Research note

Constructive improvement of the ultrasonic separation device ADI 1015

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Abstract

The use of the ultrasonic separation device is a very important step in the direction for improving animal cell bioreactor cultures. However, the normal construction of the ultrasonic separation device ADI 1015 has an inherent disadvantage in pumping the cell suspension continuously through the device by using a peristaltic pump. The cells are taken out of the reactor and are transported to the side inlet located below the separation chamber of the device. This cycling leads to cell death and a considerable reduction of the viable cell density. The modification of the configuration of the device (no circulation of the cell suspension through the retention device; during approximately 9 minutes cell-free supernatant is extracted; every 9 minute for about one minute, the volume which is equivalent to the interior volume of the chamber and the tubing connecting the device to the reactor, is flushed back in order to return the retained cells back to the reactor) allows cell densities from 10^6 to 2.7×10^6 c/ml with a viability of at least 90% (tested for the shear sensitive insect cell line High Five), whereas the maximal cell densities obtained were 0.76×10^6 c/ml for the period of continuous culture and 10^5 c/ml at the end of the use of the device in the classical mode.

Introduction

The normal construction of the ultrasonic separation device ADI 1015 has an inherent disadvantage in pumping the cell suspension continuously through the device by using a peristaltic pump (e.g. Gaida et al., 1995). The cells are taken out of the reactor and are transported to the side inlet located below the separation chamber of the device (period 1 in Figure 1, Figure 2A). This circulation is necessary for transporting, first, the cells to the device and the separation chamber, and, second, the sedimented cells back to the reactor. Without this forced circulation, the cells accumulate in the separation chamber which leads to the blocking of the chamber and thus to cell death (period 3 in Figure 1, Figure 2B). The objective of this study was to improve the use of the ultrasonic separation device in order to avoid cell damage and cell loss due to the circulation of the cell suspension and in the same time to allow a correct functioning of the device.

Experimental conditions

High Five cells were cultivated in IPL41 containing 4% fetal calf serum. The culture was carried out in a Biolafitte ICC 1L reactor (working volume: 1.6l, pH 6.2; $pO_2 = 50\%$, temperature: 27.2°C) equipped with the ultrasonic separation device ADI 1015 in a continuous or perfusion mode. The dilution rate was chosen according to the cell density (Figure 1). The separation device was used according to the recommendations of Applikon or used after modifications (see results): 3W, 75% output, 2.132 Mhz, timer: off. The following pumps were used: Watson Marlow 101GG (fresh medium pump), Masterflex Console Drive (pump R), and Ismatec IPN (pump H) (for details, see Fig. 2). The liquid level was maintained by a level sensor integrated into the Watson Marlow pump (modification by NewBrunswick Scientific). The cell density and the viability were determined using a Thoma chamber after staining the dead cells with trypan blue.

Figure 1. Culture of High Five cells in a perfusion culture system Biolafitte ICC 11 equipped with the ultrasonic separation device ADI 1015 (Applikon); (A) cell number per ml; viability (%), (B) dilution rate (1/h). Note: the actions taken at the indications 1-5 are described in Table 1.

Results and discussion

Figure 1 presents the culture of High Five cells as well as the time-points, at which the configuration of the retention device had been modified. These modifications are described in Figure 2 and Table 1. The classical configuration recommended by the producer was used during period 1 of the culture (Figures 1, 2A, Table 1): the sedimented cells were transported by the continuous flow of cell suspension from the reactor via pump (R) and the lower side entrance of the device through the vertical down-leading tubing of the device back to the reactor, however, the cells were damaged by the continuous pumping which lead

finally to a reduction of the viable cell density and of the viability. Though several cell lines might resist to continuous pumping using peristaltic pumps (Spier, pers. commun.) and can be concentrated in the reactor by means of the present retention device (Sf9 – and hybridomas – cells (Tramplé et al., 1994; Gaida et al., 1995; Zhang et al., 1998)), other cell lines, such as several insect cell lines (e.g. High Five) or cells growing on microcarriers, are known for their sensibility towards continuous pumping (Cabanié, pers. comm., Miller, pers. comm.). These differences are probably directly related to differences in the shear sensitivities observed for different cell lines (Kretzmer and Schügerl, 1991).

