Bacterial growth pattern factors

Bacteria grow in a defined pattern, determined by different factors pertaining to their nutrition and environment. The determination of bacterial growth patterns is important, especially in large scale industrial fermentation processes, where antibiotic-producing bacteria are cultured. Bacteria can be commonly cultured using two methods: the batch culture and the continuous culture. The former being of prime use in the production of secondary metabolites such as antibiotics and vitamins, it will be the one investigated.

In batch cultures, a limited amount of nutrients and oxygen is supplied to a closed system containing the microorganisms. As the oxygen and nutrients are exhausted, toxic waste products accumulate and this changed the pH and the oxygen partial pressure in the closed system. Any further cell division is therefore inhibited (Willey et al., 2008a).

The turbidity of the culture can be used as a measure of bacterial growth. As bacteria multiply in a liquid medium, the latter becomes turbid and the bacterial cells scatter light. The concentration of bacteria in the medium is therefore inversely proportional to the amount of light scattered by the cells or proportional to the absorbance (from Beer-Lambert's law). The absorbance is monitored using a spectrophotometer which converts the analog transmission of light into a digital absorbance reading (Csuros and Csuros, 1999a).

A typical bacterial growth curve, represented by plotting the logarithm of viable cells against incubation time, consists of four parts (fig. 1). The lag phase (A), corresponds to a period of no increase in cell number. At that point, the microorganisms are getting used to the growth medium and are

synthesising new enzymes and co-factors to use up the medium. In the log phase (B), bacteria divide at the maximum rate relative to the growth medium and their environmental conditions and their genetic nature (Willey et al., 2008b). At a point, the total number of viable microorganisms would remain constant; this would be the start of the stationary phase (C). The microbial population may stop dividing owing to nutrient or oxygen limitation. Growth rate becomes equal to death rate (Von Sperling et al., 2006). The number in viable cells then goes in decline in the death phase (D). During this period, there is loss of viability of the microorganisms as they are irreversibly harmed. Death rate is greater than growth rate.

Fig. 1. Components of a typical bacterial growth curve (Denyer et al., 2004a)

AIMS

The main objective of the experiment would be to use turbidity and optical density measurements to determine the growth pattern of an E. coli culture in non-shaking nutrient broth. The hypothesis would be that the curve obtained when plotting log of optical density against time after inoculation would correspond to that of a typical bacterial growth curve and that all the four different components of the curve would be identified accordingly. The generation time of the E. coli culture as well as its growth rate constant would be graphically obtained.

MATERIALS & METHODS

The methodology is as per pages 79 to 81 of the lab manual with the following changes: Escherichia coli in 12 mL of nutrient broth would be used

instead of Vibrio natriegens in 30 mL of BHI medium and no formalin would be required.

RESULTS

200 mL of nutrient broth medium was inoculated with 12 mL of an overnight culture of E. coli in nutrient broth and was thereafter left standing at 37oC without shaking. At intervals of ten minutes for 2. 5 hours, 2 mL of the culture was sampled for measurement of turbidity by optical density. The spectrophotometer was blanked using uninoculated nutrient broth and the wavelength used for all measurements was 590 nm. The mixture was swirled before removing any sample. The absorbance was recorded and its log was determined.

Table 1. Absorbance readings of E. coli culture in non-shaking nutrient agar broth medium for 2. 5 h after inoculation

Time after inoculation (min)

Absorbance at 590 nm

Log10 of absorbance at 590 nm

0

0. 041

-1. 387

10

0. 015

-1. 824

20

0. 037

-1. 432

30

0. 044

-1. 357

40

0. 067

-1. 174

50

0. 080

-1. 097

60

0. 103

-0. 987

70

0. 130

-0. 886

80

0. 163

-0. 788

90

0. 183

-0. 738

100

0. 214

-0. 670

110

0. 245

-0. 611

120

0. 287

-0. 542

130

0. 310

-0. 509

140

0. 359

-0. 445

150

0. 396

-0. 402

Fig. 2. Bacterial growth curve of E. coli culture in non-shaking nutrient agar broth medium

As time after inoculation increases, the absorbance increases as the number of bacterial cells increases. There is more scattering of light, less transmittance and absorbance reading increases. Although the log of the absorbance reading increases, it remains negative as the absorbance reading is less than 1. The second point on the graph may be erroneous as it shows a decrease in log of optical density instead of remaining stationary for the lag phase. The exponential phase is apparent but not the lag, stationary and death phases. A line of best fit was plotted and the growth rate constant and generation time was calculated for the time interval between 60 s and 120 s (exponential phase).

