intcrmig.

The Rushton disc turbine, having a diameter of one-third the fermenter diameter, has been long considered optimum for many fermentation processes. The disc turbine was considered optimum because it was shown to be able to break up a fast air stream without itself becoming flooded in air bubbles; the latter situation seriously hampers oxygen dispersal in the broth.

In contrast, the impeller and open turbine were found to have the tendency to be flooded in air at higher aeration rates. In subsequent studies, it was found that in low viscosity broths, all the four agitator types can achieve good gas dispersion provided the agitator speed is high enough.

In such broths, agitator type does not appear to be a significant factor affecting oxygen transfer efficiencies. In high viscosity broths, however, gas dispersal presents problems and is greatly reduced. In view of this, a number of agitators have been developed for high viscosity broths, e.g., Scaba 6SRGT, Prochem Maxflow T, Lightning A315 and Ekato Intermig (Fig. 14.2).

These agitators are larger, require lower power input (they do not lose as much power as the Rushton turbines when aerated), are able to handle higher air volumes without flooding, and give better bulk blending and heat transfer in more viscous media.

But they can cause mechanical problems mostly of vibrational nature. Good mixing and aeration in high viscosity broths may also be achieved by a dual impeller combination in which the lower impeller primarily dispenses the air, while the upper impeller primarily enhances mixing of the broth.

2. Stirrer Glands and Bearings:

The satisfactory sealing of the stirrer shaft assembly has been one of the most difficult problems; this is very important for maintaining aseptic conditions over long periods. Four basic types of seal assembly have been used in fermenters: (1) the stuffing box (packed- gland seal), (2) the simple bush seal, (3) the mechanical seal and (4) the magnetic drive.

Most modern fermenters use mechanical seals; these seals are more expensive, but they are more durable and less prone to leakage or contaminant entry. Magnetic drives, although quite expensive, are being used in some animal cell culture vessels.

The mechanical seal consists of two parts; one part remains stationery in the bearing housing, while the other rotates on the shaft. The two components of the seal are pressed together by springs or expanding bellows. Steam condensate is used to lubricate and cool the seals during operation and servers as a contaminant barrier.

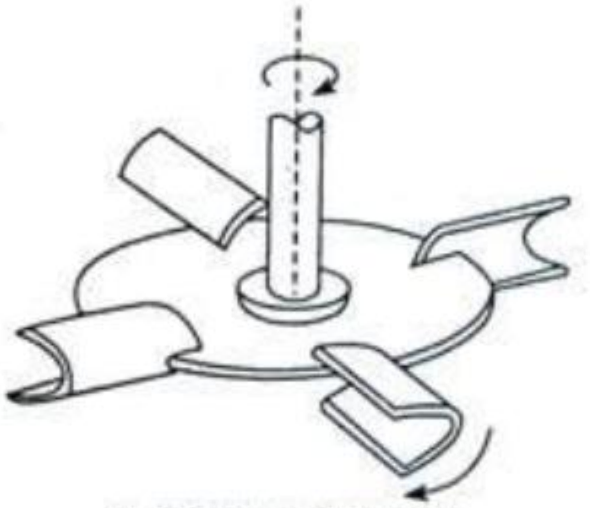
A. DISC TURBINE

B. VANED DISC TURBINE

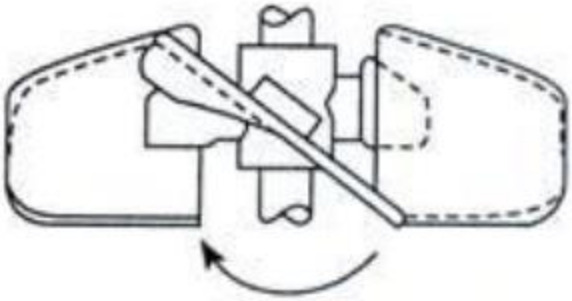
C. VARIABLE PITCH OPEN TURBINE

D. MARINE PROPELLER

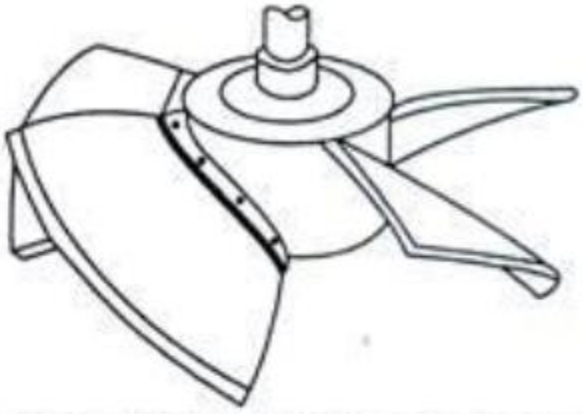
FIG. 14.1. Different types of agitators : **A.** disc turbine; **B.** vaned disc; **C.** open turbine, variable pitch; and **D.** marine propeller agitators.



