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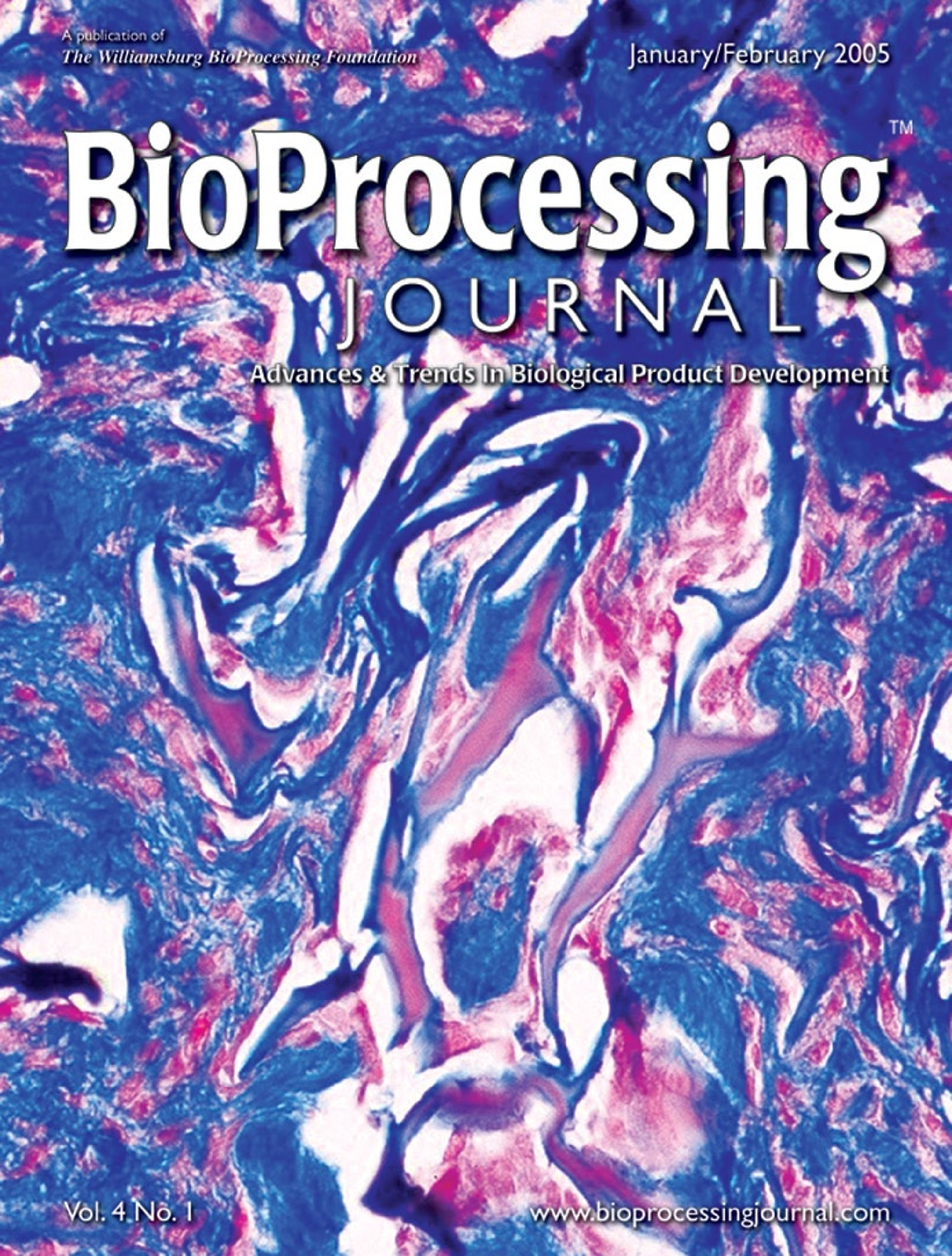

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Industrial Fermentation using Continuous Gravitational Settling

By JAMES S. WILSON

The manufacture of biological materials by fermentation is based intrinsically on established and well understood processes developed over a number of years. The fundamental basics of supplying a characterised cell line with sufficient nutrients over a period of time, with the intention of harvesting a selected protein for further processing, are similar throughout the industry. However, as a result of economic pressures and the need to control costs of an already expensive, high quality, and high compliance material, all manufacturing companies endeavour to maintain the highest level of productivity.

