Evolutionary and swarm intelligence in optimization of α-amylase from Bacillus velezensis sp.

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Abstract

Background: To optimize α-amylase synthesis from fermented broth, this research employed evolutionary and swarm intelligence-based techniques such as genetic algorithm (GA) and particle swarm optimisation (PSO). The nonlinear response surface model (RSM) of α-amylase from Bacillus velezensis sp.was used as the objective function. Results: In contrast to the enzyme activity, 418.25 U/mL, achieved by the thirteen factors OFAT technique was increased by 2.76 times using GA and PSO optimization with only nine significant parameters. The optimal process parameters predicted using GA and PSO were pH (5.37), temperature (34.18 ^{o}C), carbon source (4.12%), nitrogen source (2.04%), K_2PO_4 (0.34%), $MgSO_4$ (0.2%), NaCl (0.14%), fructose (2.0%), and $NaNO_3$ (0.47%). Conclusions: The results showed that α-amylase activity was significantly improved with both approaches, with similar predictions of optimized process parameters and α-amylase activity of 1157.2 U/ml. On the other hand, PSO surpasses GA in terms of optimized α-amylase activity and convergence rate, which might be attributed to its simple structure and efficient memory capabilities. In conclusion, the suggested GA and PSO techniques are thought to be natural and may be used instead of existing gradient-based optimization strategies in downstream enzyme processing.

Keywords: *α-amylase, Bacillus velezensis sp., Genetic algorithm, Optimization, Particle swarm optimizatio*

1.0 Introduction

Background: *α-amylase* is a starch-degrading enzyme with numerous commercial and pharmacological applications in various sectors, including washing, textiles, chocolate, detergent, and biofuel manufacturing. It is crucial in the development of digestive aids, the preparation of biodegradable polymers, and the use of cross-linked starches in tablets.

Aim: This research aims to optimize microbial fermentation process variables in enzyme recovery from the fermented broth using global evolutionary optimization algorithms, such as artificial and swarm intelligence-based approaches.

Review of literature:[1-10] Nature-inspired optimization techniques, such as ant colony optimization (ACO), particle swarm optimization (PSO), and firefly optimization (FO), have been developed to analyse problems in computational studies. Evolutionary optimization techniques are used in physiochemical parametric studies, based on biological evolution of reproduction, mutation, and other factors. Swarm optimization techniques, such as particle swarm, beehive, and ant-colony optimization techniques, are considered robust, simple, and easier to handle. Genetic algorithm (GA) is the best explanation for microorganism growth, as it involves factors such as crossover or recombination and mutation. The statistical association between the ultimate goal (*α-amylase* activity) and each of the independent variables was determined using a second-order polynomial equation. GA is an evolutionary algorithm that mimics natural evolution and involves three operators: reproduction, crossover, and mutation. Reproduction selects good strings in a population and forms a mating pool. Crossover allows for new string formation by exchanging strings with another chromosome. Mutation perturbs the child vector, achieving local search and maintaining diversity. Scale-up studies using optimization techniques are crucial for better productivity, reduced cost, and time. Advanced techniques like ANN and nature-inspired optimization are more suitable for scaling up of SmF and SSF processes, as they help understand the growth of microorganisms and their impact on the overall process.

Objective: The objective is based on a nonlinear response surface model (RSM) of *α-amylase* from *Bacillus velezensis sp.*, which is used as the objective function. Response surface methodology (RSM) optimization was applied to consider only nine significant process variables: pH, temperature, agitation, inoculum size, aeration, carbon source, nitrogen source, K_2PO4, $MgSO_4$, NaCl, incubation period, fructose, and NaNO3. This study aimed to optimize the nonlinear RSM model of *α-amylase* fermentation from *Bacillus velezensis sp.* using artificial intelligence-based GA and swarm intelligence-based PSO approaches. GA is an evolutionary algorithm that mimics natural evolution, involving reproduction, crossover, and mutation.

2.0 Materials and methods [11-20]

2.1 Materials and microorganism

All reagents used in this study were purchased from HiMedia Labs, C40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane (West) - 400 604, Maharashtra, India. *Bacillus velezensis* was obtained from School of Pharmaceutical Sciences, Lovely Professional University, Punjab, India. The chosen strain is purified using the streak plate technique and kept at 4°C as slant cultures.

