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Bioprocesses Modeling of Acidolysis and Redoxolysis Activities of $[Fe^{+3}]$ and $[Fe^{+2}]$ Iron by *Saccharomyces cerevisiae* and *Acetobacter aceti*

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ABSTRACT

The acidolysis and redoxolysis reactions regulate the oxidation and reduction of ferric $[Fe^{+3}]$ and ferrous iron $[Fe^{+2}]$ which are vital for living organisms. Bioprocesses modeling of the acidolysis along with redoxolysis activities of ferric to ferrous iron ratio $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ by *Saccharomyces cerevisiae* and *Acetobacter aceti* was studied. The bioprocess experiments were carried out for eight weeks at different temperatures as 25°C, 30°C and 35°C. Glucose, ascorbic acid, acetic acid, ethyl alcohol and vinegar were used in cultivation media as substrate and acidic purposes. The $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ ratios were determined from pH and Nernst equation, and the modeling of the bioprocesses was accomplished by employing the specific iron utilization rate $[q_{Fe^{+2}}]$ from Michaelis-Menten equation. The oxidation results of $[Fe^{+2}]$ ions were found noticeable at 35°C in the microbial environment.

$[Fe^{+3}]$ ve $[Fe^{+2}]$ Demirin Asidoliz ve Redoksoliz Aktivitelerinin *Saccharomyces Cerevisiae ve Acetobacter Aceti* ile Biyoproses Modellemesi

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ÖZ

Asidoliz ve redoksoliz reaksiyonları, canlı organizmalar için hayati önem taşıyan ferrik $[Fe^{+3}]$ ve ferröz demirin $[Fe^{+2}]$ oksidasyonunu ve indirgenmesini düzenler. *Saccharomyces cerevisiae* ve *Acetobacter aceti* tarafından ferrikten ferros demire oranı $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ redoksoliz aktiviteleri ile birlikte asidolizin biyoproses modellemesi incelenmiştir. Biyoproses deneyleri, sekiz hafta boyunca 25°C, 30°C ve 35°C farklı sıcaklıklarda gerçekleştirilmiştir. İnkübasyon ortamında substrat ve asidik amaçlarla glukoz, askorbik asit, asetik asit, etil alkol ve sirke kullanılmıştır. $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ oranları pH ve Nernst denklemlerinden belirlenmiş ve biyoproseslerin modellenmesi Michaelis-Menten denkleminden spesifik demir kullanım oranı $[q_{Fe^{+2}}]$ kullanılarak yapılmıştır. $[Fe^{+2}]$ iyonların oksidasyon sonucu, mikrobiyal ortamda 35°C'de fark edilebilir bulunmuştur.

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1. Introduction

The bioprocesses of reduction, solubility, absorption, and uptake of the ferric $[Fe^{+3}]$ and ferrous $[Fe^{+2}]$ iron ion minerals straight dependent on the acidolysis and redoxolysis reactions, which are vital for living organisms. The gastric acid lowers the pH in the stomach, and $[Fe^{+2}]$ is oxidized to the insoluble $[Fe^{+3}]$ form and resulting anemia or iron overloads in disorderly cases. The nutrition such as dietary glucose and ascorbic acid (vitamin C) can stimulate reduction, solubilization, absorption and uptake of $[Fe^{+3}]$ ions. The nutrition sourced iron is primarily in the insoluble $[Fe^{+3}]$ state, so must be reduced to the $[Fe^{+2}]$ state for absorption. There is microbial reduction of iron mechanism from the insoluble $[Fe^{+3}]$ to the soluble $[Fe^{+2}]$ form in yeasts (Yiannikourides and Latunde-Dada, 2019; Abbaspour et al., 2014; Sukru et al., 2014).

S. cerevisiae is amongst the massively used industrial microorganisms for single-cell protein and alcohol productions, and *A. aceti* is considered to be the major producer of organic acid, acetic acid, the main component of vinegar (Askwith et al., 1996; Pas et al., 2007; Peter and Goranovič, 2008).

Consequence of the preliminary literature searches for employing of *S. cerevisiae* and of *A. aceti* as bioprocess microorganisms has led to design experimental set up in cooperation with symbiotic relations of *S. cerevisiae* with the company of *A. aceti*. The key inspiring idea to design and prepare *S. cerevisiae* and *A. aceti* microbial cultures was based on the symbiotic relations between the employed microorganisms and the substrate mixtures which are employed in conjunction with microbial bioprocess studies. The point of the experimental set up *S. cerevisiae* and *A. aceti* incubation process was to have *S. cerevisiae* produce ethyl alcohol (C_2H_5OH) from glucose ($C_6H_{12}O_6$), and in nature to have *A. aceti* produce acetic acid (CH_3COOH) from the produced alcohol. *A. aceti* maintains a proton $[H^+]$ motivating force for production of CH_3COOH in microbial system (Matsushita et al., 2005).

Symbiotic work between *S. cerevisiae* and *A. aceti* and glucose conversion to alcohol ending acetic acid:

$$C_6H_{12}O_6 \xrightarrow{Saccharomyces} C_2H_5OH \xrightarrow{Acetobacter} CH_3COOH$$

The acidolysis and redoxolysis studies as part of the $[H^+]$ and the dissolved $[Fe^{+3}]$ researches included pH and Oxidation Reduction Potential (ORP) analysis. The main chemical reactions, acidolysis and redoxolysis reactions are frequently used for monitoring the biochemical processes. Microorganisms supply $[H^+]$ and $[Fe^{+3}]$ ions by acidolysis and redoxolysis reactions in the bioprocess. The protons reduce the pH of the bioprocess media and $[Fe^{+3}]$ ions produce ORP proportion with $[Fe^{+3}]$ in mV unit. ORP (mV) is the strong function of pH as well as mineral ion concentrations. In most bioprocesses, the ORP increases with decrease of pH values. Surveys of pH and ORP measurements in biooxidation process studies have confirmed that the low pH and high ORP

observations are favorable to $[Fe^{+3}]$ and $[Fe^{+2}]$ iron included bioprocess efficiency (Plumb et al., 2008).

Biooxidation of $[Fe^{+3}]$ and $[Fe^{+2}]$ ions involves primary acidic reactions, so that the bioprocess studies have been accomplished by employing acidophilic microorganisms that provide protons $[H^{+}]$ and $[Fe^{+3}]$ to reach sufficient ORP level. The biochemistry and electrochemistry of the iron minerals have been studied and significant progresses were developed to understand the modeling the mechanisms of the $[Fe^{+3}]$ and $[Fe^{+2}]$ iron bioprocess practices (Ojumu et al., 2009).

