ABSTRACT

Up to 7 % (v/v) of worldwide CO_2 emissions are caused by flue gases released by coal-fired thermoelectric facilities, with the flue gases emitted from the power plants consisting of 10 to 15 % (v/v) of CO_2. Increased CO_2 levels in the atmosphere are the cause of global warming and climate change. With a high dependence on fossil fuels, global CO_2 emissions continue to increase. An independent research perspective towards the development of a sustainable technology for CO_2 sequestration is being examined in light of the enormous need to capture and utilise CO_2 for lowering the environmental effect. Aligned with this initiative is the development of a microalgae-based carbon sequestration process, which can be attributed to the microalgae's inherent capability of growing at high CO_2 concentrations and accumulation of a high intracellular protein composition; providing an opportunity to be an attractive alternative aquafeed, thereby making the process sustainable and economically feasible. The development of an industrially practicable and economically sustainable process strategy for microalgal-based aquafeed production is currently hampered by a number of barriers. Such incipient bottlenecks can serve as motivation for the development of an aim for the selection of novel strains and the design of novel process strategies; which will boost research towards the industrialization of microalgal-based aquafeed.

The current research seeks to comprehend and address the aforementioned obstacles by screening the high CO_2-tolerant novel microalgal strain *Desmodesmus pannoniccus* CT01 and development of process engineering strategy for protein-rich high cell density cultivation. This research also focuses on real assessment of the biomass performance of novel microalgal species as an alternative raw material for aquafeed. These objectives were accomplished via the combined approach of (i) selection of potential high CO_2 tolerant microalgal strains and characterization under various nutritional and physicochemical parameters; (ii) real time evaluation of microalgae species performance as a feed supplement for aquaculture; (iii) process engineering for cultivation of high-density protein rich novel microalgal isolate and finally, (iv) demonstration of the integrated sustainable microalgal feed technology at large scale of 50 L photobioreactor.

The study begins with the screening of CO_2 tolerant strains present in the aqueous sample, collected from industrial hotspot. This was achieved by a novel CO_2 selection pressure-based screening strategy where the enriched mixed culture was exposed to sequentially elevated concentrations of CO_2 mixed with air stream starting from 5% to 25% v/v, with step-wise increase by 2.5% v/v. A unique indigenous freshwater microalgal strain *Desmodesmus pannonicus* CT01 (Accesion Number: OL470985) that could tolerate and sustain much higher CO_2 concentration of up to 25% v/v was isolated and identified. CT01 was further subjected to different suitable nutritional and growth conditions and characterization was carried out under different media compositions, various initial pH of the culture medium, nitrogen sources, and phosphate sources supporting maximum growth of CT01. Further to evaluate the application potential of the microalgal biomass as a carbon sequestration house, the influence of CO_2 concentration in the inlet gas steam on growth and CO_2 sequestration ability of the organism, experiments were performed under six different CO_2 concentration such as 0.03 (air), 5, 7.5, 10, 12.5, 15 and 20%, v/v. The strain unveiled optimal growth performance with CO2 concentration in the range of 10 -15%, v/v. The highest biomass titre and productivity of 1.42 g L-1 and 101.43 mg L-1 d-1, respectively was recorded with estimated CO_2 fixation rate of 159.91 mg L-1 d-1, when grown at 12.5% CO_2. The intracellular total protein content of 49.53 (w/w) was found to be highest in 12.5% CO_2 hinting the possible application of CT01 biomass as potential alternative aquafeed.Owing to its high protein content, CT01 biomass was

evaluated as potential alternative to commercially available conventional fish feed. Three different feedstocks were considered to evaluate growth metrics and dietetics of *Hypophthalmichthys molitrix* (silver carp): microalgae feed (MiF), that is whole cell biomass of CT01; reference feed (ReF), composition of which is equivalent to the commercially used feed for silver carp, and mixed feed (MixF) comprising both microalgae and reference feed in 1:1 ratio. Mixture of microalgae with reference feed (1:1), resulted in significant improvement in growth matrices such as final body weight (mg), average weight gain (mg day^{-1}) and specific growth rate (% day^{-1}) of fish fry as compared to the reference feed. Similar to the growth performance, dietetics of the fish fry in terms of feed efficiency, protein efficiency ratio and protein productive value was found to be higher while fed on mixed feed as compared to the reference feed. The quality of fish in terms total protein content was estimated to be highest at 60% for microalga feed, followed by mixed feed at 59% and reference feed at 54%. This suggests better digestibility of microalga protein by the fish as compared to reference diet.

