Investigating Reactions of Laccase and Small Molecule Medium as a Substrate

Zhiyu Liao
Shanghai High School
Shanghai China
e-mail: liaozhiyujonathan@outlook.com

***Abstract*—Laccase is a ceruloplasmin widely used in industrial production. It is mainly used in catalytic degradation of lignin and its catalytic process is safe and environmentally friendly. Small molecule medium is not only commonly used as a catalyst in the laccase catalytic reactions, but also can participate in the reaction as a substrate. In this study, the reactions of small molecules as substrates and laccase are investigated by the changes in the absorption wavelength of the reaction system recorded by ultraviolet spectrometer and analysis of the reaction of laccase and small molecules using MATLAB R2013a. It can be seen by the final results of hierarchical clustering analysis and calculation of the Euclidean distance that the entire medium system can be divided into three categories, namely, (i) Cumaric Acid, Sinapic Acid and HBT, (ii) Cumaric Acid and ABTS, and (iii) Ferulic Acid and Syringic Acid. The results of this study provide new ideas for finding and verifying potential medium systems.**

Keywords-laccase; small molecule medium ; principal component analysis; hierarchical clustering analysis.

I. INTRODUCTION

With the progress of science and technology, biological knowledge and technology are being gradually blended into traditional chemistry to form the more and more popular biochemistry. Currently, solving the pollution problem by a variety of enzymes is a new direction; this approach has also been shown to be an effective method. Through a variety of enzymes, the final products from decomposition of pollutants are often simple and harmless inorganic compounds. Many biological toxic, low concentration and difficultly degradable substances can often be decomposed by means of biochemical processes. Among many enzymes, laccase has the advantages of a wide range of substrates, high catalytic activity and no pollution, and thus, not only has great industry usefulness, but also has a very important position in dealing with pollution problems. Through continuous trials and studies, types and applications of the small molecule catalysts that react with laccase have begun to take shape. These small molecule mediators often play a very important role in different fields, and greatly accelerate the reaction efficiency. However, since laccase is an enzyme with a very wide range of substrates, many small molecule mediators are also within the scope of its substrates. Originally as a catalyst, small molecule mediators may cause some effect on the reaction when they act as substrates and are involved in the reaction. Further, small molecule mediators also show other characteristics in addition to catalytic properties in the reaction.

Currently, there is not a clear classification for small molecule mediators of laccase. By analyzing the nature of small molecule mediators when reacting with laccase as a substrate, small molecule mediators are classified by biological information and mathematical methods, and the role of small mediator molecules play in the reaction of laccase can be more accurately predicted. Further, the difficulty of predicting the nature of new small molecule mediators can be reduced, and testing complexity can be simplified.

The rest of the paper is structured as follows. Section II presents the experiments we performed. Section III explains principal component analysis, as well as hierarchical cluster analysis. Section IV presents the results and discussion, and we conclude in Section V.

II. EXPERIMENTS

A. Materials

Small molecules commonly used as medium in laccase reaction are selected, including ABTS, Ferulic Acid, HBT, Syringic Acid, Coumaric Acid, Caffeic Acid, and Sinapic Acid.

B. Ultraviolet spectroscopy

Absorption wavelength variation of the reaction system is recorded by Ultraviolet spectrometer and formed matrices to determine the efficiency and extent of the reaction. Data of wavelength of 240-900nm interception are the basis of analysis data set and used for reaction analysis and classification of mediators.

C. Data Analysis

Matlab R2013b [1] is used for principal component analysis of data set, and for comparison, projection and establishing response structure model diagram. The properties of small molecules in the reaction system are initially manifested. The main components of reaction are re-extracted by initial data matrix transpose, and of small molecules are classified by calculation of the Euclidean distance and hierarchical cluster analysis.