g =

From line of best fit (dotted line), t (time interval) = 120 – 60 = 60 minutes

g = (0. 301 x 60) / (-0. 542 + 0. 987) = 40. 584

Generation time of E. coli population = 40. 6 minutes

Growth rate constant (k) = 1/g

Growth rate constant of E. coli population = 1/40. 584 = 0. 025 minute-1

Table 2. Absorbance readings of E. coli culture in shaking nutrient agar broth medium for 2. 5 h after inoculation (data from group 4)

Time after inoculation (min)

Absorbance at 590 nm

Log10 of absorbance at 590 nm

0

0. 016

-1. 796

10

0. 019

-1. 721

20

0. 026

-1. 585

30

0. 036

-1. 444

40

0. 043

-1. 367

50

0. 056

-1. 252

60

0. 098

-1. 009

70

0. 101

-0. 996

80

0. 125

-0. 903

90

0. 152

-0. 818

100

0. 184

-0. 735

110

0. 235

-0. 629

120

0. 284

-0. 547

130

0. 339

-0. 470

140

0. 391

-0. 408

150

0. 446

-0. 351

Fig. 3. Bacterial growth curve of E. coli culture in shaking nutrient agar broth medium (data from group 4)

The log of the absorbance reading increases at a near linear rate but remains negative as the absorbance reading is less than 1. The 7th point on the graph shows a slight shift from the trend of the bacterial growth curve. The exponential phase is apparent but not the lag, stationary and death phases. A line of best fit was plotted and the growth rate constant and generation time was calculated for the time interval between 0 s and 110 s (exponential phase). Compared to the non-shaking broth, the growth rate is relatively more uniform.

g =

From line of best fit (dotted line), t (time interval) = 110 – 0 = 110 minutes

g = (0. 301 x 110) / (-0. 629 + 1. 796) = 28. 372

Generation time of E. coli population = 28. 4 minutes

Growth rate constant (k) = 1/g

Growth rate constant of E. coli population = 1/28. 372 = 0. 035 minute-1

DISCUSSION

Nutrient broth typically consists of beef extract and pancreatic digest of gelatin. These ingredients provide the necessary sugars, salts and carbohydrates for the metabolic activities, growth and reproduction of E. coli in an aerobic environment (Atlas, 2006a). Being one of the predominant bacteria in the human intestinal flora, it is a mesophile having an optimum growing temperature of 37-39oC (Nester, 2001a). Hence a temperature of 37oC was ideal to investigate the growth of an E. coli culture in nutrient broth.

After inoculation, the bacteria used raw materials from the broth to divide by binary fission and increase their population in the flask. The bacterial cells are assumed to have similar densities and sizes as well as similar ability to scatter light. As the concentration of bacterial cells increases, the relative scattering of light increases and the transmittance of incident light through a sample of the inoculated broth decreases, leading to an increase in the absorbance of the sample. This is shown by the increase in absorbance in table 1. According to the Beer-Lambert law, the number of the bacteria would be proportional to the absorbance of the sample. Therefore the rate increase in absorbance of the inoculated samples provides us with a measure of the bacterial growth rate.

From fig. 2, it is observed that the log of optical density drops off ten minutes after the time of inoculation before going up again. According to the theoretical bacterial growth curve (fig. 1), log of the optical density was expected to remain practically the same for some time during the lag phase as the number of viable bacteria is not expected to increase. During this time, the culture would get used to the new medium and synthesise new enzymes and co-factors for metabolic activities (Atlas, 2006b). Owing to the lack of a lag phase, it can be inferred that the medium in which the E. coli was kept before had similar characteristics to the nutrient broth, such that the bacteria did not require much time to get used to the new medium.

The unexpected drop in optical density (table 1) may be owing to inadequate shaking of the flask before sampling the culture for the recording of the optical density. This might have lead to sampling of a much less concentrated bacterial sample than the actual one. The lack of an apparent lag phase may also be because the culture was a young one. If this is the case, the culture may already be in the exponential phase, especially if similar mediums are used. There is no substantial requirement for new enzymes and the bacteria can actively divide. On the other hand, if an inoculum from an old or refrigerated culture was used, a longer lag phase would have been expected. Old cells are depleted of their ATP, essential ribosomes and co-factors. The bacteria therefore have to synthesise the necessary enzymes, co-factors and produce ATP before they can grow and replicate (Q1).

