B.Tech. Project Report - *Phase* II

Optimising the value of parameters to achieve maximum efficiency in the Reactive Extraction Process of Beta-Alanine

Submitted in partial fulfilment of requirements for the award of the degree of Bachelor of Technology from IIT (Indian Institute of Technology) Guwahati

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Certificate

This is to certify that the work presented in the report entitled **"Optimising the value of parameters to achieve maximum efficiency in the Reactive Extraction Process of Beta-Alanine"** by **Sohail-ur-Rehman**, in fulfilment of the requirements of the degree of **Bachelor of Technology in Biosciences and Bioengineering**, is a Bonafide record of the work carried out by him under the guidance of **Dr. Senthilkumar Sivaprakasam** at the Department of Biosciences and Bioengineering, Indian Institute of Technology, Guwahati. This study has not been submitted elsewhere for a degree.

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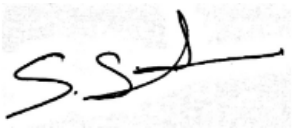
Signature of supervisor: 

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ABSTRACT

In recent years, the field of Biochemical Engineering has witnessed a growing interest in optimising processes through the utilisation of artificial intelligence (AI) techniques. This research aims to contribute to this evolving landscape by focusing on the reactive extraction process of beta-alanine, a compound of significance in pharmaceutical and industrial applications.

The primary objective of this study is to employ machine learning models to enhance the efficiency of the reactive extraction of beta-alanine. To achieve this, we adopted the responsive phase methodology, a technique designed to systematically explore the parameter space and generate comprehensive data sets for training and evaluating machine learning models.

The responsive phase methodology allows for a detailed understanding of the system's behaviour under varying conditions, providing a solid foundation for the subsequent application of machine learning models. In this project, we trained several machine learning models with the acquired data, exploring different architectures and configurations to identify the most effective model for predicting and optimising the reactive extraction process.

This introduction outlines the motivation behind the research, the methodology employed, and the overarching goal of optimising the reactive extraction of beta-alanine using machine learning models. Subsequent sections will delve into the experimental setup, data collection, and the results obtained, providing a comprehensive analysis of the contributions and implications of this study.

1. Introduction

1.1 β Alanine:

Beta-alanine is a non-essential amino acid, meaning the body can produce it naturally. It is a crucial component in the synthesis of carnosine, a dipeptide found predominantly in skeletal muscle tissues. Carnosine plays a vital role in buffering acid produced during high-intensity

exercise, thereby helping to delay the onset of muscle fatigue and improve overall exercise performance.

Unlike many amino acids that are incorporated into proteins, beta-alanine primarily exists free in the body. Its supplementation has gained attention in the fields of sports nutrition and exercise physiology due to its potential ergogenic effects. By increasing carnosine levels, beta-alanine supplementation is believed to enhance exercise capacity and performance, particularly in activities involving short bursts of high-intensity effort.

Moreover, beta-alanine has applications beyond the realm of sports nutrition. Its significance extends to various industrial and pharmaceutical processes, making it a compound of interest for research and optimization.

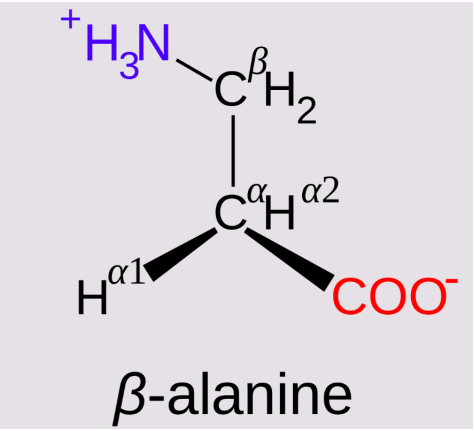


Fig 1.1: Structure of β alanine

1.2 <u>β Alanine applications</u>:

Beta-alanine holds diverse applications across sports nutrition, medicine, and industrial processes. In the realm of sports nutrition, it serves as a popular supplement, enhancing exercise performance by increasing carnosine levels in muscles and delaying the onset of fatigue during high-intensity activities. Beyond its role in sports, beta-alanine has implications in exercise physiology research, potentially improving endurance and power output. In the pharmaceutical domain, the antioxidant and anti-inflammatory properties of carnosine, synthesised with beta-alanine, make it an area of interest for therapeutic applications. Industrial processes, including reactive extraction, leverage beta-alanine's properties, showcasing its significance in chemical engineering and optimization. Additionally, beta-alanine finds utility in food additives, cosmetics, and skincare formulations, underscoring its multifaceted role in various fields.