 ISBN: 978-1-61208-409-1

III. PRINCIPLE OF PRINCIPAL COMPONENT ANALYSIS AND HIERARCHICAL CLUSTER ANALYSIS

A. Principal component analysis

In order to fully describe the system in analyzing the current problems, analysts tend to select the relevant indicators as thoughtfully as possible. In fact, many of the social, economic, and technical indicators have a synchronized growth trend. When an analyst intentionally or unintentionally describes the feature of a system by namely different indicators but actual relevant indicators, usually, he faces the issue of multiple correlated variables. Multiple correlations of variables imply artificially exaggerate certain features' position in the system analysis, which affect the objectivity of the analysis and impede decision makers' right judgment.

Principal Component Analysis (PCA) [2] is a basic method for overcoming multiple correlations of variables. PCA is method to establish as few as possible new variables for all variables originally proposed, so that these new variables are independent and uncorrelated, and these new variables should maintain information of the original proposed variables as much as possible. By means of an orthogonal transformation, PCA converts original correlated component random vectors into relevant new random vectors which are corrected to each other. This is manifested on algebra as transforming a covariance matrix of original random vectors into a diagonal matrix, and on geometry as transforming an original coordinate system into a new orthogonal coordinate system, in which sample points spread in the most open p orthogonal directions. Then, multidimensional variables are treated by reducing the dimension of the system so that variables can be converted into a low-dimensional variable system in high accuracy. The low-dimensional variable system is then further transformed into a one-dimensional system through constructing an appropriate value function.

B. Hierarchical cluster analysis

Hierarchical Cluster Analysis (HCA) [3] is one of the basic methods of exploratory research work. The so-called clustering is clustering subjects of study into classes based on the degree of closeness between the study subjects, and in accordance with the principle of "Similar Together, Different Apart".

The idea of hierarchical clustering analysis is that data with higher similarity are closer to each other, have higher tendency with similar properties, and therefore are grouped into the same category. Data can be hierarchical clustered according to the Euclidean distance of matrix, and preliminarily analyzed and classified.

IV. RESULTS AND DISCUSSION

A. Experiment results

The data collected by an ultraviolet spectrometer are expressed in a matrix and formed an original 598 × 660 matrix, wherein each row represents the instrument data measured in every 0.5s in the 190nm to 1850nm wavelength range. Finally, the original matrix is cut, and data within the wavelength range of 240-900nm are selected as the initial data set.

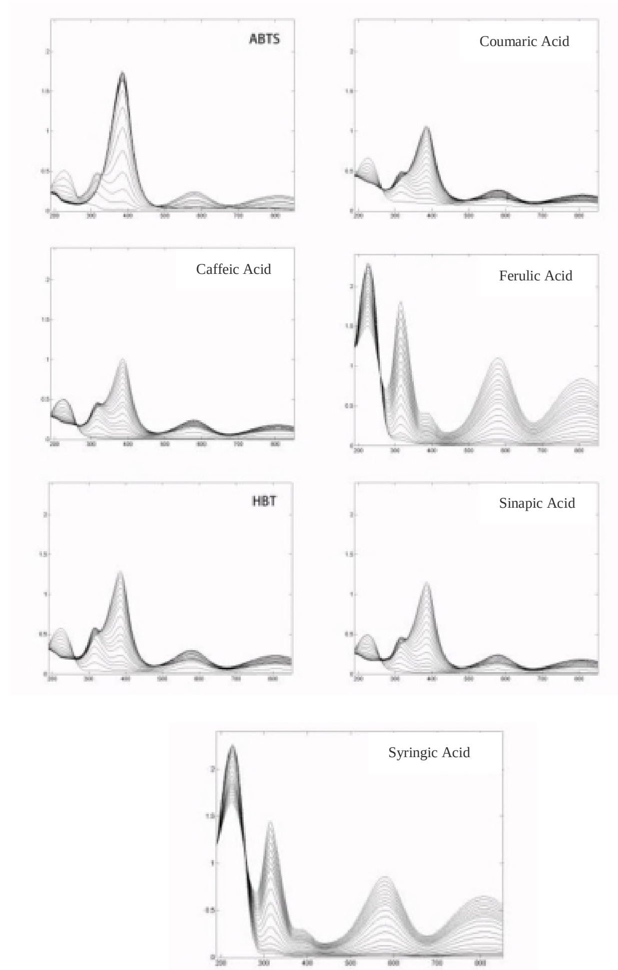

Figure 1. Figures of the raw data collected by the ultraviolet.