Serologicals Ltd. has been manufacturing monoclonal antibodies for diagnostic use since 1984. Primarily used for the blood typing industry, these antibodies must be manufactured to a high level of current Good Manufacturing Practices (cGMP) for both FDA and ISO 2001 accreditation, while operating within a market that cannot charge the premium associated with clinical products. Serologicals Ltd. addressed this constraint in 1994 by validating a manufacturing train based on a novel, continuous fermentation system that uses gravitational settling, which was known in-house as the "settler system." Subsequently, this system was patented in both the United States and the European Union, approved by FDA for the manufacture of IVD antibodies, and used for the past ten years within Serologicals Ltd. for a range of cell lines.[1]

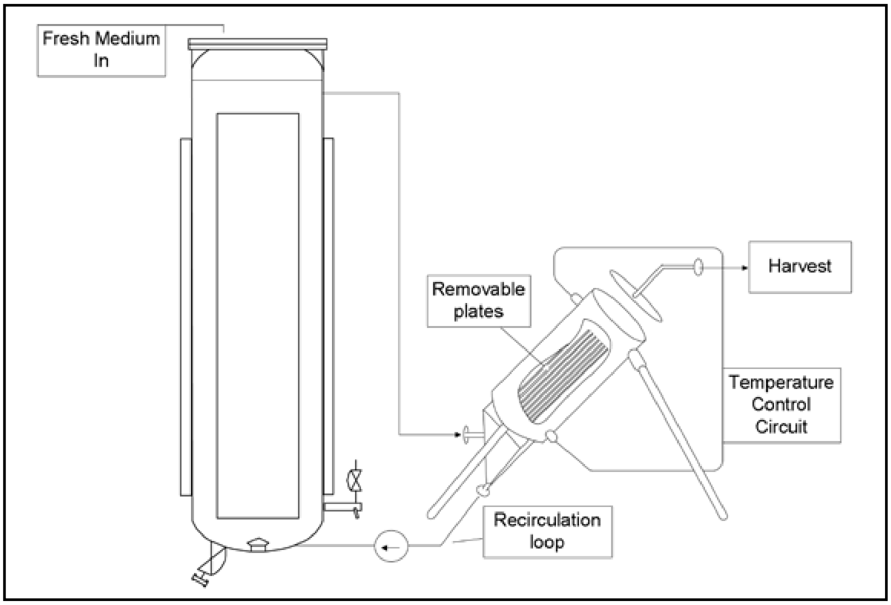

Figure 1. System configuration.

The settler system uses a series of inclined, mirror-polished plates within a sanitary, stainless-steel vessel to gently retain the cells within the fermenter without any moving parts or filtration effects. This gives a robust and readily maintained system that is free of clogging and can operate for extended periods of time with minimal intervention. The settler section is enclosed by a water jacket to allow control and stabilisation of the temperature within the system. The settler is operated as an external loop from the fermenter vessel, allowing ready attachment of the settler to any existing fermenter with minimal installation cost. Sanitary engineering design and standard industrial fittings allow a system to operate robustly and meet cGMP requirements (Figs. 1 & 2).

The theory of cell separation using the sedimentation coefficient of particles is well understood and documented.[2,3] The process has been used within the brewing industry (to separate out yeast for recycling) and within the waste-water treatment industry. A series of parallel, inclined plates is most efficient, with the liquid that contains the particles being drawn up through the plates at a constant rate. Gravity acts on the particles within the liquid and causes them to sink with the laminar flow towards the plates themselves. At some point along the plate, the particles will contact the plate surface. They slide back down the plate, in many instances collecting additional settled particles as they go, accelerating the rate of fall. When they reach the end of the plates, the particles are either collected or returned to the vessel, either on a periodic basis or by using a recirculation flow. The efficiency of the

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settler is a function of flow-rate velocity versus overall settling plate area. The system can be tuned by altering either the number or the length of the plates for the required retention efficiency or throughput rate.

Figure 2. 50L airlift system and associated settler.

Serologicals Ltd. uses settlers designed for operating at the 50L fermenter scale, with more than 95 percent retention of viable cells at a nominal dilution rate of 2.5 reactor volumes per day. The plate area has been calculated to support maximum retention (up to 5 volumes per day), allowing a higher throughput rate, if required, while maintaining the same fermentation characteristics. The fermenter system is automatically replenished with fresh medium from a dedicated, sterile holding tank. Once at a steady state, it operates with minimal intervention. This simple package gives a high throughput capability with low overhead and good reliability.