1.3 Reactive extraction:

Liquid-liquid extraction, a fundamental separation process, involves the transfer of a specific solute from the aqueous phase to the organic phase. In the context of this research, reactive extraction is a specialised form of liquid-liquid extraction tailored for solutes featuring carboxylic acid groups, such as beta-alanine. Unlike conventional liquid-liquid extraction, reactive extraction involves a distinctive aspect—the occurrence of a chemical reaction between the extractant and solute at the interface.

This process holds economic advantages, as both the diluent and extractant components utilised in the reactive extraction of beta-alanine can be recycled. This recyclability contributes to enhanced cost-effectiveness and a sustainable approach to the extraction process. Notably, the ability to reuse components is a critical factor known to facilitate superior product recovery, ensuring a final product with high purity.

Several factors intricately influence the efficiency of beta-alanine extraction through reactive methods. These factors include the concentration of **D2EHPA** (Di(2-ethylhexyl) phosphoric acid), **incubation time**, **pH levels**, **solute concentration**, **reaction temperature**, **agitation rate**, and **the choice of diluent extraction system**. The optimization of these parameters is pivotal for achieving an efficient and selective extraction of beta-alanine, a compound of significance in pharmaceutical or industrial applications. The intricate interplay of these factors will be explored in detail throughout the subsequent sections of this report, shedding light on their individual and collective impacts on the overall effectiveness of the reactive extraction process.

1.4 Responsive Phase Methodology:

In the pursuit of optimising the reactive extraction process for beta-alanine, the Responsive Phase Methodology was employed to systematically acquire data and comprehensively understand the intricate interplay of key factors. This methodological approach involves the deliberate exploration of the experimental parameter space, enabling the generation of a robust dataset. The data obtained through the Responsive Phase Methodology serves as the foundation for training and evaluating artificial neural networks (ANNs) in the subsequent phases of the research.

The predictors identified for this study encompass crucial parameters known to influence the efficiency of beta-alanine extraction. These include the concentration of **D2EHPA** (Di(2-ethylhexyl) phosphoric acid), **pH levels**, **temperature**, **solute concentration**, **incubation tim**e, and **phase ratio**. Each of these predictors plays a distinctive role in shaping the outcome of the extraction process, and their systematic variation during the Responsive Phase allows for a comprehensive examination of their individual and collective impacts on extraction efficiency.

The response variable, **extraction efficiency**, is a key metric that reflects the effectiveness of the reactive extraction process. This variable serves as the basis for evaluating the performance of different neural network models during the training and optimization phases. The utilisation of extraction efficiency as a response variable enables the identification of optimal conditions for beta-alanine extraction, facilitating the overarching goal of enhancing the efficiency of the reactive extraction process.

1.5 Machine Learning (ML) Models:

In the quest to optimise the reactive extraction process for beta-alanine, the utilisation of machine learning (ML) algorithms, particularly machine learning models, emerges as a powerful tool to model and predict the intricate relationships within the dataset obtained through the Responsive Phase Methodology.

Machine learning models are computational approaches that can capture complex patterns and non-linear relationships within datasets, making them particularly advantageous for applications in chemical engineering and process optimization.

In this study, machine learning models are employed to discern the underlying patterns between the predictors (D2EHPA concentration, pH, temperature, solute concentration, incubation time, and phase ratio) and the response variable (extraction efficiency). The training of machine learning models involves exposing the algorithm to the dataset, allowing it to learn and adapt to the intricate relationships present in the experimental data. Once trained, the machine learning model can then make predictions and optimise the extraction process by identifying the combination of parameters that yields the highest extraction efficiency.

This approach offers a data-driven and adaptive solution to the complex task of optimising the reactive extraction of beta-alanine. By harnessing the power of machine learning models, this study aims to develop a predictive model that not only accurately represents the experimental data but also provides valuable insights for enhancing the efficiency of the reactive extraction process.