Biooxidation process of $[Fe^{+3}]$ and $[Fe^{+2}]$ iron includes acidolysis and redoxolysis reactions where $[Fe^{+3}]$ is generated through the chemical and biooxidation of $[Fe^{+2}]$ by proton production. The observed pH and ORP changes by time can be used for the extracting polynomial equations which can be after derivation, applied to pH and Nernst equations to get the $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ ratios that were used in Michaelis-Menten type growth equation to model the bioprocess. Integration of the derivative equations can be used to get the predictive $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ ratio and the specific growth rate, $q_{Fe^{+2}}$ according to the modeling methods used in previous studies (Boon and Heijnen 1998, Ermurat 2013).

2. Materials and Methods

S. cerevisiae of commercial baker's yeast and *A. aceti* of traditional vinegar strains were used for the cultivations. The $FeSO_4.7H_2O$ and $FeCl_3$ were used to prepare $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ mineral iron solutions in the bioprocess experiments. Besides the mineral iron solutions, ascorbic acid (vitamin C), acetic acid, ethyl alcohol, glucose and vinegar, and controlled amount of corn steeping liquor and grape and apple syrups were used as substrates for incubation, which were carried out for eight weeks at different temperatures as 25°C, 30°C and 35°C in batch type liquid state bioreactor in an incubator. Experimental combination set up of pH and mV determinations for $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ bioprocess prepared as follows:

▶	Glucose+Ascorbic acid+Alcohol+Vinegar+Acetic acid
◀	Glucose+Ascorbic acid+*S. cerevisiae*+*Acetic aceti*
▲	Glucose+Ascorbic acid+Alcohol+*S. cerevisiae*+*A. aceti*
●	Glucose+Ascorbic acid+Vinegar+ Acetic acid+*S. cerevisiae*+*A. aceti*
▼	Glucose+Ascorbic acid+Alcohol+Vinegar+ Acetic acid+*S. cerevisiae*+*A. Aceti*

The pH and mV measurements were performed during eight weeks incubation. Matrix laboratory (MATLAB) software was used for calculation and plotting.

3. Results

The observational results of pH and mV vs. time were graphed to get polynomial equations and their derivatives, which were equalized to the derivatives of Nernst equation and adjusted in Michaelis-Menten model. The derivatives were integrated back to estimate $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ ratio and the specific growth rate ($q_{Fe^{+2}}$). Figures 1-3 present the plots of pH and mV, and tables 1-3 present the polynomial equations for pH and mV changes by time (week) at 25°C, 30°C and 35°C respectively.

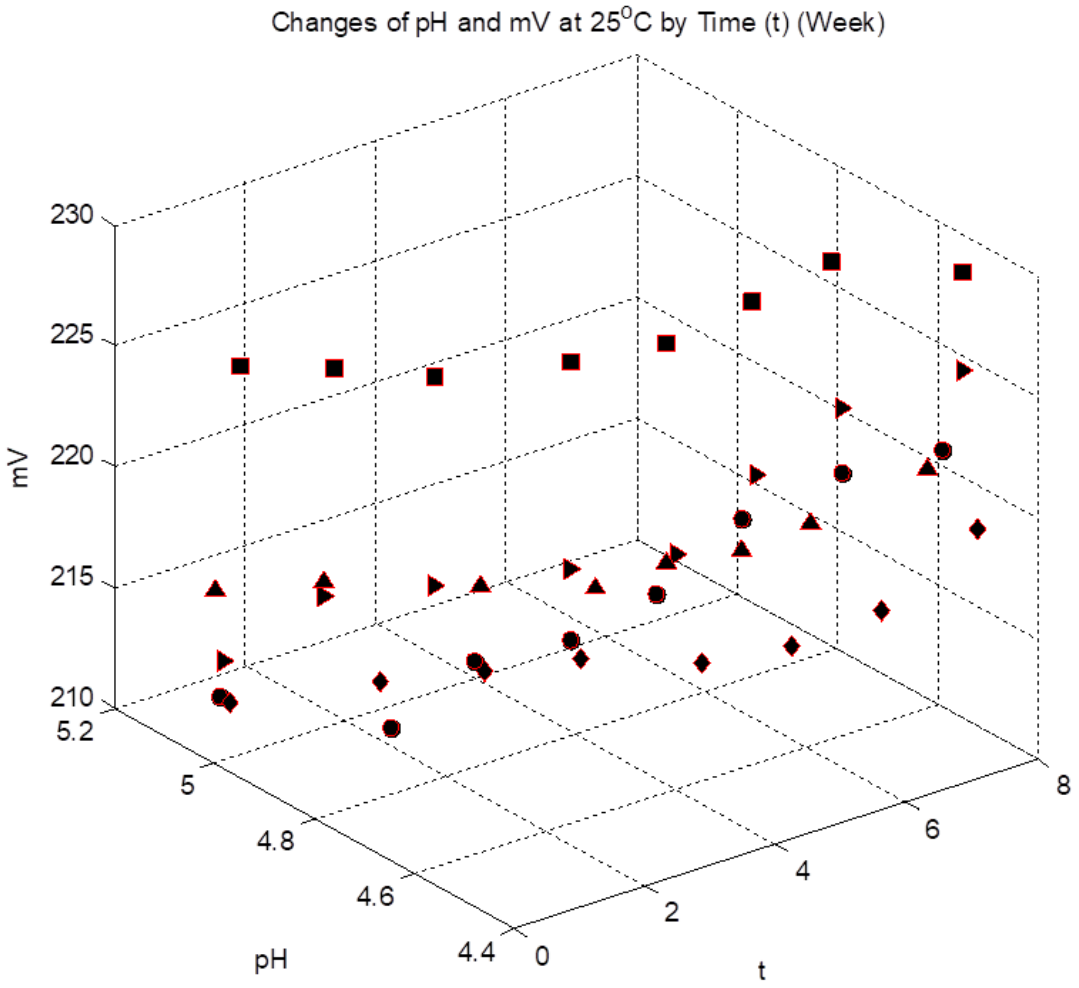

Figure 1. Changes of pH and mV by time (week) at 25°C

Table 1. The polynomial equations for pH and mV changes by time (week) at 25°C

Glucose+Ascorbic acid+Alcohol+Vinegar+Acetic acid	
pH	$y=0.00399x^2-0.115x+5.23$
mV	$y=-0.21x^2+4.22x+207$
Glucose+Ascorbic acid+*S. cerevisiae+Acetic aceti*	
pH	$y=0.00631x^2-0.0135x+5.21$
mV	$y=-\ 0.00714x^2+1.27x+205$
Glucose+Ascorbic acid+Alcohol*+S. cerevisiae+A. aceti*	
pH	$y=0.0101x^2-0.157x+5.28$
mV	$y=-\ 0.0333x^2+1.51x+204$
Glucose+Ascorbic acid+Vinegar+ Acetic acid+*S. cerevisiae+A. aceti*	
pH	$y=0.005x^2-0.11x+5.17$
mV	$y=-\ 0.00893x^2+1.07x+215$
Glucose+Ascorbic acid+Alcohol+Vinegar+ Acetic acid+*S. cerevisiae+A. Aceti*	
pH	$y=0.00196x^2-0.0914x+5.18$
mV	$y = 0.0292\ x^2+ 0.529x + 217$

