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Bacterial Cell Culture Measurement using Biowave II (and CO8000)

Biowave II can be used to give an indication of bacterial cell density, by measuring the *optical density* (OD) of a suspension of cells at 600nm (OD_{600}). Usually, the reason for doing this is so the researcher can know when cells are ready for harvesting.

It is important to note that for turbid samples such as cell cultures, the absorbance (OD) measured is due to light scattering, and not due to molecular absorption. The reading is affected by the optics of the system (distance between the cell holder and instrument exit slit, geometry of this slit and the monochromator optics). Different spectrophotometer types therefore give different readings for the same turbid sample.

1) Some users prefer that OD_{600} readings from Biowave II be comparable to those of other instruments (for example, an older instrument around which laboratory procedures have been written). To facilitate this, a correction factor is included in the Biowave II software. Results obtained by comparing Biowave II to a "typical" general purpose spectrophotometer showed OD_{600} from Biowave II to be approximately half that of the other instrument – suggesting that a correction factor of 2.0 would be appropriate in this case. For this reason, the correction factor 2.0 is the programmed default value in Biowave II. Of course, the user can change this as required via the keypad: *OD 600 – Parameters - Correction*.

To determine the precise value for Correction, simply measure the same sample in Biowave II and in the other spectrophotometer, taking care that the cells do not have time to undergo significant further growth, or fall out of suspension between the two readings (glycerol may be used for the latter). The value for Correction is found by dividing the reading of the other instrument by the reading of Biowave II. This need be done only once.

2) For those users who want Biowave II to give an indication of actual cell density (in cells/ml) rather than just an OD_{600} value, a second factor is used. This is to allow the instrument to make the correct conversion from OD to cells/ml *for the cell type being used*. To enter this value, go to *OD 600 – Parameters – Factor*. The highest number that can be entered here is 9999 so, for the user's convenience, a further number – either 1000 or 1000,000 – can be selected in the box called Multiplier. For *E.coli*, for example, an OD_{600} reading of 1.0 typically corresponds to around $8x10^8$ cells/ml. In this case, the Factor entered would be 800 and the Multiplier selected would be 1000,000.

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To determine the precise value for Factor for cells of a given organism, it may be necessary to perform a physical cell count, using a microscope. Cells should be treated, to minimise/stop further growth during the counting process. This procedure need be done only once for each organism.

3) In fact, many users do not need for the spectrophotometer to tell them about number of cells/ml – they just want to know if it's time to harvest. The best time to harvest is normally towards the end of the growth phase of the cell culture, before the cells reach such a density that their metabolites start to have a toxic effect on the population as a whole. OD_{600} measurement can be used to indicate the approach of the end of the growth phase, as cell density becomes such that light is subject to more complex scattering within the suspension, and the relationship between OD_{600} and cell density loses its linearity. For *E.coli*, the relationship between OD_{600} and cell density is typically approximately linear up to OD_{600} of around 0.6.

Many users find that the best point for harvesting is around 0.4 OD_{600}. However, there is wide variability, depending on such factors as the organism being grown, whether the requirement is simply for biomass or for some product of metabolism, etc - and Biochrom recommends that users determine for themselves the OD_{600} that signifies the best point to harvest their cells.

To determine this, it's necessary to carry out an experiment measuring OD_{600} at regular intervals during the growth phase of the cell culture. Samples are taken (every hour, for example), measured, and a graph is plotted of OD_{600} versus time. From the graph, a suitable OD_{600} at which harvesting should take place may be selected. Further analysis during downstream processing can reveal if some optimisation of the selected harvesting point is necessary.

4) Troubleshooting.
It is important to remember that the use of spectrophotometers to indicate cell density is not a precise technology and that considerable variability can exist from instrument to instrument, as has been discussed. Further variability may be introduced by other factors, including:

- the dynamics of suspensions (as different from that of homogenous solutions)
- different kinds of bacterial cell
- different strains of the same bacterial cell
- cell clumping
- the optical properties of the medium in which the cells are suspended
- the optical properties of the medium used as a blank
- temperature

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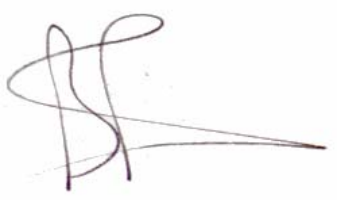
Plus, there is wide variation in the particular applications and requirements of different users. The information given above is intended as a guide only, to help users understand how Biowave II may be used to give an indication of cell density. Especially, the numbers given are for guidance only, and users should not be worried if their own experiments produce different ones.

Note for users of CO8000 cell density meter

The CO8000 cell density meter is a simple and inexpensive device for indicating cell density. It has no facility for factors to be entered by the operator, although it is designed so that the OD_{600} values reported should be similar to those given for cell density measurements by "typical" spectrophotometers.

Precise factors for standardising between instruments, and for converting OD_{600} values to cells/ml can be determined in the same way as described for Biowave II - see 1) and 2) above; the difference is that the factors and the calculations in which they are used must be recorded externally, as they cannot be stored in the CO8000.

The method described for determining when to harvest cells – see 3) above – can also be applied to CO8000.



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