1.6 Optimisation Techniques:

Optimization techniques play a crucial role in enhancing the efficiency and effectiveness of various processes, especially in fields like chemical engineering where complex interactions and dependencies exist. Leveraging machine learning (ML) algorithms presents a promising avenue for optimizing processes such as reactive extraction, as demonstrated in the study focused on beta-alanine extraction. By employing ML models, researchers can delve into the intricate relationships between key parameters like D2EHPA concentration, pH, temperature, solute concentration, incubation time, and phase ratio, and extraction efficiency. ML models

excel in capturing non-linear patterns and complex interactions within datasets, offering a robust framework for predictive modelling and optimization. Through training on experimental data, these models can discern subtle correlations and make accurate predictions, thus facilitating the identification of optimal parameter combinations for maximizing extraction efficiency. This data-driven approach not only enables the development of predictive models aligned with experimental outcomes but also furnishes valuable insights for refining and enhancing the reactive extraction process. Overall, the integration of ML techniques in optimization endeavours represents a significant advancement, providing a powerful toolset for tackling the complexities inherent in chemical processes and driving improvements in efficiency and performance.

2. PREVIOUS WORK

A machine learning model, capable of accurately predicting the efficiency of the beta-alanine reactive extraction process was trained.

Linear Regression Model:

The results obtained from these initial linear regression models are as follows:

Metric	Value
Training Mean Squared Error (MSE)	25.43
Training R-squared	0.91
Testing Mean Squared Error (MSE)	25.75
Testing R-squared	0.92

The coefficients and intercept for the linear regression model are as follows:

Parameter	Value
D2EHPA Concentration	0.774
pH	4.200
Temperature	-0.572
Solute Concentration	-0.005

Incubation time	0.019
Phase Ratio	-3.299
Intercept	12.699

Going forward, this model will be used for optimisation.

3. OBJECTIVES

The primary objective of this endeavour is to harness the power of optimization algorithms to augment the efficiency of beta-alanine production. Leveraging a well-trained Linear Regression Model serves as the cornerstone for this pursuit. By employing sophisticated optimization techniques, we aim to identify the optimal values for various parameters that govern the production process, thereby maximizing output while minimizing resource utilization and associated costs.

In pursuit of this objective, the Gekko Python library emerges as a pivotal tool. Gekko is renowned for its versatility in solving optimization problems, particularly in the realm of dynamic systems and control engineering. Its robust capabilities make it an ideal choice for our endeavour, allowing seamless integration with our existing Python environment and facilitating the optimization of complex systems with ease.

4. LITERATURE REVIEW

A method of reactive extraction aimed at isolating amino acids from a mixture. This process utilises four acidic extractants (DNNSA, D2EHPA(S), D2EHPA, and Versatic 10) dissolved in toluene to extract a range of amino acids (arginine, phenylalanine, alanine, glycine, and aspartic acid) from a solution. The extractive efficiency of the organic phase displays a defined hierarchy for amino acids, and separation factors are detailed for various amino acid pairs. Furthermore, the extractive and loading capabilities of the extractants exhibit a specific diminishing order, with DNNSA identified as a particularly promising extractant for both amino acid extraction and fractionation [1].
The study using di(2-ethyl-hexyl) phosphoric acid in aqueous solutions found that amino acid extraction is pH-dependent and influenced by the amino acids' hydrophobic nature. Higher pH values enhance extraction efficiency, especially for amino acids with longer carbon chains or cyclic structures. Conversely, those with additional polar groups exhibit lower extraction efficiency. Furthermore, successful amino acid extraction from cottonseed meal hydrolysate

was achieved through microbial fermentation and HCl hydrolysis, with optimal results at an initial feed pH of 6.5. The use of D2EHPA reactive extraction was emphasised for its ability to simplify the process, avoiding multiple purification stages and yielding purer back-extracts. This approach streamlines the separation and purification of amino acids effectively [2].

The extraction equilibria of L-phenylalanine using liquid ion-exchange extractants, specifically Aliquat 336 and di-2-(ethylhexyl) phosphoric acid (DEHPA), in the diluent methyl isobutyl ketone. The results compare the extraction efficiency of each single extractant against a mixed extractant system combining equimolar amounts of Aliquat 336 and DEHPA. While the mixed system shows lower loading values than the single-extractant systems, it maintains uptake capacity across a broader pH range (1.8 to 9.2) [3].

The use of di-(2-ethylhexyl) phosphoric acid (D2EHPA) in reactive extraction for separating amino acids, reveals the potential for selective separation based on pH and the acidic or basic nature of each amino acid. Through multistage extraction, distinct groups of amino acids (neutral, basic, acidic) are successfully separated at specific pH ranges [4].