Figure 1. shows wavelength changes for small mediator molecules in the reaction system. Among them, there is no significant similarity between each two data sets.

B. Principal component analysis[4]

- Data with time information
  Table 1 summarizes the first and second principal component contribution rates and their sum of each mediator by principal component analysis of the experimental data containing time information. It can be seen from the table that the sums of all of the first and second main component contribution rates are in excess of 95%. The results analysis illustrates that the first and second principal components can be a good representative of the relevant data sets.

 ISBN: 978-1-61208-409-1

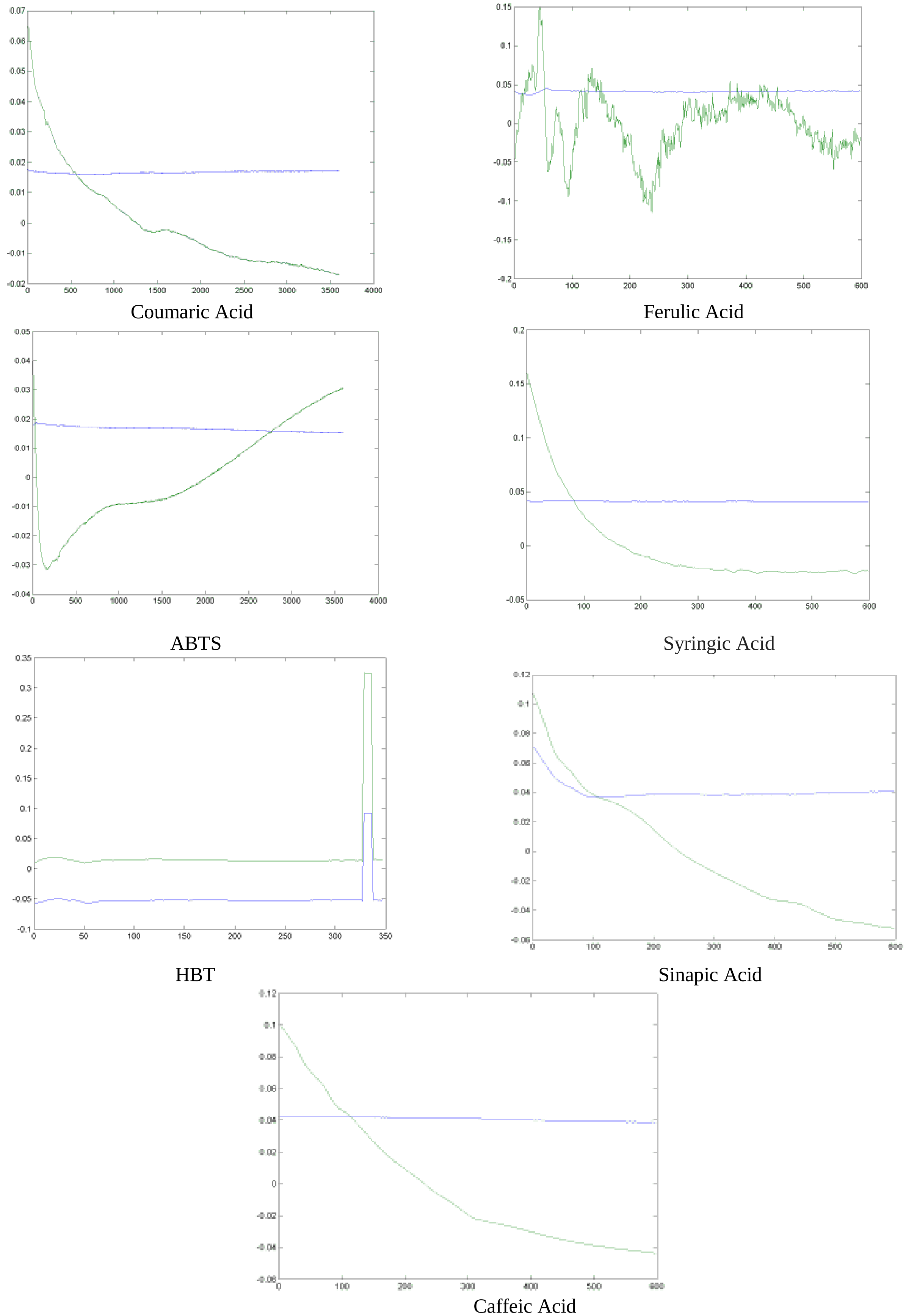

Figure 2. Curves of the first two principal components of the changes of reactions between the small molecule mediums and laccase.

After initial overlap comparison, it can be observed that there is no significant similarity among the above images. The corresponding one-dimensional matrixes of the first and second main components are combined to form a two-dimensional vector. Figure 3 shows the two-dimensional vectors of the tested small molecule mediators.