2.2 Methods

Submerged fermentation

In order to evaluate the growth pattern, *Bacillus velezensis sp.*, a potential α-amylase-producing bacterial strain, is cultured in seed media containing Luria-Bertani broth. A 2% inoculum of the 16-hour-grown seed medium is added to basal media containing (g/L) starch 5.0, peptone 20.0, $MgSO_4$ 1.0, and K_2HPO_4 3.0, and the incubation process is repeated. Every hour, turbidity at 600 nm and the 3,5-dinitro salicylic acid test (DNSA) are used to determine the rate of bacterial growth and the synthesis of the enzyme -amylase. To get the crude extract, which serves as an enzyme source for the *α-amylase* test, the medium is centrifuged at 5000 RPM for 15 minutes. Each test is run three times, and the average results are reported. The bacterium strain with the highest α -amylase activity is then cultured in a seed medium that has been specially designed for it.

2.3 Enzyme Assay

The 3, 5-dinitro salicylic acid test is used to measure α -amylase activity. At pH 5.5 and 55 °C, one mL of 1% starch is incubated with 0.05 mL of enzyme-containing supernatant for 8 minutes before adding 0.5 mL DNSA to the reaction mixture. After 10 minutes in a boiling water bath, the reaction mixture is cooled and 3.45 mL of distilled water is added. The released reduced sugar is measured at 540 nm by using Miller's method. Blank is prepared without the use of enzyme and the concentration of protein is estimated by Lowry's method.

3.0 Optimization methodology

3.1 Statistical optimization of α-amylase production process parameters by RSM

The second-order polynomial response surface model RSM design was developed at three levels in the selection of each independent bioprocess parameter. Thirteen variables were found to be potent among the total parameters evaluated for their significance on *α-amylase* activity in the OFAT method (results are not shown here), namely pH, temperature, agitation, inoculum size, aeration, carbon source, nitrogen source, K_2PO_4, $MgSO_4$, NaCl, incubation duration, fructose, and $NaNO_3$. The response function was estimated by a second-degree polynomial with quadratic and interaction effects using the least squares approach (Rajulapati et al. 2011). The Definitive Screen (DSD) Design was used to assess pH (A), temperature (B), carbon source (C), nitrogen source (D), K_2PO_4 (E), $MgSO_4$ (F), NaCl (G), fructose (H), and $NaNO_3$ (I) ranges for experimental investigation.

Table 1 shows the true ranges of coded factors based on the results of the OFAT technique (data is not provided). The statistical association between the ultimate goal (*α-amylase* activity) and each of the independent variables was determined using a second-order polynomial equation.

$$Y=1085.35+1.15A+0.25B+1.0C+1.5D+17.5E+6.0F+17.5G+1.0H+5.0J-1.69A^2-5.53C^2-24.33D^2-20.86E^2-16.33F^2-35.09G^2-5.34H^2-13.64J^2 \quad \text{Eq. } \mathbf{(1)}$$

Where, Y is the level of *α-amylase* activity.

Use of RSM produced the ensuing quadratic regression equation for the final response of *α-amylase* activity [Eq. (1)]. The ultimate objective's optimised bioprocess parameter values were identified as 5.20, 35.92, 4.22, 2.06, 0.34, 0.14, 0.23, 1.48, and 0.53, respectively, for pH, temperature, carbon source, nitrogen source, K_2PO_4, $MgSO_4$, fructose, and $NaNO_3$.

Table 1: Variables used in experimental design

Factor Code	Name	Lower limit (-1)	Upper limit (+1)
A	pH	4.0	6.0
B	Temp(^{o}C)	32.0	36.0
C	Carbon source (%)	3.0	5.0
D	Nitrogen source (%)	1.0	3.0
E	K_2HPO_4 (%)	0.20	0.4
F	$MgSO_4$ (%)	0.05	0.2
G	NaCl (%)	0.1	0.3
H	Fructose (%)	1.0	2.0
J	$NaNO_3$ (%)	0.3	0.7

3.2 Evolutionary and swarm intelligence-based optimization

This study aims to optimize the nonlinear RSM model of *α-amylase* fermentation from *Bacillus velezensis sp.* using artificial intelligence-based GA and swarm intelligence-based PSO approaches.