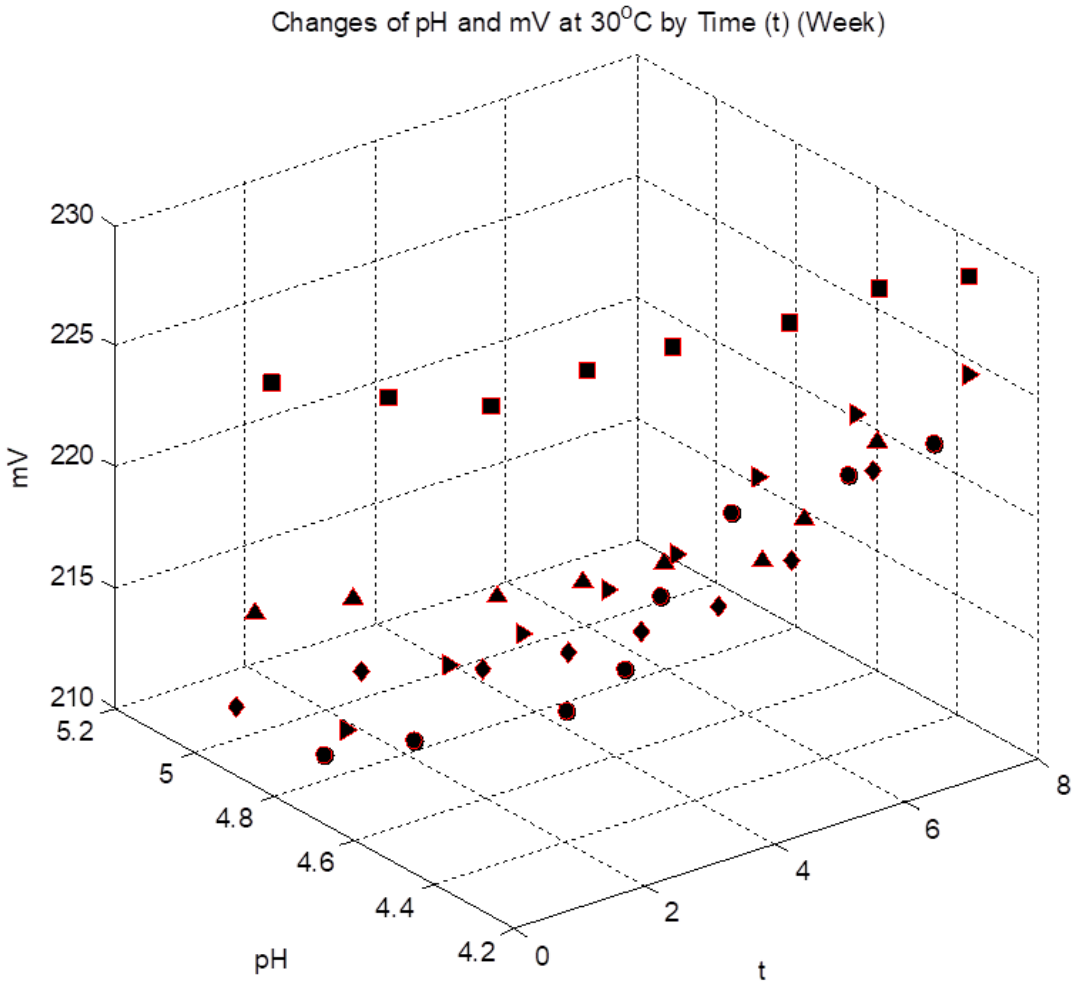

Figure 2. Changes of pH and mV by time (week) at 30°C

Table 2. The polynomial equations for pH and mV changes by time (week) at 30°C

Glucose+Ascorbic acid+Alcohol+Vinegar+ Acetic acid	
pH	$y=-0.00381x^2+0.0155x+4.77$
mV	$y=0.0952x^2+0.812x+212$
Glucose+Ascorbic acid+*S. cerevisiae+A. aceti*	
pH	$y= 0.0109x^2-0.155x+5.18$
mV	$y=0.0905x^2+0.0167x+211$
Glucose+Ascorbic acid+Alcohol+*S. cerevisiae+A. aceti*	
pH	$y= 0.014x^2-0.185x+5.2$
mV	$y=0.0411x^2+0.141x+215$
Glucose+Ascorbic acid+Vinegar+ Acetic acid+*S. cerevisiae+A. aceti*	

pH	$y= 0.00381x^2-0.0783x+4.88$
mV	$y=0.0607x^2+1.03x+209$
Glucose+Ascorbic acid+Alcohol+Vinegar+ Acetic acid+*S. cerevisiae+A. Aceti*	
pH	$y=0.00286x^2-0.11x+5.06$
mV	$y = 0.0208x^2+1.139x+211$

