Abstract

Lipopeptides are ecofriendly green surfactants with myriad applications in the areas of healthcare, energy and environment. Their increasing demand compounded by poor yields call for the development of improved and efficient bio-manufacturing processes. In this regard, the present thesis endeavors to design and optimize a bioprocess for enhanced production of lipopeptide biosurfactants from marine bacterium *Bacillus megaterium*. Firstly, critical process variables were optimized to increase lipopeptide production from food waste by a combined artificial neural network–particle swarm optimization (ANN-PSO) technique. Batch cultivation of *B. megaterium* under the optimized process conditions in 3.7 L stirred tank reactor resulted in a lipopeptide production of 6.58 g L^{-1} at a rate of 148 mg L^{-1} h^{-1}. The productivity was further improved by employing two nutrient feeding strategies namely, DO-stat feeding and cyclic feeding. The DO-stat feeding increased the lipopeptide productivity to 177 mg L^{-1} h^{-1}, while cyclic feeding enhanced it to 224 mg L^{-1} h^{-1}. The resulting crude product composed of three lipopeptide families such as iturin, fengycin and surfactin, with each family comprising a mixture of homologues. In order to recover and separate lipopeptide families from crude mixture, a simple and efficient pH-solvent double gradient elution strategy was devised in macroporous resin column chromatography (MRCC). Consequently, lipopeptides were effectively resolved and purities up to 68.3% (iturin), 77.6% (fengycin) and 91.6% (surfactin) were achieved. Finally, a rapid RP-HPLC method was developed and optimized using analytical C18 column for efficient purification of homologues of the three lipopeptide families. The optimized conditions of analytical HPLC were then systematically scaled-up to semi-preparative HPLC without affecting purification efficiency and product quality. The partially purified surfactin product obtained after MRCC was evaluated for its ability as encapsulating and stabilizing agent in microbubble synthesis. Thus, the thesis showcases strategic development and optimization of an improved bio-manufacturing process for enhanced production and purification of lipopeptides and application of one purified lipopeptide in microbubble generation and stabilization. To the best of our knowledge, the thesis adds new scientific values to the relevant world literature in terms of application of ANN-PSO as an optimization strategy, development of dual gradient MRCC for simultaneous recovery and purification of individual lipopeptide families, semi-preparative purification of lipopeptide homologues and use of lipopeptide as stabilizing agent for microbubbles.