3.2.1 Genetic algorithm (GA)

GA is an evolutionary algorithm that mimics natural evolution. It involves three operators: reproduction, crossover, and mutation. Reproduction selects good strings in a population and forms a mating pool. Crossover allows for new string formation by exchanging strings with another chromosome. Mutation perturbs the child vector, achieving local search and maintaining diversity. The process is repeated until a termination criterion is met. In this study, a binary-coded GA was used to optimize *α-amylase* extraction from fermented broth for enhanced *α-amylase* activity. The minimization problem is converted to a maximization problem using negative sign before enzyme activity.

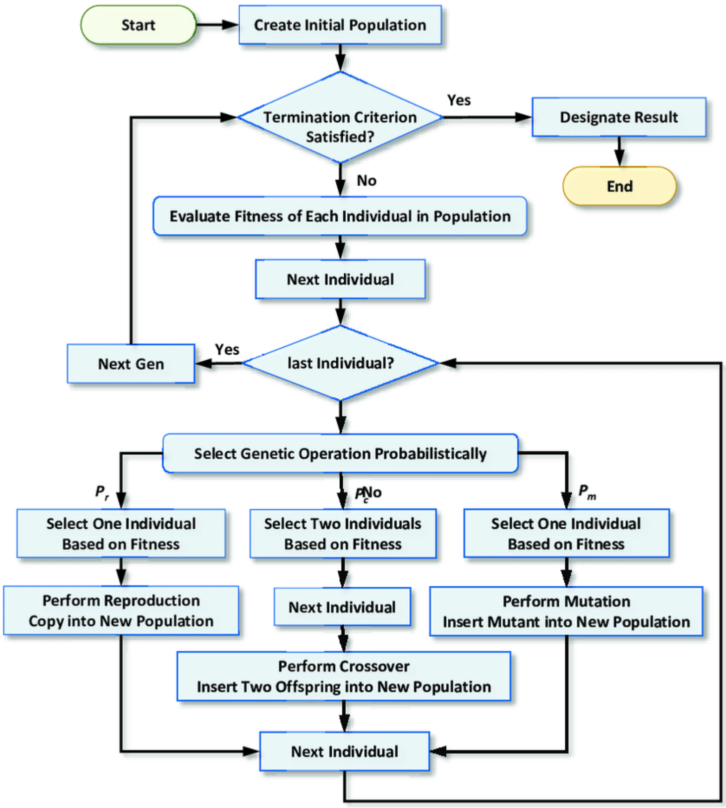

Figure 1. Flow chart of a simple GA.

3.2.2 Particle swarm optimization (PSO)

The swarm intelligence-based optimization (PSO) approach, developed by Kennedy and Eberhart, overcomes drawbacks of generalized optimization (GA) such as convergence toward local optima and difficulty in dynamic sets. PSO involves a population of particles, each with a memory for its previous best position. It has advantages over GA, such as easier implementation, fewer parameters needed for adjustment, and higher memory capability. However, PSO can sometimes suffer from premature convergence, leading to suboptimal solutions. This study uses the multi-objective PSO-crowding distance (MOPSO-CD) approach to solve the optimization problem of *α-amylase* extraction. MOPSO-CD incorporates the crowding distance operator, which affects global best selection criteria by deleting non-dominated solutions. The algorithm's mutation operator is adapted for exploratory capability, initially performing mutations on the entire population and rapidly decreasing coverage over time to prevent premature convergence. The MOPSO-CD algorithm's working procedure is shown in a flow chart in Fig 2.

To compute the new velocity, V[*i*]:
$V[i] = W * V[i] + R_1 * [Pbest (i) - P (i)] + R_2 * [A(Gbest) - P (i)]$

Where, W is the inertia weight, which is equal to 0.4, R1 and R2 are the random numbers in the range of (0-1), $P_{best}(i)$ is the best position reached by particle *i* and $A(G_{best})$ is the global best guide for each dominated solution.
To calculate the new position of P[*i*]:
P[*i*] = P[*i*] + V[*i*]
The MOPSO-CD algorithm was used to optimize lipase extraction by exploring the nonlinear RSM model and particle size parameters.