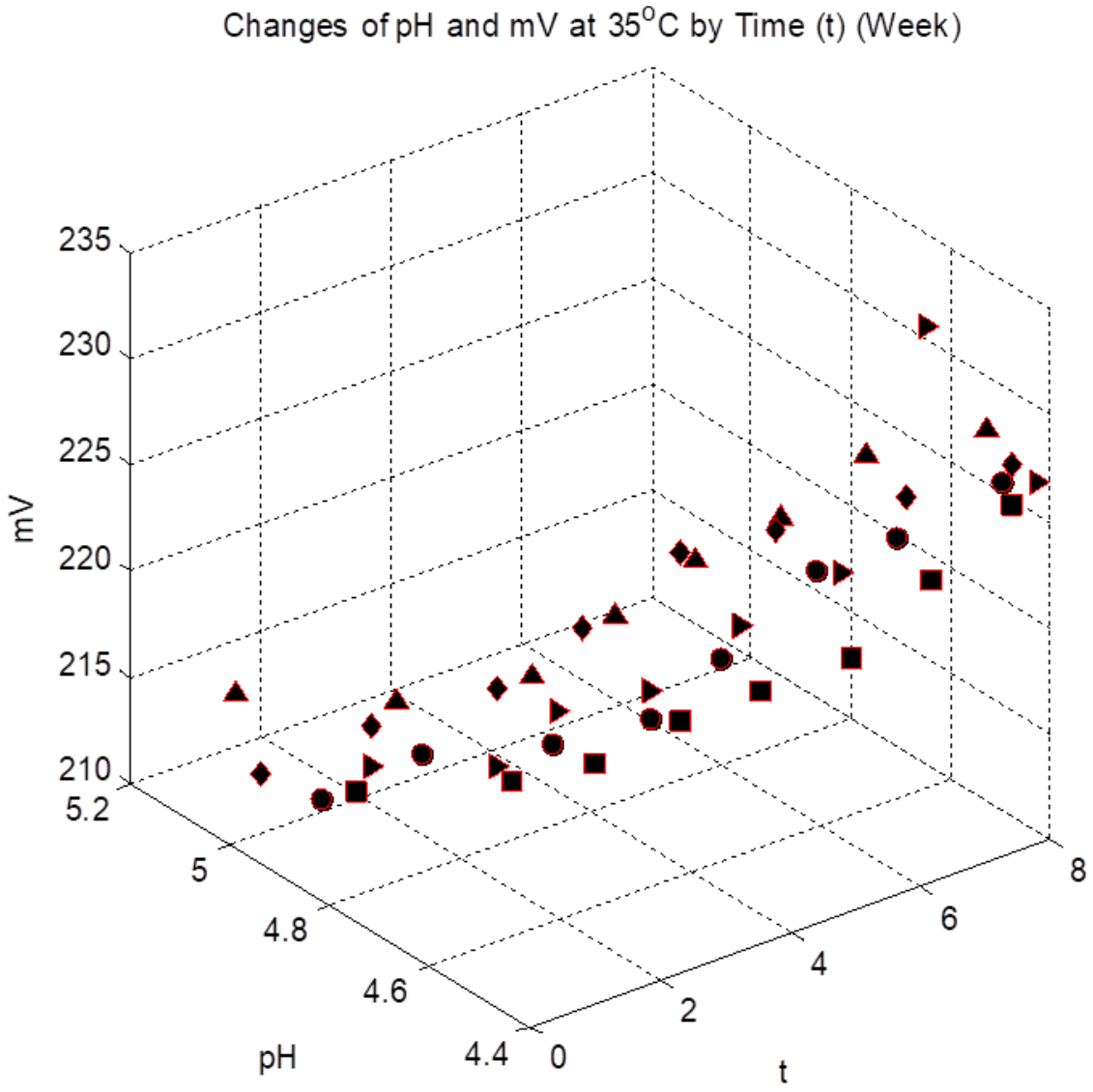

Figure 3. Changes of pH and mV by time (week) at 35°C

Table 3. The polynomial equations and their derivatives for pH changes by time (week) at 35°C

Glucose+Ascorbic acid+Alcohol+Vinegar+Acetic acid	
pH	$y=-0.00143x^2-0.0417x+4.87$
mV	$y=0.0863x^2+0.848x+213$
Glucose+Ascorbic acid+*S. cerevisiae+Acetic aceti*	
pH	$y=-0.000655x^2-0.0752x+5.13$
mV	$y=-0.0869x^2+3.02x+208$
Glucose+Ascorbic acid+Alcohol+*S. cerevisiae+A. aceti*	
pH	$y=0.0181x^2-0.144x+5.21$
mV	$y=0.00714x^2+1.87x+212$
Glucose+Ascorbic acid+Vinegar+ Acetic acid+*S. cerevisiae+A. aceti*	
pH	$y=0.00464x^2-0.102x+5.04$
mV	$y=0.0899x^2+1.14x+211$
Glucose+Ascorbic acid+Alcohol+Vinegar+ Acetic acid+*S. cerevisiae+A. Aceti*	
pH	$y = 0.00643x^2-0.107x+4.94$
mV	$y = 0.158x^2+0.798x+214$

Figures 4-6 show the plots of changes of $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{pH}$ vs. $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{mV}$ by time (week) at 25°C, 30°C and 35°C respectively.