Table 1. Culture periods, modifications of the use of the retention device and effect of these modifications on the culture

Culture periods	Configuration	Specifications, remarks and problems associated with the configuration used
1. 31.5–143.25 h	typical (Fig. 2A)	Pumping rate of the cell suspension pump (R) is 3 times higher than that of the harvest pump (H). The cell suspension is continuously taken out from the reactor and is pumped to the side inlet of the device. The cells are aggregated by the ultrasonic standing wave field and sediment. Periodic shut-off of the ultrasonic field allows aggregates to settle. However, they are only efficiently returned to the reactor by means of continuous pumping. Problem: The circulation of liquid through the device is necessary for transporting the concentrated cells back to the reactor. However, continuous circulation of cells through peristaltic pumps leads to cell damage.
2. 150.5 – 295.25 h	device not used	Classical continuous culture. Problem: loss of biomasse
3. 295.25 – 359.5 h	modified (Fig. 2B)	Pumping rate of the harvest pump (H) equals that of the fresh medium pump, pump (R) is not in use. Problem: The cells accumulate in the retention device because they sediment to its bottom and the upflowing medium prevents the return of the cells to the reactor. Thus, 80%–90% of the cells accumulated at the bottom of the device. These difficulties are the cause of cell loss and of reduced viability.
4. 368.25 – 506 h	modified (Fig. 2C)	The cell suspension is pumped into the cell retention device and the cell free supernatant is further pumped to the harvest vessel by using pump (H). The pumping rate depends on the cell density in the reactor and equals that of the fresh medium pump supplemented by the rate of pump (R) (10.5 ml/min). Every 9'16.75" pump (R) is used to add PBS or medium to the device in order to return the sedimented cells back to the reactor. This pump displaces the volume of the device for 67" (10.5 ml/min). Problem: Dilution of the supernatant by the PBS/medium added to push back the cells to the reactor – therefore a modified improved configuration should be used (Fig. 2D, see text).
5. 506 h	device not used	Infection of the culture with a rec. baculovirus.

The negative effect of the recommended use of the retention device on cell viability has conducted us to study the effects of modifications of the device on its performance, viability, and cell density during their uses.

The use of the classical continuous culture mode lead to a slow increase of the cell density from about 1.85×10^5 to 7×10^5 cells/ml at 288 h accompanied by a considerable increase of the viability (period 2). However, because of cell wash out due to higher dilution rates, no really high cell densities could be obtained. Therefore, a modified configuration (1 = configuration 1 without using pump (R)) was used (period 3, Figure 2B). This lead to a fast but only transient increase in the cell density. It was not possible to work without pump (R) for more than several days because the cells accumulated at the bottom of the retention device and were inhibited from sedimenting back to the reactor by the outcoming supernatant flow, in particular, after having increased the perfusion rate

(Figure 1B). This accumulation lead to cell death and finally to a reduction of the cell density in the culture system (from 336 to about 360 h).

In order to avoid the continuous circulation of the cell suspension through the retention device and back to the reactor and, in the same time, assure the return of the sedimented cells back to the reactor, configuration 3 was tested (period 4, Figures 1, 2C, Table 1). During 9'16.75" cells were extracted from the reactor via the retention device by using pump H. In order to completely push back the cells to the reactor, pump H was stopped and pump R was started for adding PBS or medium displacing the cell suspension back to the reactor (during 67"). The volume displaced was equivalent to the volume of the lower part of the retention device and the connection tube of the device to the reactor. Using this configuration, it was possible to increase the cell density as well as the viability from 1.03×10^6 to 2.4×10^6 c/ml and from 55 to 86%, respectively. During this period (136 h) the per-