The optimization of the culture medium played a pivotal role in significantly enhancing the production of human interferon alpha 2b (huIFNα2b) in glycoengineered P. pastoris. The application of a Design of Experiments (DoE)-based medium optimization strategy not only improved efficiency but also demonstrated its effectiveness at both shake flask and bioreactor levels. This successful scale-up underscores the practical applicability of the optimised conditions in larger-scale industrial settings. Furthermore, the confirmed N-glycosylation and biological activity of the purified huIFNα2b highlight the importance of optimization in achieving a structurally characterised and functionally potent protein. The study's findings underscore the critical role of optimization in maximising productivity and ensuring the potential therapeutic relevance of huIFNα2b, particularly in the context of cancer therapy [5].

This study addressed a critical challenge in hyaluronic acid (HA) production related to the highly viscous nature of the fermentation broth, making real-time monitoring with physical sensors challenging. The objective was to non-invasively track the state variables involved in HA production and deduce critical process parameters using a hybrid model. By leveraging online bioreactor data, including pH, dissolved oxygen percentage (DO%), %CO2 evolved, feed rate, and agitation rate, the hybrid model enabled real-time tracking of HA bioprocess dynamics. The study utilised a comprehensive dataset comprising historical batches and newly performed fed-batch runs at various specific growth rate set-points (μsp). The hybrid model, trained on this dataset, demonstrated accurate predictions of biomass and HA concentration for test runs, with a mean squared error of prediction ranging from 0.018 to 0.049 $(g/L)^2$. Additionally, recurrent neural networks were evaluated for forecasting specific growth rate (μ) and HA productivity rate (qHA) to observe desired process trajectories. This research contributes to the field by showcasing the applicability of a hybrid model-based soft sensor in predicting the trend of process parameters in HA fermentation, offering valuable insights for optimising and controlling this complex bioprocess [6].

The initiation of a synthetic chemist's training involves conducting experiments based on traditional recipes found in textbooks and manuscripts, fostering the development of practical skills and chemical intuition through successful reactions. This approach often persists throughout a researcher's career, with new recipes evolving from established protocols and

deviations guided by experiential learning from failed experiments. However, this paper underscores that when delving into the understanding of chemical systems, model-based, algorithmic, and miniaturised high-throughput techniques surpass human chemical intuition. They achieve reaction optimisation with superior efficiency in terms of both time and materials, a topic explored comprehensively within this review. Notably, many synthetic chemists lack exposure to these advanced techniques during undergraduate education, leading to a significant number of scientists desiring reaction optimization without the knowledge or utilisation of these methodologies. This literature review illuminates both the fundamentals and cutting-edge aspects of modern chemical reaction optimization, highlighting its implications for process scale-up. By providing a comprehensive reference, it aims to empower and inform scientists, serving as a valuable resource for those inspired to explore and apply these techniques in their research endeavours [7].

5. MATERIALS AND METHODS

Extraction:

For the extraction of beta-alanine, the reactive extraction technique was employed. This method involves the simultaneous extraction and chemical reaction of the target component from the liquid phase into an immiscible solvent phase. The choice of reactive extraction aimed to optimise the process for beta-alanine extraction.

Dataset Generation:

To generate the dataset for this study, a combination of the Responsive Phase Methodology and experimental data obtained in the laboratory was employed. The Responsive Phase Methodology systematically explores the experimental parameter space, allowing for a comprehensive understanding of the system's behaviour. This hybrid approach, incorporating both methodologies, facilitated the collection of data crucial for training and evaluating the subsequent Machine Learning (ML) Models.

Data Analysis:

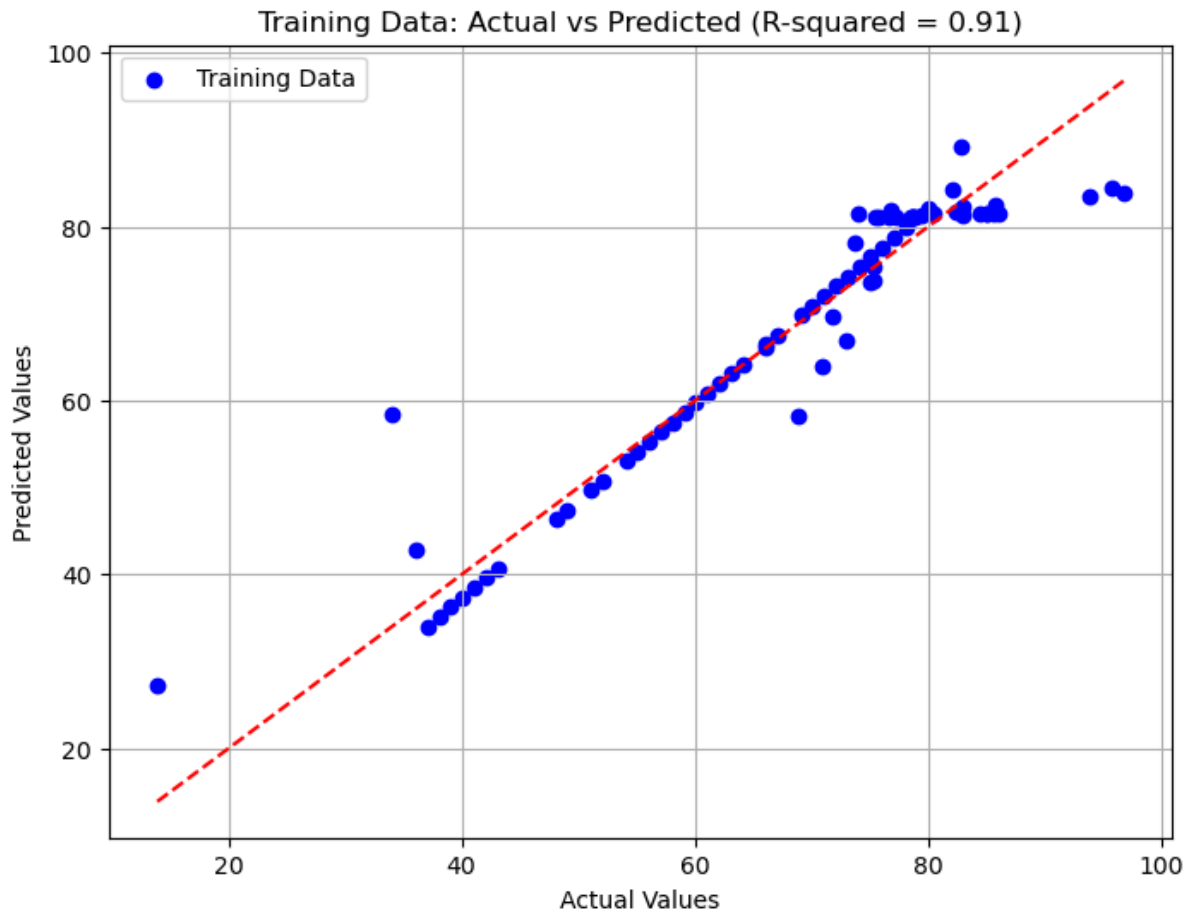
The collected data were then analysed using various Python libraries, including NumPy, Pandas, Scikit-learn, Gekko, TensorFlow, and Keras. TensorFlow, a widely used machine learning library, facilitated the implementation and training of artificial neural networks. Gekko was used for the optimisation task. The analysis was conducted within a Jupyter notebook, providing a collaborative and interactive environment for exploring and interpreting the results. The integration of these diverse libraries allowed for a comprehensive

examination of the data and the application of machine learning techniques to the reactive extraction process of beta-alanine.