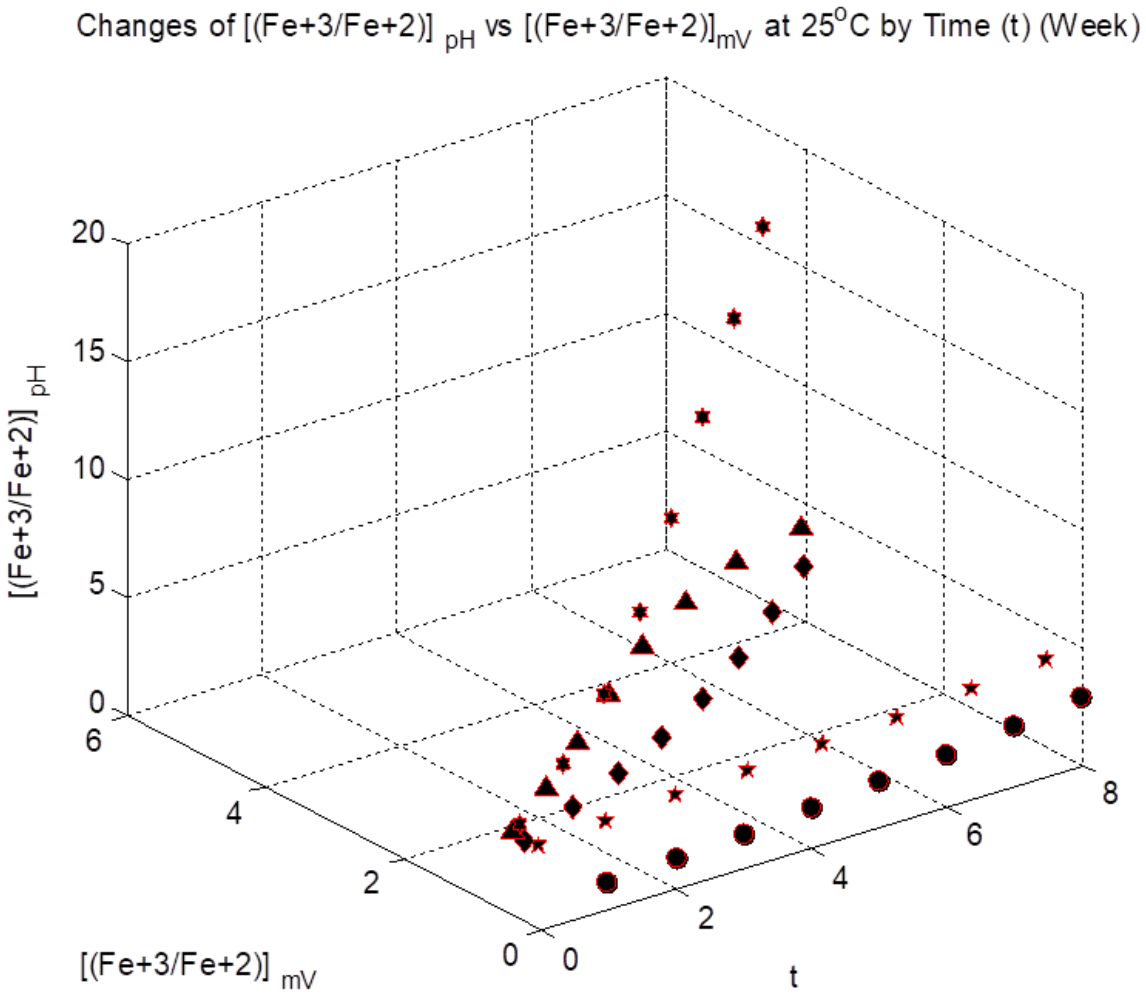

Figure 4. Changes of $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{pH}$ vs. $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{\mathrm{mV}}$ by time (week) at 25°C

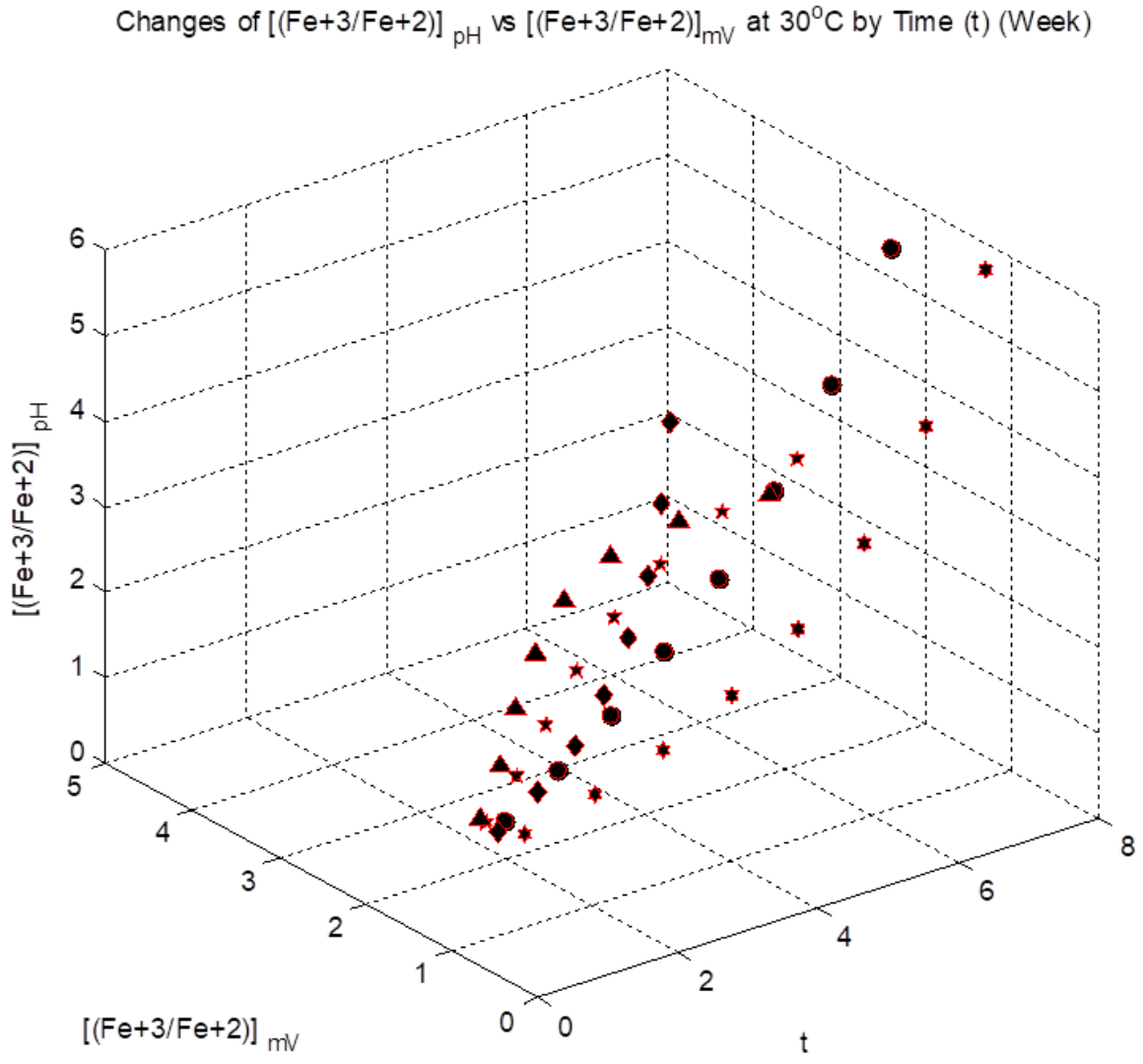

Figure 5. Changes of $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{pH}$ vs. $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{\mathrm{mV}}$ by time (week) at 30°C

