Production of poly-β-hydroxybutyrate: poly-β-hydroxyvalerate copolymers

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In this paper I shall discuss some of the factors that are important in the large scale production by fermentation of poly-β-hydroxybutyrate: poly-β-hydroxyvalerate (PHB/HV) copolymers. Many of the points discussed are common process features in any scale up procedure.

1. CURRENT PRODUCTION PROCESS

Alcaligenes eutrophus is grown in a fed batch mode. The primary substrate for the cell growth stage is glucose. The medium for this stage contains an excess of all nutrients except phosphate which is added in an amount calculated to give a particular dry weight of cells. The culture grows and becomes phosphate-limited. At that point a feed of glucose and propionic acid is started and continued until the final polymer content of about 70–80% is attained. The HV content of the PHB/HV is controlled by variation of the glucose to propionate ratio in the feed. HV contents at particular values in the range 5–30% are normally made. Homopolymer is only manufactured for biomedical applications.

Polymer is separated in an aqueous based process that solubilises cell components and washes the polymer free of contaminants. The resulting white powder is melt-extruded and converted to the chips that is the usual form in which PHB/HV is supplied to fabricators.

2. FACTORS IMPORTANT IN LARGE SCALE PRODUCTION

The current production capacity is of the order of 300 tonnes per annum. In order to reach this level of production it has obviously been necessary to scale up both the fermentation and polymer harvesting processes. Many problems are met in the course of scaling up a process and a number of them are discussed below.

2.1. Interdisciplinary nature

Personnel with many different skills and disciplines are required to complete a successful scale up project. It is essential that they all interact well, appreciate each other’s needs for information and recognise how that information should be presented in order that all parties have a common understanding of the process.

Over the years we have adopted a protocol that assembles a multidisciplinary project team.
very early in progressing towards scale up. In that way the team develops with the project and has a detailed knowledge of the process which aids the transition to full scale plant design and operation.

2.2. Time

Time is a much underestimated factor for the unwary in scale up. I do not mean the time taken to develop a process, etc., but the difference in the time taken between the laboratory and a full scale plant to carry out simple operations such as mixing, filling and emptying vessels, transferring solutions, processing product.

2.2.1. Mixing time. In a shaken flask or a laboratory fermenter mixing time, the time taken to fully distribute an added aliquot of substrate, is virtually instantaneous. In contrast the mixing time in a 1000–2000-litre vessel is 20–30 s and at the 100000-litre scale can be of the order of minutes, 2–3 min being not unusual.

The implications for substrate distribution, pH control and oxygen concentration are obvious. Organisms are bound to experience variation of all the above and the effect of this on their performance needs to be considered during scale up.

2.2.2. Emptying of vessels. Laboratory fermenters can be emptied very quickly. This cannot be done at large scale. It will take several hours to empty a 100000-litre fermenter. Consideration has to be given to the stability of the organism and product over that period. If the organism lyses a viscous, hard to pump gel-like mass can result. In the case of polyhydroxyalkanoate (PHA) fermentation, molecular mass stability and retention of polymer content are important factors at this stage.

2.3. Unit operations

Any processing step that has been developed in the laboratory must be capable of transfer to large scale if the overall process is to be successful. Operations that are simple in the laboratory with small volumes of culture can be impractical at large scale or at least impractical at economic cost. Rapid and sequential temperature changes over relatively large ranges with incubation periods between are a good example of operations that are facile in the laboratory but difficult at scale.

Purification protocols have to be devised that give the required product quality but are simple unit operations. Environmental impact is also a key parameter. The aqueous based PHB/HV harvesting process has these characteristics. It was developed in response to a need to change from the solvent based extraction process that was the process first used in the laboratory to extract the polymer. It was rejected for two main reasons: (i) high cost at large scale – solvent inventory, solvent recovery plant; and (ii) environmental concerns – large amount of chlorinated solvents.

2.4. Contamination

The time taken to empty large fermenters has been mentioned above. The culture from the fermenter will often be stored in a holding tank prior to processing since few operations are fully and continuously integrated. The holding tank is unlikely to be a steam sterilisable pressure vessel for cost reasons. The culture is a biological fluid suitable for growing microorganisms. Contaminants will grow in the culture and on the walls of the vessel. Cleaning operations can keep the contaminant levels down but care has to be taken to monitor the situation.

2.5. Culture stability

Laboratory fermenters will be inoculated with the contents of a shaken flask. The number of cell generations to give a final cell concentration of 10 g/l may be as few as 8–10. To reach an equivalent cell concentration in a 100000-litre vessel will take about 25–30 generations, and at that point there will be approximately $10^{21}$ cells present. Real production cell concentrations are higher than this. Any strain used for production of, e.g., PHB/HV, therefore has to be very stable, otherwise it will be outgrown by faster growers or lower producers, etc. Fortunately PHA production has an advantage over many other fermentations in that there is a relatively short cell growth phase followed by a polymer accumulation phase when the cells are not growing.
There is, therefore, opportunity for reversion/mutation only in the first stage.