The fermenter suite in the Livingston (Scotland, UK) facility has been designed around continuous systems, so that three 50L airlift and medium handling packages are placed next to medium preparation areas. The medium is manufactured and sterile filtered into the holding vessels at point-of-use, then chilled to 2–8º C. The medium tanks are pressurised, which provides the motive force to transfer the liquid into the fermenters when the automated level system detects a decrease in volume (for example, because of harvesting the product through the settler). Once the system is at full harvest rate, ongoing maintenance becomes simply a medium-handling issue, in which the tanks are "topped up" every four to five days. Product harvested from the settler flows into presterilised plastic bags, simplifying the need for multiple sterile holding tanks downstream of the fermenter. It is likely that if Serologicals Ltd. were to install such a system in the current climate, medium addition would be based around disposable technology as well, because it significantly reduces capital expenditure and initial validation.

A typical hybridoma fermentation operated for eight weeks continuously, results in around 5,000L of antibody-containing supernatant. In capacity terms, this gives a system that compares in volume with a 1,000L-batch vessel without the initial capital expense of the larger system.

During fermentation, the fermenter moves through the following steps: inoculation, cell growth to steady-state, full flow harvest, and final shut down. The cell lines grown in Serologicals Ltd., which are a combination of murine and

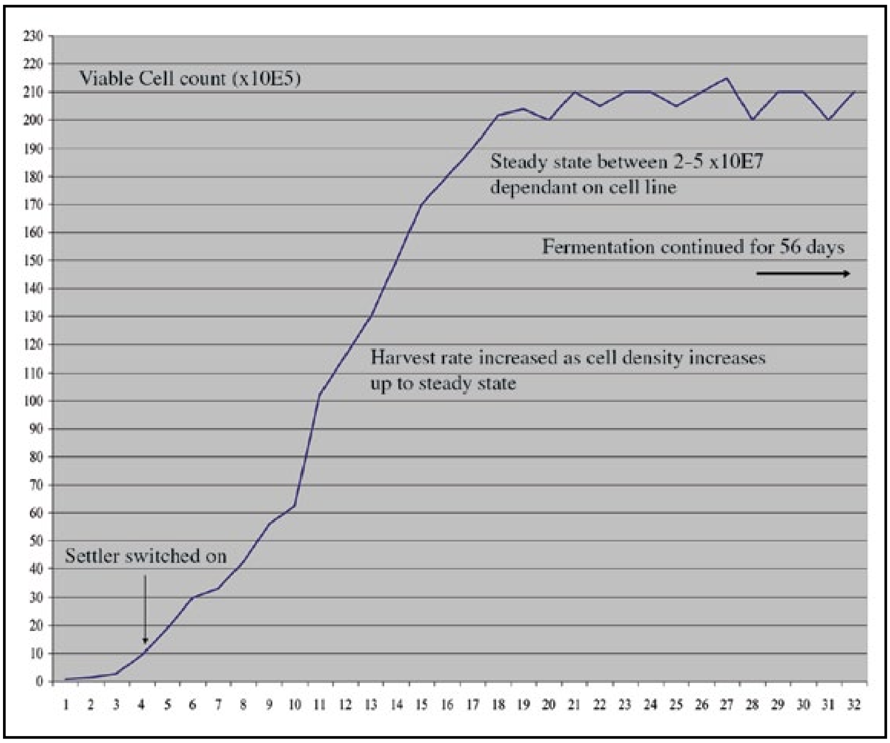

Figure 3. Typical growth curve during settler operation.

human hetero-hybridomas, achieve cell densities at steady states varying from 2–5 x 10^7 viable cells per ml (Fig. 3). Product concentration within the supernatant is comparable to that obtained in batch mode. In addition, the high cell density reduces the dependency of the cells for growth supplements; once at a steady state, the consumables cost is also reduced.

The Serologicals Ltd. settler system is one of many alternatives to maximise the throughput of an existing system. During the development of the settler, we also investigated hollow-fibre systems, encapsulation of cells with various gel matrices, entrapment onto microcarriers and glass beads, spin filters, both internal and external, and continuous centrifuge systems.[4–7] These alternatives offer various advantages and disadvantages that need to be considered while selecting a preferred method. Serologicals Ltd. felt that most of the systems failed to provide the level of robustness needed for a high throughput, commercial application, and developed the settler to address these deficiencies.

In more recent years, other systems have now become available, such as the Applikon BioSep acoustic filter.[8,9] This system uses an acoustic chamber to aggregate the cells and accelerate the settling process, clearing the cells from the product stream more rapidly. For new systems, this is a very attractive design, but to realise the maximum potential, the integration of the separator into the fermenter should be carried out during the fermenter design stages. In comparision, the settler system can be retrofitted to existing fermenters with minimal cost and simple connection, facilitating a rapid move towards continuous operation and improved productivity.

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