The exponential phase of the E. coli culture is apparent as from 40 minutes after the inoculation until 2. 5 hours after inoculation. During this time the

bacteria are actively dividing by binary fission and the nutrients in the broth are not limited. The rate of binary fission is at its maximum for the conditions provided and the growth rate was calculated from the slope during the exponential phase from one hour to two hours after inoculation, the interval showing the most uniform growth rate. A linear relationship between the log of the optical density and the time after inoculation is observed as the growth follows the pattern of a first order reaction. A generation time of 40. 6 minutes is obtained for the non-shaking broth. It implies that 40. 6 minutes is required for a single E. coli bacterium in the flask to divide and mature (Atlas, 2006c). This is typical of neither an E. coli culture at its optimum, which has a generation time of about 15 minutes, nor that of typical bacteria, which have generation times of one hour to three hours (Atlas, 2006d) (Q4). It can therefore be concluded that the culture inoculated had a growth rate much lower than what was expected and that E. coli, however are faster at dividing than most typical bacteria. The lower generation time than expected was perhaps because of non-optimum conditions. The pH of the nutrient broth may not have been at pH 7, which is the optimum pH for mesophiles such as E. coli. The growth rate of the bacteria was also very sensitive to the concentration of salts in the medium. The growth rate constant of 0. 025 minute-1 was determined as per the first order equation of the E. coli growth rate.

Errors might be involved in the determination of the generation time as well as the growth rate constant. As the determination of the optical density depends on the scattering of light by the bacterial cells, viable cells as well as dead cells which are not yet lysed would be taken into account when

recording the optical density. A higher optical density may be obtained, giving the false impression of a greater number of bacteria present than there are actually. The growth rate constant and generation time may have been underestimated (they are higher than the determined ones) (Csuros and Csuros, 1999b).

The fact that the flask was non-shaking meant that the little oxygen dissolved at the surface (oxygen is not very soluble) was not uniformly distributed throughout the flask. E. coli are obligate aerobes and require oxygen to grow and replicate. Without shaking, the oxygen dissolved in the broth was rapidly depleted and not replaced. A reduction in the availability of oxygen meant that the rate of growth of the bacteria was less than the optimum, explaining the greater than expected generation time. Compared to the non-shaking flask, the shaking broth flask displayed a generation time of 28. 4 minutes and a growth rate constant of 0. 035 minute-1. Hence, the bacteria in the shaking flask divided at a much faster rate compared to the non-shaking flask, confirming the effect of shaking on the distribution of oxygen and the importance of the latter in the adequate growth of E. coli (Csuros and Csuros, 1999c).

Both the shaking and the non-shaking cultures showed no signs of the stationary phase, which should have been shown by a plateau in the graphs. No such plateaus are seen in fig. 2 and 3. Both cultures, after 2. 5 hours after inoculation, are still in their exponential phase. The stationary phase occurs when the nutrients in the medium start becoming limited and the death rate of the bacteria becomes equal to the growth rate, explaining the plateau. It can therefore be affirmed that the nutrients did not become limited in either

case. However, the graph of the shaking medium tends off at a less steep slope after 110 minutes after inoculation, meaning that the stationary phase is not after a very long time. In order to observe the stationary phase of the E. coli culture, the latter could have been left in the water bath for a longer time with the optical density being recorded every 10 minutes, until the stationary phase became apparent in the graphs. It would have been expected that the stationary phase in the shaking flask would be reached faster than that in the non-shaking flask since the growth rate is higher in the former and the nutrients would be depleted faster (Q3).

Considering that the growth rate in the inoculated flask is far from the optimum, it would be interesting to note the different methods employed in the industry to achieve near-optimum growth rates. Typically, the higher the growth rate constant, the lower is the doubling time of bacteria, meaning that they replicate more rapidly. The affinity of bacteria to their substrate also determines an increase or decrease in the doubling time of a bacterial population. This is usually defined by KS, the substrate concentration in the exponential phase corresponding to half the maximum growth rate constant. A higher KS would lead to a higher doubling time while a lower KS would lead to a lower doubling time (Nester, 2004b). This is an analogy of the Michaelis-Menten constant in enzyme kinetics (Q2).

In the industry, especially in pharmaceutical industries or bio-fertiliser producing industries, growth rates and doubling times have to be maintained at the optimum level in the exponential phase. Bacteria and fungi are the raw materials and are cultured in biofermentors. Thermostats are used to ensure that the optimum temperature. The contents of biofermentors are

continuously mixed to ensure that all the cells have the right amount of oxygen and nutrients. Moreover, oxygen, temperature and pH probes are used to ensure that the correct parameters are in place for the optimum growth of the microorganisms (Denyer et al., 2004b).

CONCLUSION

The generation time of the E. coli inoculated non-shaking nutrient broth was found to be 40. 6 minutes and the growth rate constant was found to be 0. 025 minute-1. When using the shaking broth, generation time was reduced to 28. 4 minutes and growth rate constant was increased to 0. 035 minute-1. Reproduction rate was higher in the shaking broth. Oxygen was an important factor in determining growth rate. Only the exponential stage was clearly observed out of the four theoretical stages owing to a too short lag phase and non-reaching stationary and death phases.