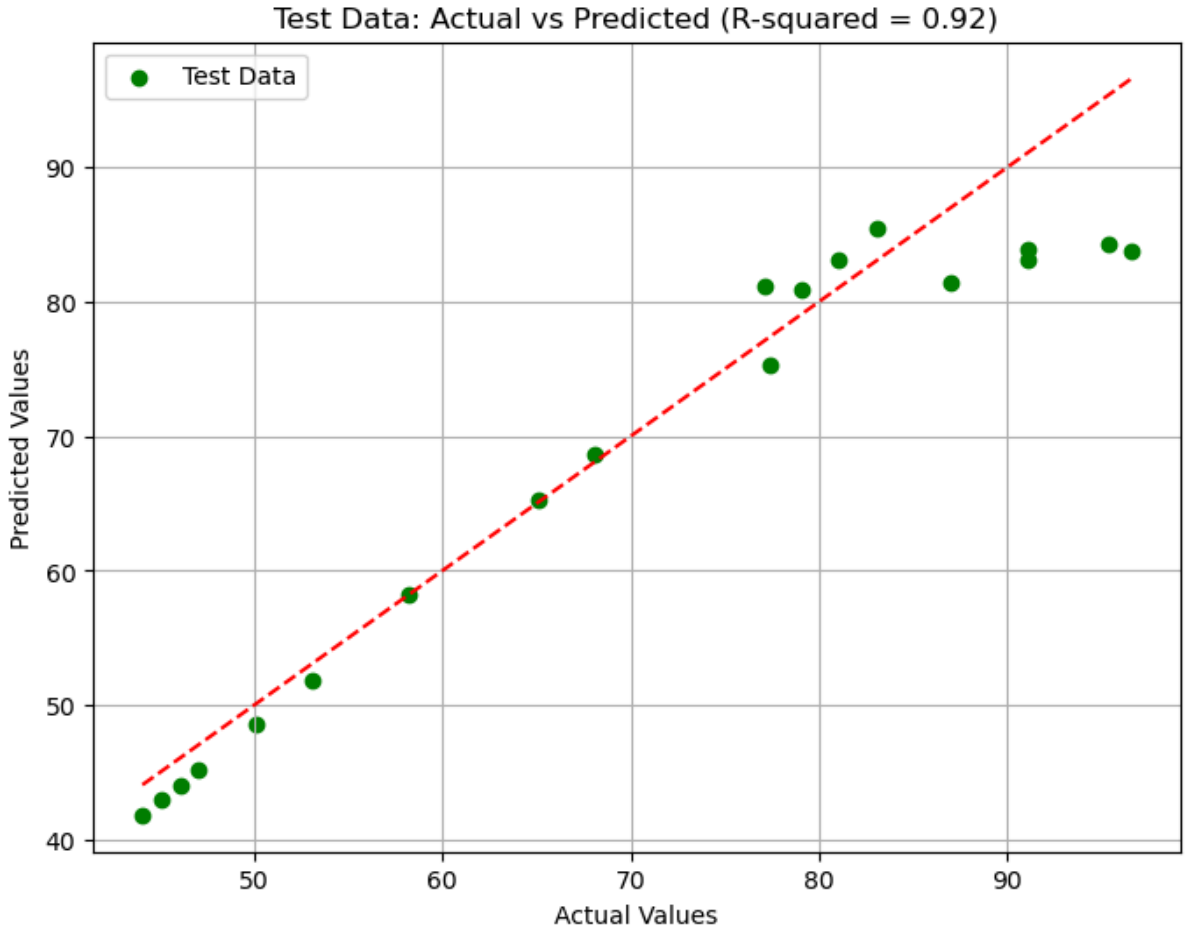
6. RESULTS AND CONCLUSIONS

In this section, we assess the performance of our regression model on both the training and test datasets. The model employed is a linear regression model, trained using the training data. To evaluate its performance, we utilized two key metrics: Mean Squared Error (MSE) and R-squared (R^2). MSE quantifies the average squared difference between the predicted and actual values, providing a measure of the model's accuracy. R-squared, on the other hand, indicates the proportion of the variance in the dependent variable that is predictable from the independent variables. Higher R-squared values suggest a better model fit. The generated graphs depict the relationship between the actual and predicted values for both the training and test datasets. Additionally, the R-squared value is displayed on each graph to provide a clear understanding of the model's goodness of fit.