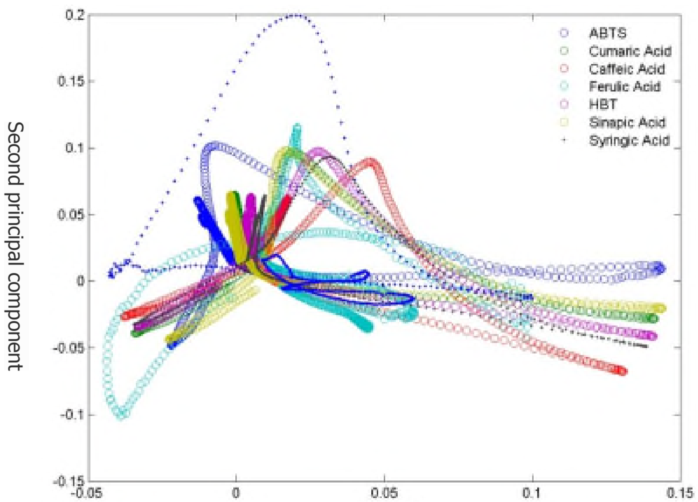

Figure 3. Put all of the seven figures of Figure 2 together.

It can clearly be seen through the above figure that in all the data, some parts are intensively overlapped, while the rest is very discrete. There is no significant similarity and obvious trend among the data.

- Data with wavelength information
  Table 2 summarizes the first and second principal component contribution rates and their sum of each mediator by principal component analysis of the experimental data containing wavelength information. It can be seen from the table that the sums of all of the first and second main component contribution rates are in excess of 95%. The results analysis illustrates that the first and second principal components can be a good representative of the relevant data sets.

Wavelength data of the first and second principal components are also plotted to obtain seven different curves, respectively representing the properties of the selected seven small molecule mediators in the reaction of laccase. These seven curves are drawn together to get Figure 4:

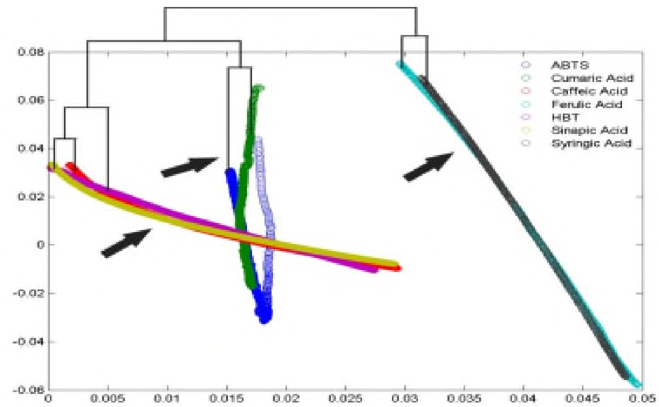

Figure 4. Combination of the seven curves.