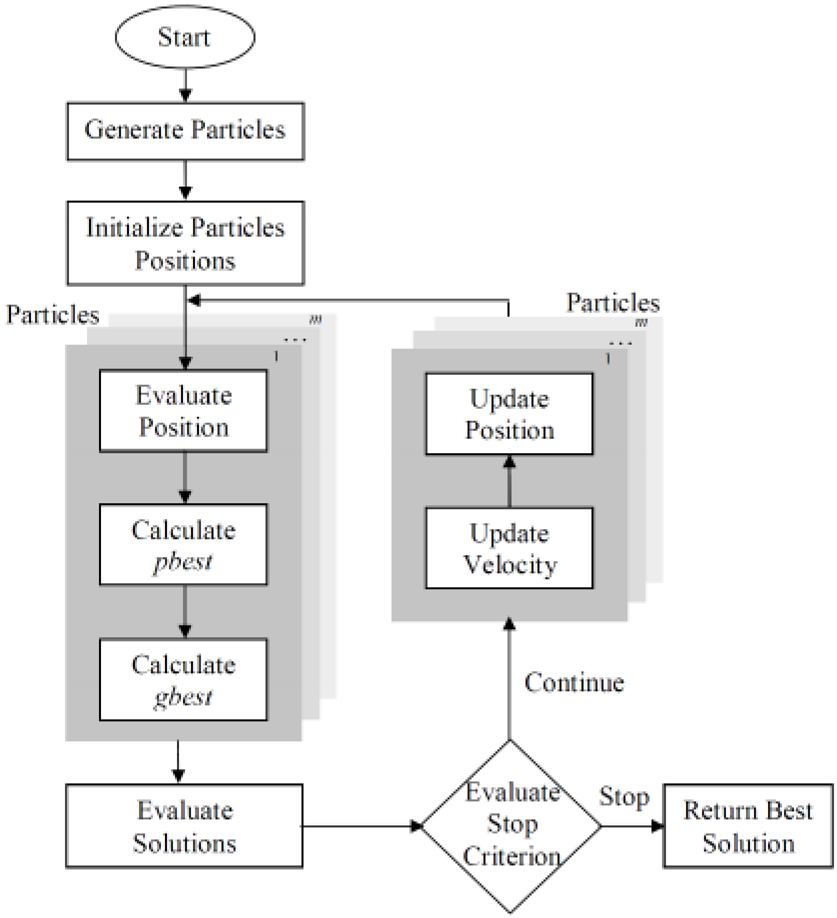

Figure 2. Simplified illustration of a sequence of events in the PSO approach.

4.0 RESULT [21-26]

Evolutionary and swarm intelligence-based optimization
α-amylase production process optimization using artificial and swarm intelligence-based algorithms enhances recovery and activity, offering flexibility, ease of operation, and global perspective.

4.1 Optimization by GA

GAs are an alternative to traditional optimization methods that struggle to predict optimal conditions in complex optimization problems with numerous local optima. The selection of population size, number of generations, mutation probability, and crossover mechanism play a crucial role in exploring the input space of the problem of interest by GA.

In this study, the RSM model of *α-amylase* extraction is posed as an optimization problem for maximizing amylase activity. A systematic study was conducted to determine the GA parameters responsible for an optimal value of amylase activity. The results showed that the majority of the population was in the group of 1150.0–1160.0 U/ml, with over 75% consistently above this value. The optimum values of the process parameters were found to be pH (5.37), temperature (34.18 ^{o}C), carbon source (4.12%), nitrogen source (2.04%), K_2PO_4 (0.34%), $MgSO_4$ (0.2%), NaCl (0.14%), fructose (2.0%), and $NaNO_3$ (0.47%). The maximum amylase activity for *Bacillus velezensis sp.* was found to be 1157.2 U/ml, with the maximum *α-amylase* activity of *Bacillus velezensis sp*. being 1157.2 U/ml. This optimized set of variables was chosen for experimental model validation. The experimental amylase activity under the stated conditions was found to be 1155.1 U/ml, which is in good agreement with the GA-optimized value. The OVAT approach was used to obtain amylase activity of 418.25 U/mL, indicating a significant improvement (2.8 folds) in *α-amylase* activity.