Training Dataset



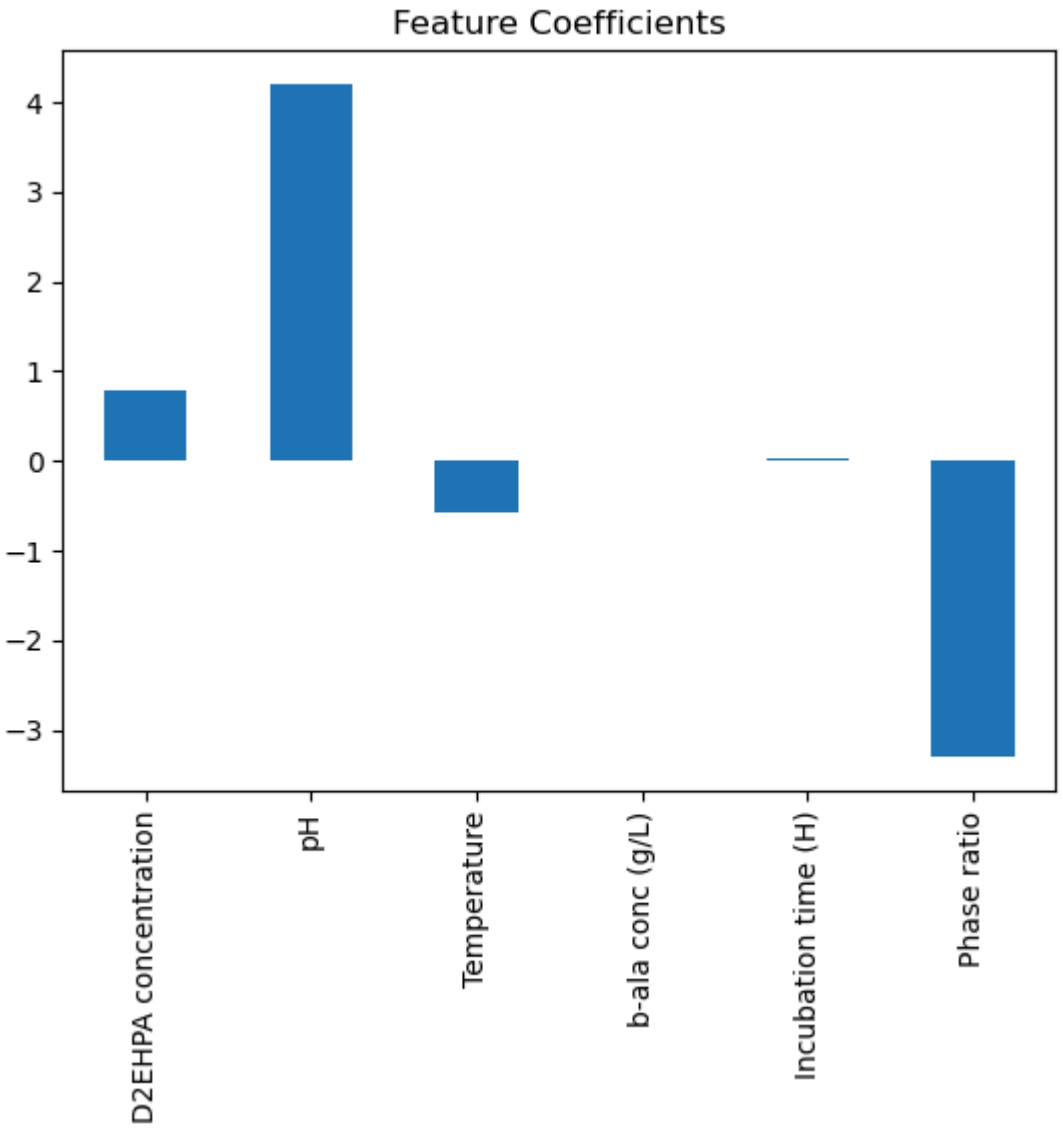
Test Dataset



Feature Coefficient Plot:

In a linear regression model, the coefficient of each feature indicates how much the target variable is expected to change when that feature increases by one unit, assuming all other features remain constant. A positive coefficient suggests a positive correlation with the target variable, meaning an increase in the feature value leads to an increase in the target variable, and vice versa for negative coefficients.

By examining the coefficients, you can identify which features have a significant impact on the target variable and which ones are less influential. Features with larger absolute coefficients are considered more important, as they contribute more to the prediction.



The optimization process utilizing the GEKKO library successfully yielded optimal values for the variables, resulting in enhanced system efficiency. The objective function, which encompassed various parameters including D2EHPA concentration, pH, temperature, b-ala concentration, incubation time, and phase ratio, was effectively maximized. The constraints were determined from the nature of the data.

The following constraints were used on the parameters:

Parameter	Value
D2EHPA Concentration	> 10
pH	>= 3 & <= 14
Temperature	> 0
Solute Concentration	> 0
Incubation time	> 0
Phase Ratio	> 0

Under the given constraints, the optimal values of the parameters are:

Parameter	Value
D2EHPA Concentration	10
pH	3
Temperature	10
Solute Concentration	9.99
Incubation time	5.57e-06
Phase Ratio	10

These findings underscore the efficacy of the optimization approach in bolstering system efficiency. By adhering to defined constraints and systematically exploring the parameter space, the optimization process unearthed configurations that maximize productivity and resource utilization. The discerned optimal values represent a culmination of computational prowess and domain expertise, poised to drive tangible improvements in beta alanine production. In essence, this endeavour not only showcases the power of optimization algorithms but also underscores their pivotal role in advancing industrial processes towards heightened efficiency and performance.