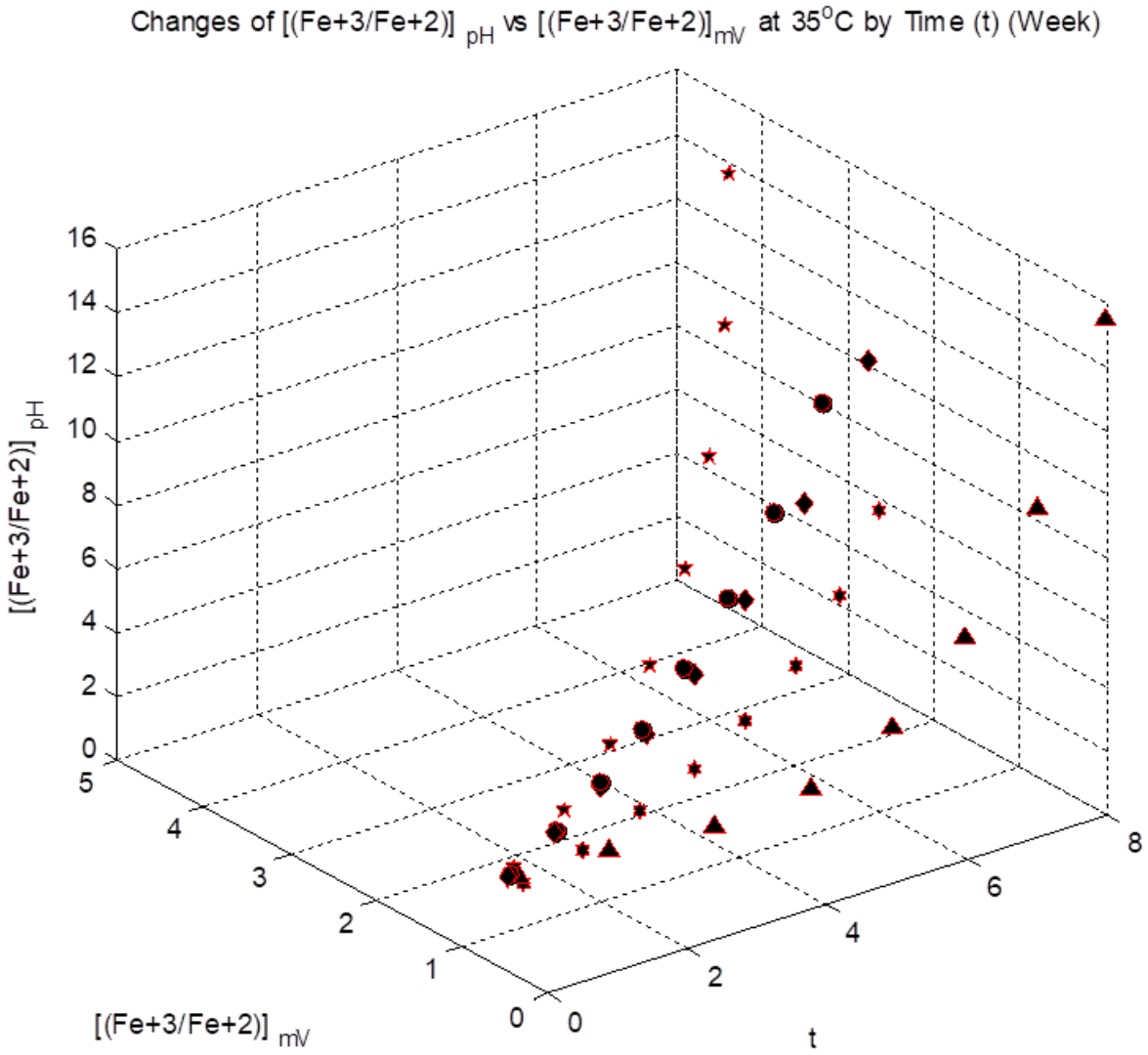

Figure 6. Changes of $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{pH}$ vs. $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{\mathrm{mV}}$ by time (week) at 35°C

Figures 7-9 present the graphs of the predicted $\lfloor q_{Fe^{+2}} \rfloor_{pH}$ vs. $\lfloor q_{Fe^{+2}} \rfloor_{\mathrm{mV}}$ changing by time (week) at 25°C, 30°C and 35°C respectively.

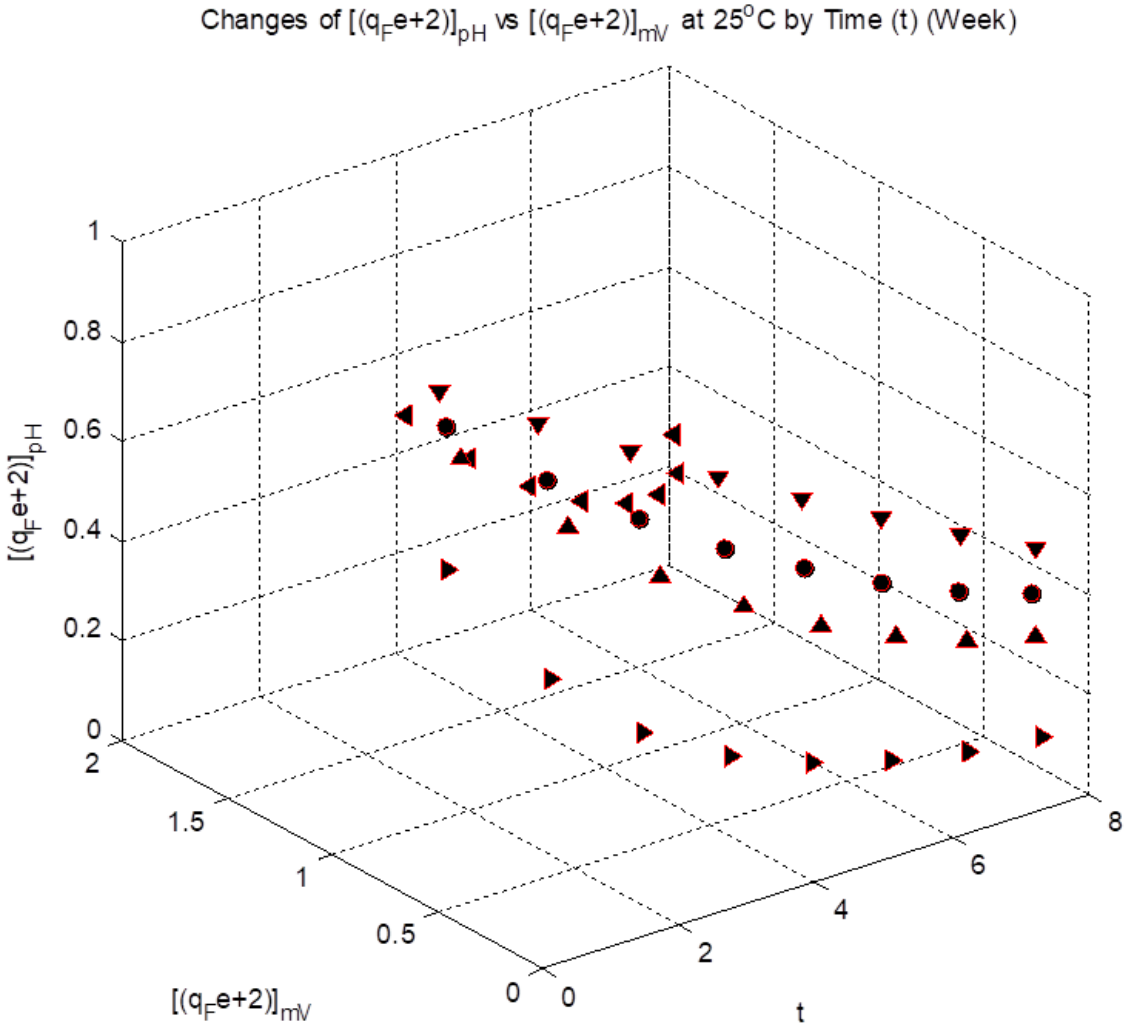

Figure 7. Changes of $\lfloor q_{Fe^{+2}} \rfloor_{pH}$ vs. $\lfloor q_{Fe^{+2}} \rfloor_{mV}$ by time (week) at 25 ^{o}C