Figure 2. Different configurations of the ultrasonic separation device ADI 1015 (Applikon); A) typical configuration 1 (period 1 in Figure 1, Table 1), B) modified configuration 2 without using pump (R) (period 3 in Figure 1, Table 1), C) modified configuration 3 (period 4 in Figure 1, Table 1), D) improved configuration 4 by using pumps H_1 and H_2 (discussed in text).

fusion rate was increased from 0.0469 to 0.1215/h. The cells were infected at 506 h with recombinant baculovirus without medium change. During period 5 the perfusion was stopped and the accumulation of the

recombinant protein was equivalent to that in spinner cultures. Using an inducible or a lytic expression system without perfusion during the production phase, as shown here, configuration 3 (Table 1, Figures 1, 2C) is

very useful because the addition of PBS or medium for pushing back the cells to the reactor has no negative effect on the final product concentration in the reactor. In order to avoid product dilution in continuous production systems, a modified configuration (not used in this study, Figure 2D) should be used, which was already suggested for configuration 1 (Figure 2A) by Gaida et al. (1996) for perfusion cultures with very high cell densities for assuring a complete removal of accumulated cells from the separation chamber. The side entrance of the retention device which is normally used for the circulation of the cell suspension (Figures 1, 2A, Table 1, configuration 1) is not in use and the flushing back of the sedimented cells is done by pump H₂ which pushes back the interior volume of the device and the connecting tube (device – reactor) every 9'16.75" for 67" (here chosen for pump H₂ working with a rate of 10.5 ml/min). During this time, pump H₁ is stopped. A pump working in both directions, of course, can replace pumps H₁ and H₂. In a more general case, the indicated time periods for extracting supernatant and backflushing have to be chosen according to the cell density as well as to the rate of the pump; e.g. a higher cell density will need a more frequent backflushing, and the duration of backflushing depends directly on the pumping rate of pump H₂. In addition, a maximum allowable period between backflushes will be necessary to assure periodic removal of all cells from the device back into the reactor. This configuration was not tested in this study because for lytic production systems it is not necessary, however, in preliminary tests configuration 4 showed satisfying results.

In conclusion, the ultrasonic separation device ADI 1015 is very useful for increasing the cell density in perfusion reactor systems, however, for very sensitive cell lines as well as for microcarrier cultures the standard configuration has to be modified. The modified configurations 3 or 4 (Table 1, Figures 2C, 2D) are recommended for the retention of sensitive cell lines and a correct retention of cells (shear sensitive or not) with a high viability can be obtained.

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References

- Gaida Th, Doblhoff O, Strutzenberger K, Katinger H, Burger W, Gröschl M, Handl B and Benes E (1995) Scale-up of ultrasonic resonance field cell separation devices used in animal cell technology. In: EC Beuvery et al. (eds) *Animal Cell Technology: Developments towards the 21st Century* (pp. 699–703), Kluwer Academic Publishers, Dordrecht.
- Gaida Th, Doblhoff O, Strutzenberger K, Katinger H, Burger W, Gröschl M, Handl B and Benes E (1996) Selective retention of viable cells in ultrasonic resonance field devices. *Biotechnol Prog* 12: 73–76.
- Kretzmer G and Schügerl K (1991) Response of mammalian cells to shear stress. *Appl Microbiol Biotechnol* 34: 613–616.
- Trampl F, Sonderhoff SA, Pui PWS, Kilburn DG and Piret JM (1994) Acoustic cell filter for high density perfusion culture of hybridoma cells. *Bio/Technology* 12: 281–284.
- Zhang J, Collins A, Chen M, Knyazev I and Gentz R (1998) High-density perfusion culture of insect cells with a BioSep ultrasonic filter. *Biotechnol Bioeng* 59: 351–359.