Chapter 5

Conclusion

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Hyaluronic acid production was performed using *Streptococcus zooepidemicus* MTCC 3523 procured from IMTECH, Chandigarh, India. Microbial fermentation was achieved using a defined medium with growing conditions of 100 rpm, 6.8 pH, and 37 ºC temperature in the shake flask.

The fermentation medium was optimized for the improved production of hyaluronic acid using one variable at a time (OVAT) method. The agro-industrial waste molasses proved an excellent carbon source for better hyaluronic acid production. Molasses improved the growth of *Streptococcus zooepidemicus.* The enhanced production of hyaluronic acid was due to the carbohydrate source and solid interactions among medium components.

The results of the ANOVA analysis gave the optimized value for the hyaluronic acid production utilizing molasses. The optimum combination of different parameters for purification of hyaluronic acid obtained from surface plots for pH, molasses, temperature, and agitation was 6.46, 9.76%, 36.2 ºC, and 207 rpm, respectively. The maximum predicted percentage of production of HA was 3.45 g/L. To confirm the expected response, experiments were conducted in triplicates. Experimentally, the maximum output of HA was found to be 3.36 g/L, which was close to it. The results of this study should be used to design and enhance hyaluronic acid production.

Hyaluronic acid production was then carried out in a 3.7 L stirred tank bioreactor with two impellers. Kinetic analysis was performed for the production of hyaluronic acid. Because the culture was active, the growth phase began immediately after inoculation and lasted for 28 h, during which time the maximum rate of hyaluronic acid was achieved.

The process parameters, such as agitation and aeration rates, were also optimized for better production of hyaluronic acid. Bench-scale fermenters were used to study aeration and agitation rate impacts on the hyaluronic acid production from *Streptococcus zooepidemicus*. HA production enhanced with increasing agitation and aeration rates at the batch fermentation but decreased with increased agitation in the bioreactor due to the shear thinning feature. Therefore, the relevant criteria for scaling up the fermenter are maintaining DO above the critical value and agitating the fermenter mildly for homogeneity. HA maximum yields were obtained at pH 7 and 37°C at 2.0 vvm aeration rate and 250 rpm agitation speed. Using the dynamic gassing-out method, the volumetric oxygen transfer coefficient k_La was studied concerning aeration and agitation rates. The volumetric oxygen transfer coefficient enhanced with a rising aeration rate (13.43 to 15.08 h^{-1}) and agitation rate (14.64 to 19.02 h^{-1}). The oxygen uptake rate (OUR), primarily controlled by k_La, should be used as the online control parameter for scaling up HA production. Volumetric oxygen transfer coefficients were used to scale up the pilot experiment successfully.

Because of its outstanding precision and resilience, the GRNN is recognized as the preferably appropriate ANN model based on modeling findings. Due to its reliable and accurate testing findings, another acceptable alternative model is the SVM. Repeated trials show that the GRNN and SVM exhibit 100% prediction accuracy when the tolerance is 30%. According to the results, the GRNN and SVM can be used to optimize for the HA fermentation conditions. The GRNN and SVM provide various benefits over the MLFNs and other algorithms offered by previous research, notably low RMS error, time efficiency, and user-friendliness. A wide scale of training data can prevent over-fitting because it can eliminate the local over-fitting phenomena, a feature of machine learning models.

Therefore, a more significant sample size may enhance the prediction results. One can logically expect that in future practical applications, more data collected from industrial mass production can guarantee the resilience of a model and higher availability for improving the HA production environment.

The results of the purification process demonstrated that the precipitation, adsorption, and diafiltration under the selected operating settings are well suited for HA purification from a complex mixture. By utilizing ethanol diluted with distilled water (3:2) and 1 M NaCl solution, together with centrifugation, it was possible to remove the cell debris and other insoluble contaminants. Endotoxins, high molecular weight proteins, nucleic acids, and extraneous color were all eliminated from the fermented broth after further processing with adsorbents. While DARCO® was found to be the best for removing endotoxins, nucleic acids, and proteins, XAD-7 helped in adsorbing both the contaminants and the removal of charcoal. The diafiltration process helped to attain the maximum purity of HA. After seven diavolumes, a purity value close to 90% was obtained, producing a yield of 79.16% with an approximate molecular weight of 600 kDa. FTIR, XRD, and NMR spectroscopic analysis revealed that the HA generated in this investigation had chemical characteristics identical to the standard HA. The antioxidant capacity of the HA showed that the refined HA polymers are an outstanding contender for numerous biocompatible applications. The encouraging findings obtained with PI in this investigation can be scaled up for purification.

Studies were conducted on hyaluronic acid synthesized with the help of *S. zooepidemicus* to determine its antibacterial and antioxidant properties. This compound can potentially have antibacterial properties and be a valuable source for designing and developing new anti-infectives. Because of these qualities, hyaluronic acid can be employed in a wide range of products for cosmetic purposes. Two key targets were subjected to docking studies in order to determine a possible mechanism of action for this drug. Molecular docking and simulations indicated a relationship between the inhibitory activity and the tyrosyl-tRNA synthetase and topoisomerase II DNA gyrase in *S. aureus* and *E. coli.* Based on the findings, it may be concluded that hyaluronic acid could be suitable for anti-infective gels and other skin-care applications.

The current study attempts to conduct a comprehensive investigation into the synthesis of hyaluronic acid utilizing *Streptococcus zooepidemicus* by exploiting agro-industrial waste molasses through various optimization processes for production and strategizing its purification procedure. A detailed study of the characterization of hyaluronic acid and its antioxidant and antibacterial properties were also investigated.

Hyaluronic acid has a very extended market presence. Mostly, it is extracted from animal tissues, but in the last 2-3 decades, its microbial production has been practiced. Still, there is room for sustainability, accessibility, and efficacy. Inefficient procedures perform poorly and are often linked to increased errors and subpar products, which drives up the expense of punitive actions. There is a huge demand for HA in the market these days. To meet this demand, the candidate's research looks into unique cultural conditions that can increase the production of HA. Using new technologies in outdated methods can create a clear path with fewer potential errors. A detailed study of advanced analytical techniques to monitor the synthesis and purification of HA, utilization of agro-industrial wastes for the production process, and improving the quality and yield of the product most sustainably.

The investigation will expand in the future by studying metabolic flow analysis, which could provide information on modifying biosynthetic pathways to increase hyaluronic acid production. The future scope also aims to create microbial-based nanogels that can treat infections in burn wounds. The market is filled with many formulations of topical gels. However, this gel's main characteristics are the green synthesis of the ingredients used and the scalable manufacturing procedures for these gels, which use hyaluronic acid's hydration and tissue regeneration capabilities, curcumin's antibacterial qualities, and lavender oil's cooling effect. These are all in their nano-conjugation, resulting in a high and sustained absorbance rate. One possible way to replace animal-based products is to use agricultural biomass waste to produce metabolites and nano-formulations through biological techniques.