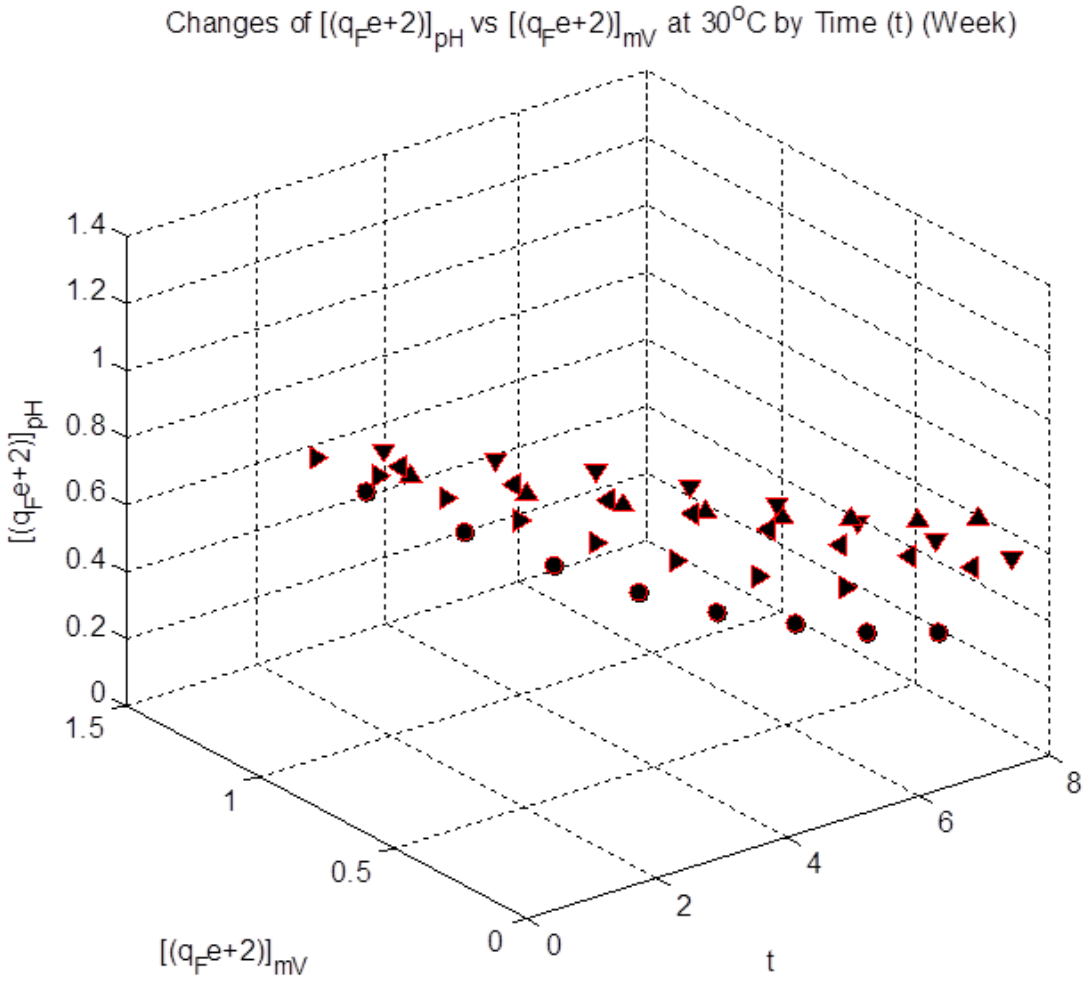

Figure 8. Changes of $\lfloor q_{Fe^{+2}} \rfloor_{pH}$ vs. $\lfloor q_{Fe^{+2}} \rfloor_{mV}$ by time (week) at 30°C