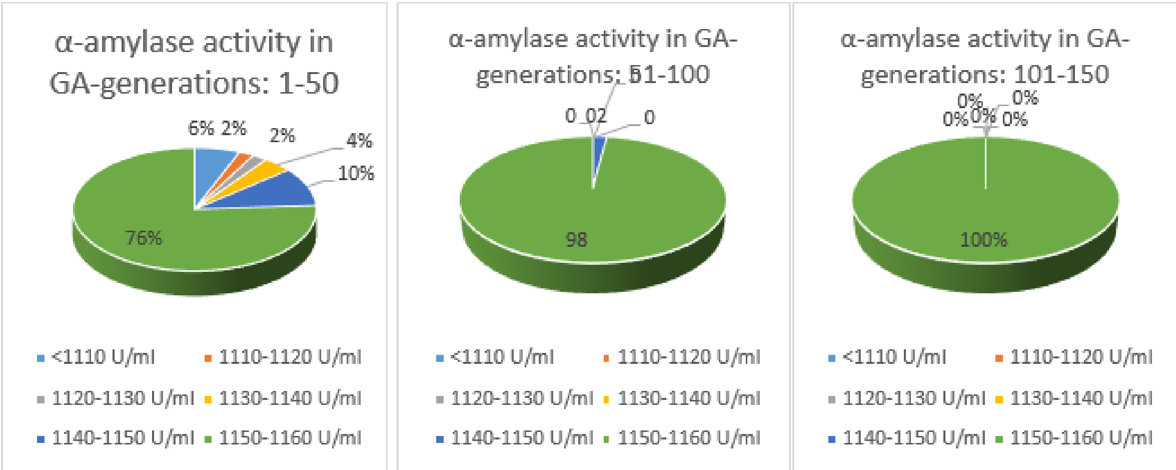

Figure 3. Population profile at different generations in the GA.

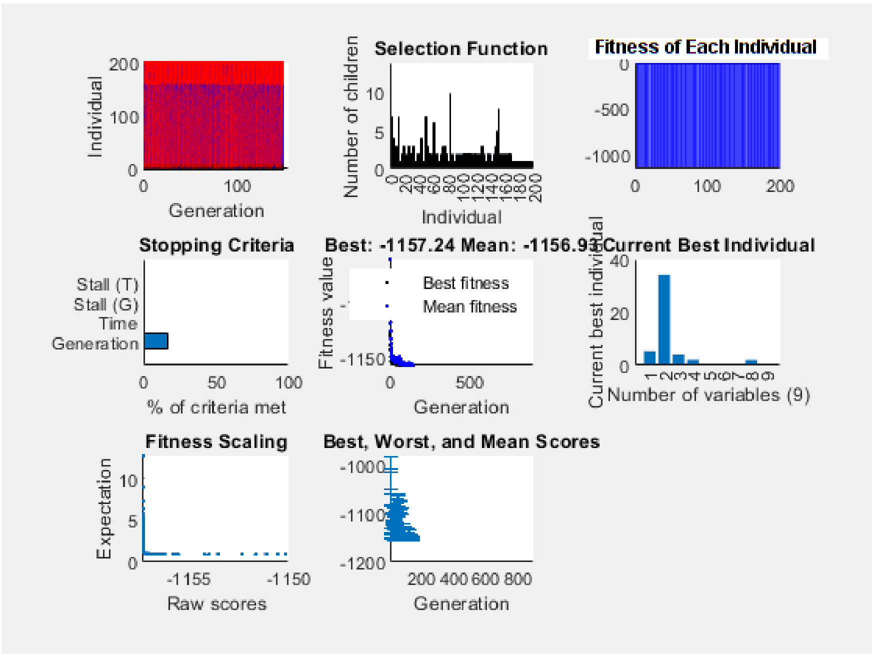

Figure 4. Results of a parametric study of GA.

4.2 Optimization by PSO

PSO is a computer-based approach that uses parameters like particle size, generation number, inertia weight, and cognitive and social components. Randomly generated particles with a specific size were used to generate optimal amylase activity. The optimal amylase activity of 1157.2 U/ml was obtained with the process variables of pH (5.37), temperature (34.18 ^oC), carbon source (4.12%), nitrogen source (2.04%), K_2PO_4 (0.34%), $MgSO_4$ (0.2%), NaCl (0.14%), fructose (2.0%), and $NaNO_3$ (0.47%). Confirmation experiments were conducted in triplicate with the PSO-predicted conditions, resulting in an experimental amylase activity of 1155.1 U/ml, which is in good agreement with the predicted value.