Further to enhance the cell density and protein content of CT01, a process engineering strategy was developed. At the onset, the media components, concentrations of initial phosphate, initial nitrate and trace and micro elements, were optimized for improved biomass titre and productivity using response surface methodology (RSM) based central composite design (CCD). Validation of the model was confirmed by comparing the predicted biomass concentration (0.9778 g L^{-1}) with the experimental value (0.9649 g L^{-1}) and predicted biomass productivity (32.11 mg L^{-1} d^{-1}) with the experimental value (31.39 mg L^{-1} d^{-1}) at optimized concentration of nitrate (0.79 g L^{-1}), phosphate (0.185 g L^{-1}) and TME (0.73-unit L^{-1}). CCD-RSM based optimization of the process variables resulted in 28 % increase in biomass titer and 27% increase in biomass productivity while compared to un-optimized condition. With the optimized medium, the CT01 was subjected to different light wavelength and intensity to improve the total protein content in the biomass composition. Experiments were performed

under exposure to seven different wavelength of light combining mono and multi wavelengths *i.e* white, blue, green, red, red-blue, red-green and blue green and subsequently under exposure to four different light intensity ranging from 50, 100 ,150, 200 µE $m^{-2}s^{-1}$ to enhance the biomass productivity, total protein content and carbon sequestration ability of the strain. The biomass concentration and biomass productivity were found to be highest when exposed to combination of red-blue light wavelength and attain the maximum value of 1.38 g L^{-1} and 81.7 mg L^{-1} d^{-1}, respectively. As the CO_2 sequestration ability of the microalgae has a liner correlation with the growth kinetics, the highest CO_2 fixation rate of 144.15 mg L^{-1} d^{-1} was found when exposed to red-blue light. Total protein 54.6 % (w/w) was found to be highest with red-blue light wavelength enhancing the credibility for biomass as a suitable candidate for alternate aquafeed. Total protein of the biomass and carbon sequestration rate of CT01 were also found to be higher with the light intensity of 150 µE $m^{-2}s^{-1}$. The interdependent dynamics of light intensity, growth and culture pH indicated that the process engineering strategy, based on on-demand supply of CO_2 under optimized light wavelength and intensity, may results in improved biomass concentrations and productivity by maintaining optimal culture pH and eliminating CO_2 limitation. Two parallel batches of CT01 were performed where all the growth parameters were kept identical except in one batch, the pH of the culture was maintained at optimal value of 8 through cascade driven intermittent purging of CO_2 and in another batch, culture pH remained uncontrolled. The batch with controlled pH resulted in maximum biomass concentrations of 1.93 g L^{-1} , productivity of 145.4 g L^{-1} day^{-1}, CO_2 fixation rate of 259 mg L^{-1} day^{-1} shows improvement as in when compared with the batch with uncontrolled pH.

With the view of evaluation of developed process engineering strategy for CT01 at large scale, cultivation was carried out in a novel customised airlift photobioreactor (APBR) of 50 L volume. The scaling of APBR up by volume while trying to maintain sufficient CO_2, light availability to cells and minimal shear forces was the key areas to be considered for. The design of novel APBR was targeted to minimal the risk of scaling up without compromising the growth and intracellular properties of the strain. The biomass composition obtained from both batches suggest the reactor performs well and also successful in minimising the risk. Next to it, the high protein rich fresh microalgal biomass was subjected as aquafeed for the model fish *Cirrhinus mrigala* (mrigal carp). The fish growth performance was evaluated in terms of growth metrices and biomass composition. The live fresh biomass of CT01 showed no negative impact on the fish as compared to reference feed suggesting its potential to be an alternative aquafeed.