Chapter 5

General conclusions and future perspectives

Monoclonal antibodies (mAbs) have tremendous applications in the field of clinical research, diagnosis and immunotherapy. Commercially, mAbs are produced in vivo cultivation as ascites fluid in mouse and in vitro cell culture in flasks or bioreactors. Nowadays in vitro production technology plays a vital part than in vivo production due to the animal ethical concerns. The small-scale suspension cell culture production of mAb utilizes devices such as spinner flasks, roller bottles and shake-flasks. The major drawbacks of the above systems are its low cell density (due to oxygen limitations) and batch method where the concentration of mAb released in the culture medium varies between 10 and 100 μg/ml. For large-scale mAb production, different types of cell culture bioreactor systems are available, for example, stirred tank bioreactor, hollow fiber systems, wave bioreactor and fixed-bed reactors.

High cell density and good long-term culture stability are two key factors in developing a continuous bioreactor system. Nevertheless, hybridoma cells are usually difficult to culture as suspension in a bioreactor because of their sensitivity to shear and bubble damage. Thus, various immobilization techniques have been studied. Among them, recently (Nilsang et al., 2007) have described a supermacroporous polyacrylamide gel, called cryogel (pAAM-cryogel), matrix as a polymeric support for the cultivation of mouse hybridoma cells.

The aim of the present study was to develop a mini-bioreactor system using different dimensions of macroporous cryogel (as column and discs) as a novel supporting material for hybridoma cell culture for continuous monoclonal antibody production. Comparison of antibody production from cryogel-based bioreactors with conventional T-flask batch culture method was also carried out. In a recent study, we have reported a successful separation of IgG1 monoclonal antibody from ammonium sulphate precipitated and dialyzed cell culture supernatant by pseudobioaffinity chromatography, using a novel
monolithic convective interaction media (CIM)-Histidine disk (Premkumar et al., 2012). Here, we extended the application of the above method for purification of mAbs under this study. In this context, the monolithic CIM-Histidine disk was used for fast pseudobiospecific separation and purification of monoclonal antibody from cell culture supernatant. The results obtained and conclusions made out of this research work are summarized as follows:

- A clone producing anti-beta lactoglobulin monoclonal antibody (H9E10) was obtained from Prof. Anjali A. Karande, Dept. of Biochemistry, Indian Institute of Science (IISc), Bangalore and further characterized in terms of their class and subclass determination, light chain variable, antibody titer, antigen sensitivity and specificity.

- The mAbs produced by the above clone was subjected to isotyping and it was found that mAb belongs to IgG₁ having kappa (κ) light chains.

- The antibodies possessed high sensitivity towards antigen and it was observed that the antibody titer was high for the clone with a titer of 1: 32768.

- In addition to the ELISA based experiments, the Western blot analysis demonstrated the reactivity / specificity of the anti-β lactoglobulin mAb with the membrane blotted commercially available β-lactoglobulin antigen.

- Initially, the growth pattern and mAbs production in serum supplemented media using conventional T-flasks were analyzed. The cell density reached maximum on day three and the specific antibody secretion was observed to be maximum on the ninth day of culture.

- The mAb concentration from the sample taken during the peak production in T-flask culture was found to be ~ 26.57 μg/ml.
• A rapid and efficient purification of the mAbs 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.

• Moreover, it also showed good purity of IgG1 with higher specific activity based on SDS-PAGE analysis and ELISA.

• In the present study, mini-bioreactor setup was developed by us with cryogel matrix for hybridoma cell immobilization and continuous production of monoclonal antibody. For this study, cryogels of two different dimensions were utilized.

• The initial experiments carried out with smaller volume gel (a single monolith column of 1.27ml bed volume; dimensions, 9mm dia and 2cm ht) showed that the mAb concentration and overall productivity of hybridoma cells during medium reservoir 2 was higher than during medium reservoir 1.

• The glucose consumption rate of the second reservoir was found to be 3.06mM/day and the production rate of lactic acid was 4.06mM/day which was also found to be higher than that of the first reservoir.

• The mAb production in the tube shaped smaller gelatin-cryogel matrix was found to be the maximum on day 14 where the concentration reached 81.4 μg/ml.

• Moreover, the experimental results revealed that after cell grew for 14 days in cryogel column, and thereafter the toxic by-products that produced from cells induced apoptosis and limited antibody production even when fresh medium (reservoir 3) was replaced in the system on 15th day.
• The cell density and morphology on the cryogel matrix for different periods was studied using scanning electron microscopy. The micrographs showed that cells were attached to the matrix and grow more and more with time inside the gel.

• Experiments were carried out with the mini-bioreactor designed by us using the bigger gel (disc shaped gel of 35.32ml bed volume; dimensions, 75mm dia and 8mm ht) for the production of monoclonal antibodies.

• We found that the hybridoma cells got immobilized on the gelatin-cryogel disc over 12 h of incubation after seeding of cells.

• On fourth day, the glucose concentration in the medium reservoir was found to be 50% of the initial concentration and the medium was replaced with fresh medium (reservoir-2).

• The glucose consumption rate of the reservoir-2 was found to be higher than from that of the reservoir-1. The production rate of lactic acid and monoclonal antibody were also found to be higher than that of the reservoir-1.

• Then onwards, the medium was changed every 2 days as the glucose concentration reached below 50% of the initial concentration after every 2 days and the reactor was operated continuously for 26 days.

• The results of the bioreactor run showed that the maximal mAb concentration and overall productivity of hybridoma cells during medium reservoir-9 was higher than that from other medium reservoirs.

• The maximum secretion of mAb was observed on twentieth day with a concentration of 236 μg/ml.
• Moreover, when compared to the mAb production in T-flask batch cultivation which was ~ 26.57 μg/ml, the production in the mini bioreactor set up was nine times greater (observed on twentieth day).

• The major benefit of this cryogel based system can be its use as a disposable bioreactor for process development of monoclonal antibody production. 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 purification strategy employed in our study for mAb purification is an efficient and rapid method which may be a useful alternative to the conventional gel or membrane base systems or the bioaffinity ligands such as Protein A / G. Moreover, faster separation of mAb under non-denaturing buffer conditions could be achieved with CIM-disks, which will be more economical when compared to the conventional methods of purification. It may be worth exploring this method for the purification of monoclonal antibodies in general. The results obtained from the present mini-bioreactor study may be utilized and extended further in future for different cell cultures for production of other molecules. The mini-bioreactor system developed in the present study may also be tried for the growth of other cell systems. In addition to that, the use of even more bigger size with larger volume of medium reservoir might be carried out for larger scale production of mAbs.