The code for the work can be found here

7. FUTURE WORK

Moving forward, there are several avenues for expanding upon the current study's findings and methodologies. One promising direction involves leveraging larger datasets to enhance the robustness and generalizability of the machine learning models. With a larger dataset, the models can capture a wider range of patterns and variations, potentially leading to more accurate predictions and insights into the extraction process. Additionally, exploring more sophisticated machine learning algorithms and advanced optimization techniques can further refine the predictive capabilities of the models. Techniques such as ensemble learning, deep learning, or reinforcement learning could be investigated to uncover deeper insights and improve the efficiency of the extraction process optimization. Moreover, incorporating domain-specific knowledge or expert insights into the model development process could enhance the interpretability and applicability of the results. Overall, by embracing these advancements and exploring new avenues, future research can continue to push the boundaries of understanding and optimizing extraction processes using machine learning techniques.

8. APPENDIX

Code for the Regression Model

```
import numpy as np
import pandas as pd
from sklearn.model_selection import train_test_split
import matplotlib.pyplot as plt

data = pd.read_excel("data.xlsx")

X=data.iloc[:,:-1]
Y=data.iloc[:,-1]

X_train, X_test, y_train, y_test = train_test_split(X, Y,
test_size=0.2,random_state=1)

from sklearn.linear_model import LinearRegression
```

```
model = LinearRegression()
model.fit(X_train, y_train)

y_train_pred = model.predict(X_train)
y_test_pred = model.predict(X_test)

y_pred = model.predict(X)

train_r2 = r2_score(y_train, y_train_pred)
test_r2 = r2_score(y_test, y_test_pred)
```

Code for the Actual vs Predicted Values (Training Data)

```
plt.figure(figsize=(8, 6))
plt.scatter(y_train, y_train_pred, color='blue', label='Training
Data')
plt.plot([min(y_train), max(y_train)], [min(y_train),
max(y_train)], color='red', linestyle='--')
plt.title(f'Training Data: Actual vs Predicted (R-squared =
{train_r2:.2f})')
plt.xlabel('Actual Values')
plt.ylabel('Predicted Values')
plt.legend()
plt.grid(True)
plt.show()
```

Code for the Actual vs Predicted Values (Test Data)

```
plt.figure(figsize=(8, 6))
plt.scatter(y_test, y_test_pred, color='green', label='Test Data')
plt.plot([min(y_test), max(y_test)], [min(y_test), max(y_test)],
color='red', linestyle='--')
plt.title(f'Test Data: Actual vs Predicted (R-squared =
{test_r2:.2f})')
plt.xlabel('Actual Values')
plt.ylabel('Predicted Values')
plt.legend()
plt.grid(True)
```

```
plt.show()
```

Code for The Feature Coefficient Plot

```
coefficients = pd.Series(model.coef_, index=X.columns)
coefficients.plot(kind='bar')
plt.title("Feature Coefficients")
plt.show()
```

Code for optimisation using GEKKO library

```
from gekko import GEKKO
m = GEKKO()
# Initialize variables
x1 = m.Var(value=1,lb=0,ub=10)
x2 = m.Var(value=1,lb=0,ub=10)
x3 = m.Var(value=1,lb=0,ub=10)
x4 = m.Var(value=1,lb=0,ub=10)
x5 = m.Var(value=1,lb=0,ub=10)
x6 = m.Var(value=1,lb=0,ub=10)

# Init the coefficients
c = m.Const(value=12.699)
m1 = m.Const(value=0.774)
m2 = m.Const(value=4.20)
m3 = m.Const(value=-0.572)
m4 = m.Const(value=-0.005)
m5 = m.Const(value=0.019)
m6 = m.Const(value=-3.299)

# Inequality constraints from input data
m.Equation(x1 > 10)
m.Equation(x2 >= 3)
m.Equation(x2 <= 14)
m.Equation(x3 > 0)
m.Equation(x4 > 0)
m.Equation(x5 > 0)
```

```
m.Equation(x6 > 0)

#setting the objective function
m.Obj(m1*x1+m2*x2+m3*x3+m4*x4+m5*x5+m6*x6+c) # Objective
m.options.IMODE = 2
m.solve(disp=False)

print('Results')
print('x1(D2EHPA concentration): ' + str(x1.value))
print('x2(ph): ' + str(x2.value))
print('x3(temp): ' + str(x3.value))
print('x4(b-ala conc): ' + str(x4.value))
print('x5(incubation time): ' + str(x5.value))
print('x6(phase ratio): ' + str(x6.value))
```

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