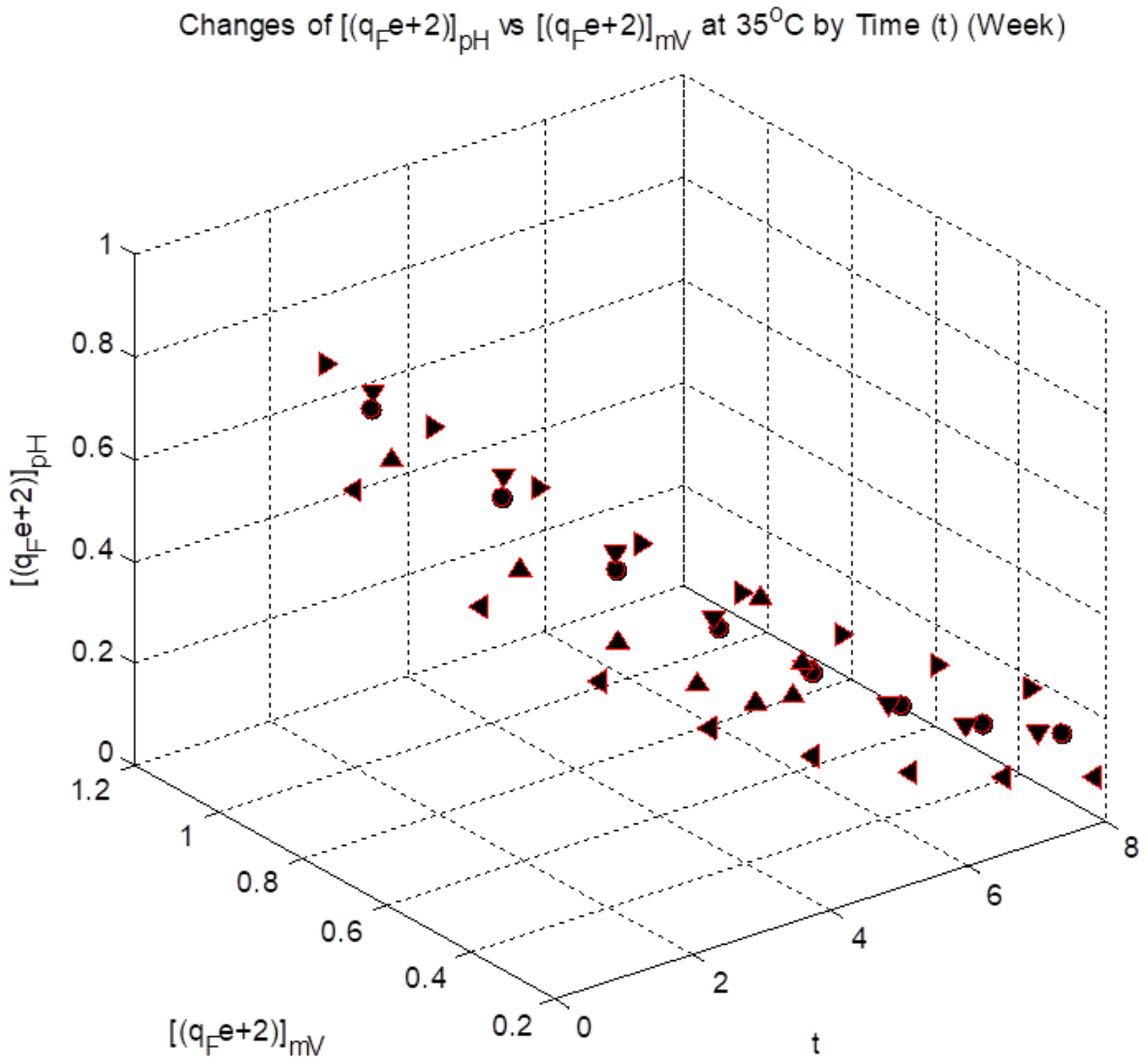

Figure 9. Changes of $\lfloor q_{Fe^{+2}} \rfloor_{pH}$ vs. $\lfloor q_{Fe^{+2}} \rfloor_{\mathrm{mV}}$ by time (week) at 35°C

4. Discussion

The acidolysis and redoxolysis reactions of the $[Fe^{+3}]$ and $[Fe^{+2}]$ iron minerals in bioprocess of *S.cerevisiae* and *A. aceti* have shown that the chosen experimental parameter conditions offer promising potentials for the microbial growth with the iron minerals. It has been shown experimentally in this study that the cultivability of *S. cerevisiae* and *A. aceti* was successfully observed in relation with the the $[Fe^{+3}]$ and $[Fe^{+2}]$ minerals through the experiment. The mixed cultures of *S. cerevisiae*+*A. aceti* in the iron mineral bioprocess displayed a cooperative symbiotic living and no notice of lethal effect was recorded.

There was no notice of microbial colonization of *S. cerevisiae* and *A. aceti* however some of the microorganisms were well immobilized on the surface of substrate particles. The bioprocess

consequently was presented three defined categories of bioprocess with no lethal harmful results, that one of them was direct bioprocess by immobilization of *S. cerevisiae* on the surface of the substrate particles, another was indirect bioprocess by no attachment of *A. aceti* to the particles and cooperative the bioprocess by symbiotic living of *S. cerevisiae* and *A. aceti*. The most part of the glucose as main substrate was supposed to be consumed by the microorganisms and by the iron ions oxidation.

The initial pH of the bioprocess medium, in the presence of the acetic acid and vinegar was decreased to about 5, besides the acetic acid secretion, further lowering the pH 5 to 4. The initial ORP of the medium was above 200 mV than was raised to around 240 mV during the bioprocess in relation with the decrease in pH values. The inconsequential changes in pH and mV show a few reductions of iron ions and consequently sulfur elements.

The observed pH and mV data of the $[Fe^{+2}]$ to $[Fe^{+3}]$ iron bioprocess expectedly have shown mild conditions like common bioprocesses that primarily results acidic (pH>4) with redox potential (mV<240) conditions. Therefore, it has been shown that the *S. cerevisiae*+*A. aceti* microbial bioprocesses had the capability of the acidolysis tendency. Briefly, it has been indicated that the controlled number of microbial mixtures might be used as pH lowering agents due to their acidolysis capacity and nontoxic aspects, instead of using chemical neutralizing agents in every stage of the related fermentation processes. Though the used microorganisms *S. cerevisiae* with *A. aceti* were recognized as effective in the bioprocess, inconsistently the pure or mixed cultures of the bioprocess have displayed likewise less effective function in $[Fe^{+3}]$ iron conversion. This is primarily liable to having very limited oxygen in the incubation media, yet the bioreactors had not been shaken or mixed through the experimental observations. Regarding the $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ bioprocess medium where there was no manually aeration or mixing, the foremost result of the neutralizing pH and lessening mV is likely consequence of the very limited quantity of oxygen naturally dissolved as a result of less mixing in the bioprocess media, therefore the less chemical interactions occurred between the $[Fe^{+3}]$ and $[Fe^{+2}]$ ions and sulfur and oxygen molecules. As all other microorganisms, *S. cerevisiae* and *A. aceti* have affinity of heavy metals, so that most possibly some of $[Fe^{+3}]$ and $[Fe^{+2}]$ ions were adsorbed by the microorganisms.

The effect of the glucose in some biobatches had not confidently shown on the extent of iron oxidation results. In some extend, the increase in the bioprocess temperature had shown encouraging results for iron immobilization in this study. As far as the trivial changes in pH and mV measurements were recorded, the selected parameters of the bioprocess were encountered as the other accountable constraints.

Figures 5-7 show that the pH and mV originated predictive $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ ratios were found numerically comparable by the variation of log constant 2,303 in mV originated oxidation ratio as presented in the

plots of $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{pH}$ vs. $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]_{\mathrm{mV}}$ changes by time (week) at 25°C, 30°C and 35°C temperatures respectively.

Figures 7-9, the ratios of the pH and mV originated predictive specific growth rates $q_{Fe^{+2}}$ consequently have presented the depiction of the slight amount of oxidation of $[Fe^{+2}]$ ion in the microbial environment as shown in the plots of changes of $\lfloor q_{Fe^{+2}} \rfloor_{pH}$ vs. $\lfloor q_{Fe^{+2}} \rfloor_{\mathrm{mV}}$ by time (week) at 25°C, 30°C and 35°C respectively.

5. Conclusions

The acidolysis and redoxolysis mechanisms consist of biochemical reactions for the reduction, solubility, absorption and uptake of the $[Fe^{+3}]$ and $[Fe^{+2}]$ ions by living organisms. The microbial processes of *S.cerevisiae* and *A. aceti* with the selected parameters have shown that the acidolysis and redoxolysis mechanisms of the $[Fe^{+3}]$ and $[Fe^{+2}]$ offered some promising potentials for biooxidation process of the iron minerals, microbial growth and subsequently sulfur conversion. The observed changes in pH and the ORP of the bioprocesses present insignificant decrease in pH, and a quantity of rise in redox potential. The models provided approvable prediction of $\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$ and $\lfloor q_{Fe^{+2}} \rfloor_{pH}$ vs. $\lfloor q_{Fe^{+2}} \rfloor_{\mathrm{mV}}$ sourcing from pH and the ORP observations with minor amount of oxidation of $[Fe^{+2}]$ ions in the microbial environment.

Conflict of Interest

There is no conflict of authors in this work.

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