The production strain used by ICI to manufacture 'BIOPOL' is a mutant that assimilates propionate more efficiently into HV than the wild type. It is stable in use since propionate is only present in the fermentation in the non-growing polymer accumulation phase when selection for revertants to wild type propionate utilisation characteristics cannot take place.

2.6. Measurement

Measurements that are simple and reliable in the laboratory are more problematic at large scale. Probes to sense pH and dissolved oxygen tension become fouled rapidly in large fermenters with time. The location of the probes can significantly alter the read out value obtained (see above, Mixing). Items that can be measured directly in the laboratory can often only be estimated or measured indirectly on a full scale plant, e.g., addition of substances to the fermenter must be estimated from pump rates and measured concentrations in vessels.

2.7. Raw materials

Substrates such as glucose obtained from laboratory suppliers can be assumed to be the same from batch to batch and to give the same organism performance. This cannot be assumed at large scale. Raw materials vary and can give significant differences in performance. At large scale powdered glucose is replaced with glucose syrups of different dextrose equivalents (DE). DE is essentially a measure of free glucose. The syrups can vary, in DE and also vary in analysis from manufacturer to manufacturer. In addition to DE the inorganic ions and non-glucose sugars in the syrups can vary. Different brands need to be tested to determine the optimum for a particular fermentation and to chose an alternative in the event of problems with supply. Inorganic medium components and chemicals used in harvesting and purification procedures are also subject to variation from supplier to supplier.

2.8. Other factors

Many other factors have an influence on the performance of a process or the ability to scale up a project. These include sterilisation, containment, materials of construction, heat removal, effects of overpressure, CO₂ removal, additives, inoculum development. This list is not complete and it seems that given the complications of scale up it is amazing that a fermentation industry has developed at all. However, a successful industry does exist and PHB/HV is one of the latest products to be added to the list of those made biologically. All of the factors described above have been important in the development of the manufacturing process. Additional considerations are mentioned briefly below.

3. CHOICE OF ORGANISM

*Alcaligenes eutrophus* was selected as the 'BIOPOL' production organism after screening a number of candidates.

*Azotobacter* was the first organism to be grown by ICI for PHB synthesis. It was rejected for further development since it diverted carbon to polysaccharide (EPS) synthesis (we did not have a suitable EPS⁻ mutant) and was difficult to control.

*Methylobacterium*, a methanol utilising organism was attractive at first sight. ICI is a major methanol producer, methanol is a soluble, easy to handle, cheap substrate. However, the fermentation was slow, had low polymer content, the PHB was difficult to extract and the molecular mass was low.

*Alcaligenes eutrophus* has high polymer content, good molecular mass and the PHA can be relatively easily extracted. A glucose utilising mutant was isolated from the original H16 strain. This organism can be regarded as the first generation. Molecular genetics now offers the possibility to transfer the genes for PHA synthesis to a wide variety of organisms. The number of options available is bewildering and careful thought would have to be given to any development in this area.

The other option that has been presented is to abandon bacteria and produce PHB in plants. Poirier (this volume) has shown that this is feasible but that there is still a significant development required to produce economic yields of
polymer by this route. One major problem that has to be overcome is the synthesis of copolymer (PHB/HV) of controlled HV content in these systems. Scale up concerned with polymer purification will be an issue also.

4. SUBSTRATE

Many of the arguments that govern choice of substrate have been well rehearsed so I will make only a few points here.

4.1. Availability

The substrate needs to be available in large quantities and to be politically stable – that is as free as possible of the vagaries of political influence on price/supply.

4.2. Concentration

A concentrated feedstock is needed since unless a plant is built 'over the fence' from the source large quantities of water will have to be transported with the substrate. Equally important is the fact that dilute substrate addition to a fermenter increases the size of the vessel required, and hence its cost, dramatically.

4.3. Consistency

Within the limits discussed above the substrate needs to be consistent. The manager of a plant does not want to have to contend with great batch to batch variation giving changes in plant performance. For the above reasons the ‘BIOPOL’ process has been designed to operate with glucose as the growth substrate for the culture.

5. APPLICATIONS OF ‘BIOPOL’

A number of applications have now been commercialised for ‘BIOPOL’. These include bottles for cosmetics and other personal care products in Germany, Japan and the USA. A bottle for biodegradable motor oil has also been launched. Long term developments in biomedical applications of PHB homopolymer are also in progress.