ABSTRACT

In this present study, we have used macroporous cryogel column and discs as a novel supporting material for hybridoma cell culture for continuous monoclonal antibody production. A hybridoma clone producing anti-beta lactoglobulin monoclonal antibody (H9E10) was obtained and characterized in terms of their class subclass determination, light chain variable, antibody titer, antigen sensitivity and specificity. Initially, the growth pattern and mAbs production using conventional T-flasks were analyzed.

Purification of the mAbs based on pseudo-bioaffinity chromatography was carried out using a novel convective interaction media (CIM)-Histidine monolithic column. It was observed that the peak fractions eluted with 0.2M NaCl in 25 mM MOPS buffer, pH 6.5, had a purification fold of about 5.5. Initially, bioreactor experiments were carried out using smaller volume cryogel matrix (a single monolith column of 1.27ml bed volume; dimensions, 9mm dia and 2cm ht) and it showed that the mAb production was maximum on day 14 with mAb concentration of 81.4μg/ml. The study of cryogel with scanning electron microscopy showed that cells were attached to the matrix and grows more and more with time inside the gel.

Later, experiments were carried out using the bigger cryogel matrix (disc shaped gel of 35.32ml bed volume; dimensions, 75mm dia and 8mm ht) based mini-bioreactor system. The results of the bioreactor run showed that the maximal mAb concentration and overall productivity of hybridoma cells during medium reservoir-9 (day 20) was higher than that from other medium reservoirs. The maximum secretion of mAb was observed on twentieth day with a concentration of 236μg/ml. Moreover, when compared to the mAb production in T-flask batch cultivation which was ~ 26.57μg/ml, the production in the mini bioreactor set up was nine times greater (observed on twentieth day). The unique advantage of this mini bioreactor set up is simple operation with the added benefit of continuous control over production levels during a run. The detailed procedures of the methods involved and the discussion of the results obtained from the experiments including characterization, purification and the cryogel-based bioreactor studies carried out have been discussed in the following chapters.