A. SCABA AGITATOR



B. LIGHTNING A315 AGITATOR



C. PROCHEM MAXFLO T AGITATOR

FIG. 14.2. Diagram of **A.** Scaba agitator; **B.** Lightning A315 agitator (four blades) and **C.** Prochem Maxflo T agitator (four, five or six blades).

3. Baffles:

Baffles are metal strips roughly one-tenth of the vessel diameter and attached radially to the fermenter wall (Fig. 14.3). They are normally used in fermenters having agitators to prevent vortex formation and to improve aeration efficiency.

Usually, four baffles are used, but larger fermenters may have 6 or 8 baffles. Extra cooling coils may be attached to baffles to improve cooling. Further, the baffles may be installed in such a way that a gap exists between the baffles and the fermenter wall. This would lead to a scouring action around and behind the baffles, which would minimise microbial growth on the baffles and the fermenter wall.

4. Aeration System (Sparger):

The device used to introduce air into the fermenter broth is called sparger. Spargers are of the following three basic types: (1) porous spargers, (2) orifice spargers and (3) nozzle spargers. Porous spargers may be made of sintered glass, ceramics or a metal.

They are used primarily on a laboratory scale in non-agitated vessels. The bubble size from such spargers is always 10 to 100 limes larger than the pore size of the sparger. These spargers have low air throughput because pressure drops across the sparger, and the fine holes often become blocked by microbial growth.

Orifice spargers consist of perforated pipes arranged in various ways, e.g., the sparger pipe forming a ring below the impeller. In most cases, air holes are drilled on the underside of the pipe and the holes are arranged in the form of ring or cross.

It is desirable that the holes are at least 6 mm in diameter to avoid clogging by microbial growth. These spargers (without agitation) have been used to a limited extent in yeast manufacture, effluent treatment and in air-lift fermenters used for single-cell protein (SCP) production.

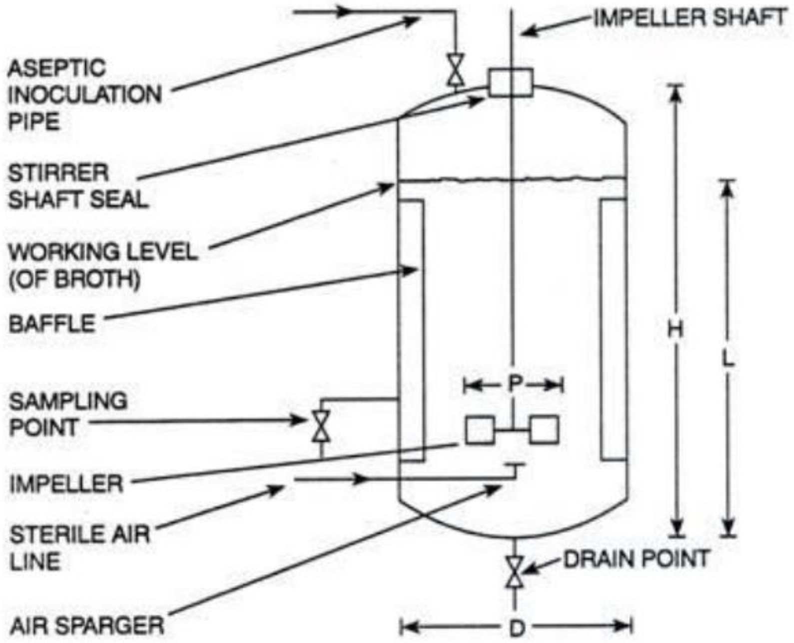


FIG. 14.3. Diagram of a fermenter with one multi-bladed impeller. H, fermenter height; L, liquid height; D, tank diameter; P, impeller diameter.

Nozle sparger consists of an open or partially closed pipe. Most modern fermenters (laboratory to production scale) have a single open or partially closed pipe as a sparger that is ideally placed centrally below the impeller.

It provides a stream of air bubbles. The sparger should be as far below the impeller as possible to avoid flooding of the impeller in a stream of air bubbles. These spargers cause a lower pressure loss than the other spargers and they are not easily blocked.

In small fermenters, a combined sparger-agitator may be used. In this case, the air is introduced via a hollow agitator shaft, and it comes out through holes drilled in the disc between the blades and connected to the base of the main shaft. This design gives a good aeration in baffled vessels over a range of agitator speeds.

Temperature Regulation:

The fermenter must have an adequate provision for temperature control. Both microbial activity and agitation will generate heat. If this heat generates a temperature that is optimum for the fermentation process, then heat removal or addition may not be required.

But in most cases, this may not be the case; in all such cases, either additional heating or removal of the excess heat would be required. Temperature control may be considered at laboratory scale, and pilot and production scales.