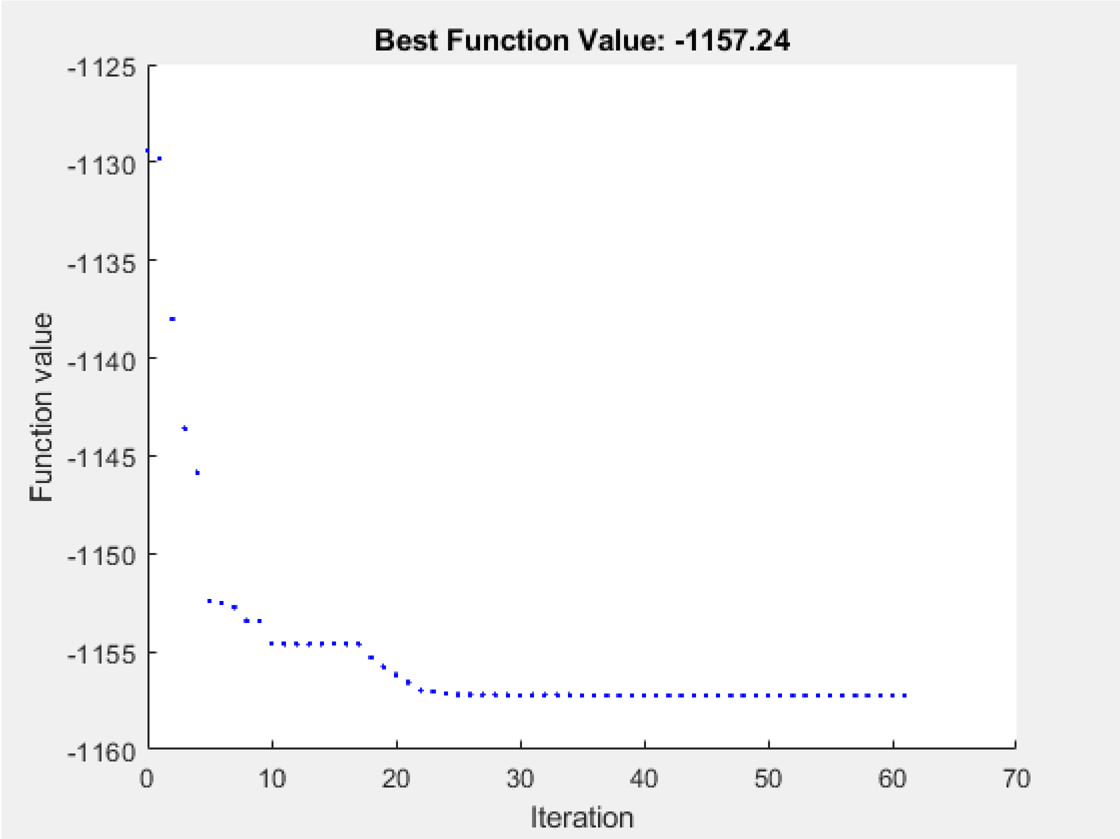

Figure 5. Variation of the objective function value with the number of generations in PSO.

5.0 DISCUSSION

5.1 Comparison between GA and PSO

The study analyzed amylase extraction optimization using GA and PSO approaches in fermented broth. Results showed that PSO converges faster than GA, with an experimental amylase activity of 1157.57 U/mL, which is in agreement with the predicted value. The PSO approach's optimal amylase activity and parameters are slightly better than GA due to easier implementation, fewer parameters, and higher memory capability.

Table 2: Experimental and predicted α-amylase activity (U/ml) with optimal variable conditions of OVAT, GA and PSO approaches.