C. *Hierarchical cluster analysis*

From Figure 4, the tendency of each image and the similarity between each other can be seen more clearly. Among them, caffeic acid, Sinapic Acid and HBT as a group, p-coumaric acid and ABTS as a group, and ferulic acid and syringic acid as a group， have high degree of overlap of curves within each group, indicating that the nature of the reactions of the small molecule mediators within each group has high similarity. On the other side, there is significantly different tendency between each groups, indicating that the grouping of mediators is qualitative and efficient.

The wavelength changes during the reaction of small molecules with laccase are shown in the reaction structure diagram, wherein horizontal coordinate is the first principal component and vertical coordinates is the second principal component. Reaction structure diagram represents the full nature of small molecules react with laccase, but there is a large number of irregular curves overlap, and there is no clear tendency. Therefore, principal components are re-analyzed by transpose of the original data matrix, and the first and second principal components are combined into a vector. Then, hierarchical clustering analysis is done by calculating the Euclidean distance. The results are shown in the Figure 4, wherein smaller Euclidean distance indicates the nature of the reaction chemistry and the catalytic mechanism are similar. The slopes of curves in the figure are similar, and the degree of correlation is high.

V. CONCLUSION

When small molecule mediators as a substrate are involved in the reaction with laccase, Coumaric Acid, Sinapic Acid, HBT; Coumaric Acid, ABTS; Ferulic Acid and Syringic Acid respectively have substantially the same catalytic mechanism, and can be divided into three groups. The selected molecules form different catalytic systems with laccase and are divided to different categories of medium, although many medium molecules may contain similar functional group (such as benzene ring) or specific chemical elements (such as nitrogen). Medium molecules may not have the same catalytic reactions with laccase, although they may have the same or similar groups. Medium molecules may impact the catalytic reaction of laccase very differently, there are belong to different groups. These three types of catalytic medium in the catalytic system with laccase exhibits different catalytic mechanism, changes in specific wavelengths are also different. This new discovered classification system can help study the reaction mechanism of laccase and small molecule medium. The screening method used in the study is based on principal component analysis and cluster analysis, and can be used for classification and clustering of unknown reaction medium system of laccase. New small molecule medium systems can also be incorporated into the above classification, and their properties can be predicted by the categories they are belonging to. Further, the classification can also be used as a screening tool for small molecules that are suitable in certain reactions. This rapid screening method can also be extended to other fields, such as study of the reactions of other enzymes and prediction of the properties of other reactants.

REFERENCES

[1] Yilei Zhao, "Basic tutorial of Matlab", unpublished.

[2] Yanke Bao, "Data Analysis", Tsinghua University Press, Sep. 2011, pp. 68-75, ISBN: 978-7-302-26596-2.

[3] Yanke Bao, "Data Analysis", Tsinghua University Press, Sep. 2011, pp. 202-216, ISBN: 978-7-302-26596-2.

[4] 梁逸曾，许青松,《复杂体系仪器分析:白、灰、黑分析体系及其多变量解析方法》, 化学工业出版社, 第 1 版, 2012, Part 1-Chapter 3-Section 2.

TABLE I. THE FIRST AND SECOND PRINCIPAL COMPONENT CONTRIBUTION RATES

	ABTS	Ferulic Acid	HBT	Syringic Acid	Coumaric Acid	Caffeic Acid	Sinapic Acid
First principal component	0.9633	0.9993	0.8944	0.9992	0.8647	0.9035	0.9021
Second principal component	0.0361	5.585×10^{-4}	0.1055	5.380×10^{-4}	0.0922	0.0964	0.0978
Total of First and Second principal component	0.9994	0.9999	0.9999	0.9997	0.9569	0.9999	0.9999

TABLE II. THE FIRST AND SECOND PRINCIPAL COMPONENT CONTRIBUTION RATES OF DATA WITH WAVELENGTH INFORMATION

	ABTS	Ferulic Acid	HBT	Syringic Acid	Coumaric Acid	Caffeic Acid	Sinapic Acid
First principal component	0.9423	0.9099	0.9206	0.9434	0.9956	0.7904	0.9174
Second principal component	0.0543	0.0900	0.0793	0.0565	0.0042	0.1791	0.0825
Total of First and Second principal component	0.9967	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999