1. In laboratory scale fermentations, normally little heat is generated. Therefore, heat has to be added to the system; this can be achieved in the following ways: (a) the fermenter may be placed in thermostatically controlled bath, (b) internal heating coils may be used, (c) water may be circulated through a heating jacket, or (d) a silicone healing jacket may be used. The silicone jacket consists of two silicone rubber mats, and heating wires between these mats. This jacket is wrapped around the fermenter and is held in place by Velcro strips.

2. In case of larger fermenters beyond a certain size, excess heat is generated, and the fermenter surface becomes inadequate for heat removal. The size at which fermenter surface becomes inadequate for heat removal will depend on the fermentation process and the ambient temperature at which fermentation is being carried out. In such cases, internal coils have to be used to circulate cold water through them for removing the excess heat.

The cooling surface area necessary for temperature control will depend mainly on the following factors: (i) temperature of cooling water, (ii) the culture temperature, (iii) the type of microorganism, and (iv) the energy provided by stirring. The average cooling area for a 55,000 l fermenter may be considered to be around 50-70 m^2; if the cold water temperature were 14°C, the broth temperature would cool down to 30°C from 120°C in 2.5 to 4 hours without stirring. The cooling water consumed during bacterial fermentation in a vessel of this size would range between 500 to 2,000 l h^{-1}. Fungal fermentation, however, may need 2,000 to 10,000 l cooling water per hour as they have a lower optimum temperature for growth.

The heating/cooling requirements for a specific fermentation process can be accurately estimated by taking into account the overall energy balance of the process, which is described by the following formula.

$Q_{met} + Q_{ag} + Q_{gas} = Q_{acc} + Q_{exch} + Q_{evap} {}^{+} Q_{sen}$

where, Q_{met} – the rate of heat generated by microbial metabolism;

Q_{ag} = the rate of heat produced by mechanical agitation;

Q_{gas} = the rate of heat generated by aeration power input; Q_{acc} = the rate of heat accumulation in the system;

Q_{exch} = the rate of heat transfer to the surroundings and/or heat exchanger, i.e., heating/cooling device;

Q_{evap} = the rate of heat loss due to evaporation; and

Q_{sen} = the rate of sensible enthalpy gain by the flow streams (exit-inlet). This equation may be arranged as follows.

$Q_{exch} = Q_{met} + Q_{ag} + Q_{gas} - Q_{acc} - Q_{sen} - Q_{evap}$

In this equation, Q_{exch} provides the estimate of heat that has to be removed by the cooling system. The values for Q_{met} are experimentally determined for different substrates, while those of Q_{ag}, Q_{gas}, Q_{evap},and Q_{sen} are computed using appropriate methods/formulae. For example, estimates of these values for Bacillus subtilis grown on molasses in one study are summarised in Table 14.7. Among these values, contributions due to Q_{evap} and Q_{sen} are quite small. In a steady-state system, Q_{acc} is zero; further, Q_{evup} can be eliminated by using a saturated air stream that has the same temperature as the broth. In large fermenters, Q_{evap} will depend on operating temperature and flow conditions. Similarly, Q_{ag}, will be determined by the choice of agitator and the speed of agitation, while aeration rate and sparger design will determine Q_{gas}.

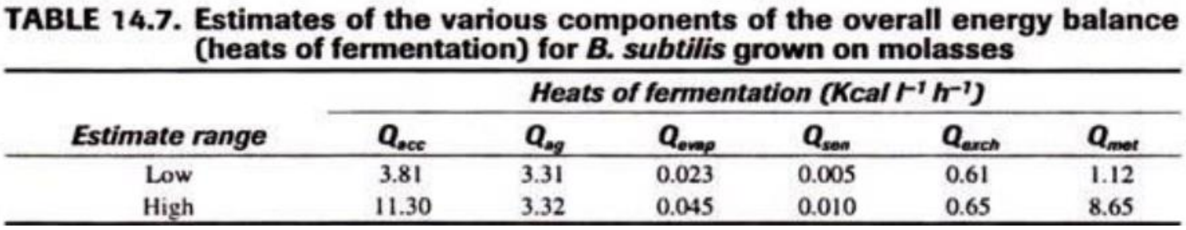


TABLE 14.7. Estimates of the various components of the overall energy balance (heats of fermentation) for *B. subtilis* grown on molasses

	Heats of fermentation (Kcal l^{-1} h^{-1})					
Estimate range	Q_{acc}	Q_{ag}	Q_{evap}	Q_{sen}	Q_{exch}	Q_{met}
Low	3.81	3.31	0.023	0.005	0.61	1.12
High	11.30	3.32	0.045	0.010	0.65	8.65

Once the value of Q_{exch} is estimated, the cooling requirement (jacket and/or pipes) can be computed using appropriate formulae. The factors that will affect the heat transfer surface area may be summarised as follows: vessel geometry, fluid properties, flow velocity, wall material and thickness, the temperature difference between the cooling agent and the broth, and of course the value of Q_{exch}.