	***α-amylase* production process parameters**									***α-amylase* activity (U/ml)**	
Approach	pH	Temp.	Carbon	Nitrogen	K_2HPO_4	$MgSO_4$	NaCl	Fructose	$NaNO_3$	predicted	Experimental
OVAT	5	34	4	2	0.3	0.1	0.2	1.5	0.5	---	418.25
GA	5.38	34.18	4.12	2.04	0.34	0.2	0.14	2.0	0.47	1157.24	1155.13
PSO	5.38	34.18	4.12	2.04	0.34	0.2	0.14	2.0	0.47	1157.24	1155.15

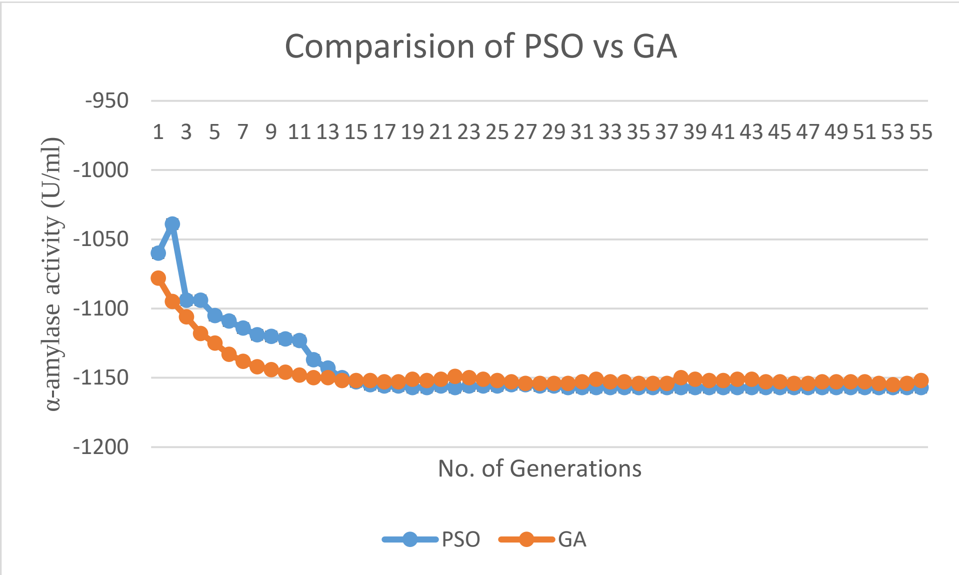

Figure 6: Comparison of PSO versus GA

6.0 CONCLUSION[27-34]

The optimization of *α-amylase* production using evolutionary and swarm intelligence-based algorithms improves recovery and activity, offering flexibility, ease of operation, and global perspective. Evolutionary (GA) and swarm intelligence-based (PSO) optimization is an alternative to traditional optimization methods that struggle to predict optimal conditions in complex optimization problems with numerous local optima. In this study, the RSM model of *α-amylase* extraction was posed as an optimization problem for maximizing amylase activity. A systematic study was conducted to determine the GA parameters responsible for an optimal value of amylase activity. Most of the population was in the 1150.0–1160.0 U/ml group, with over 75% consistently above this value. The optimum values of process parameters were pH (5.37), temperature (34.18°C), carbon source (4.12%), nitrogen source (2.04%), K_2PO_4 (0.34%), $MgSO_4$ (0.2%), NaCl (0.14%), fructose (2.0%), and $NaNO_3$ (0.47%). The maximum amylase activity for *Bacillus velezensis sp.* was found to be 1157.2 U/ml.

Optimization by PSO is a computer-based approach that uses parameters like particle size, generation number, inertia weight, and cognitive and social components.
Randomly generated particles with a specific size were used to generate optimal amylase activity. The optimal amylase activity of 1157.2 U/ml was obtained with the process variables of pH (5.37), temperature (34.18°C), carbon source (4.12%), nitrogen source (2.04%), K_2PO_4 (0.34%), $MgSO_4$ (0.2%), NaCl (0.14%), fructose (2.0%), and $NaNO_3$ (0.47%). The PSO approach's optimal amylase activity and parameters are slightly better than GA due to easier implementation, fewer parameters, and higher memory capability.

List of abbreviations

RSM-Response Surface Methodology
ACO- Ant-Colony Optimization
$CaCl_2$- Calcium chloride
DNSA-3,5-dinitro salicylic acid assay
$FeCl_3$-Ferric chloride
FO- Firefly Optimization
GA- Genetic Algorithm
K_2HPO_4-Dipotassium hydrogen phosphate
$MgSO_4$ -Magnesium sulfate
MOPSO-CD- Multi-Objective Particle Swarm Optimization -Crowding Distance
NaCl-Sodium chloride
$NaNO_3$-Sodium nitrate
NH_4NO_3-Ammonium nitrate
OVAT - One-Variable-At-A-Time
PSO- Particle Swarm Optimization

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