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DOI: 10.2225/vol16-issue2-fulltext-1 *RESEARCH ARTICLE*

# Modeling the kinetics of pyrite ash biodesulfurization by *Saccharomyces cerevisiae* and *Acetobacter aceti* in liquid state bioreactors

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## Abstract

**Background:** Modeling the kinetics of the biodesulphurization bioprocess for the refining of pyrite ash by *Saccharomyces cerevisiae* and *Acetobacter aceti* have been studied in batch-type liquid- state bioreactors.
**Results:** The biodesulphurization experiments were performed at varying temperatures of 25ºC, 30ºC and 35ºC for eight weeks. Glucose, acetic acid and ethyl alcohol were used in the incubation media as substrates and acid sources. pH and oxidation reduction potential (ORP) observations have been determined weekly and the dissolved sulphur was measured at the end of the eight weeks trials. An equation calculating pH was derived from the iron oxidation reaction containing the ferric to ferrous iron [$Fe^{+3}/Fe^{+2}$] ratio as a variable. The Michaelis-Menten predictive specific growth rates ($q_{Fe}^{+2}$), which were estimated from pH and ORP observations, were compared by plotting $[q_{Fe}^{+2}]_{pH}$ vs. $[q_{Fe}^{+2}]_{mV}$. The highest ratio of dissolved sulphur over total sulphur ($S_d/S_t$) was found to be 0.5 in the biodesulphurization processes.
**Conclusions**: The model provides predictions of ferric to ferrous iron rates and specific growth rates $[q_{Fe}^{+2}]_{pH}$ vs. $[q_{Fe}^{+2}]_{mV}$ and can be used for the determination of oxidized and reduced ions. The ratios of dissolved sulphur to total sulphur ($S_d/S_t$) have shown some promising results for *S. cerevisiae* to be used as a biodesulphurization and refining microorganism for pyrite ash and the other sulphide minerals.

**Keywords:** *Acetobacter aceti*, biodesulfurization, kinetics, modeling, pyrite ash, *Saccharomyces cerevisiae.*

## INTRODUCTION

A mineral waste ash, pyrite ash is a largely unused waste product of calcination process of the pyrite mineral for sulphuric acid production. The high iron content of pyrite ash makes it a valuable mineral ash, which nevertheless cannot be used for iron production due to its very low sulphur content. Using biodesulfurization methods to dissolve the sulphur bound to the iron is one potential way to employ bioleaching and thus allow for the purification and effective use of the mineral ash. *Saccharomyces cerevisiae* was used in this research for two main purposes. First, *S. cerevisiae* is used as a biodesulphurization microorganism for its significant capabilities of metabolizing inorganic and organic sulphur sources. *S. cerevisiae* consumes sulphur compounds for the metabolism of sulphur-containing cysteine, methionine amino acids and hydrogen sulphide (Piotrowska and Paszewski, 1990; Thomas and Surdin-Kerjan, 1997; López del Castillo-Lozano et al. 2007; Mendes-Ferreira et al. 2009). Second, to minimize effects that would decrease the iron amount in the mineral ash, substantial biooxidation of

ferrous iron ($Fe^{+2}$) was avoided in pyrite ash biodesulfurization process. Besides *S. cerevisiae* has a high affinity to pyrite and other sulphide minerals, thus is used in mineral bioremediation processes for theremoval of the minerals in coal flotation (Janetski et al. 1977; Kawatra and Eisele, 1999). *Acetobacter aceti* is used for its proton ($H^+$) motive force for acetic acid production (Matsushita et al. 2005). In fact, *S. cerevisiae* and *A. aceti* are among the most widely used industrial microorganisms, though they have not been employed in bioleaching and in biodesulfurization studies. The preliminary investigation of the use of *S. cerevisiae* as a biodesulfurization microorganism has also led to the design of experiments examining the symbiotic relations between *S. cerevisiae* and *A. aceti* (Krisch and Szajani, 1997).

Common similar techniques have been applied in bioleaching and biodesulfurization processes. Bioleaching studies have generally been conducted by observing pH and reduction potentials because of their effects on microbial growth and thus enzymatic activities (Rand and Woods, 1984; Brandl, 2001; Kumar and Nagendran, 2007). The major chemical reactions, protonolysis and redoxolysis, are regularly used to monitor biotechnological processes; therefore, the parameters of the bioleaching studies have been applied to biodesulfurization trials. Microorganisms supply protons ($H^+$) and ferric iron ions through protonolysis and redoxolysis reactions in pyrite ($FeS_2$) bioleaching. Protons decrease the pH of the bioleaching medium and ferric iron ions generate the ratio of the oxidation reduction potential (ORP) with ferrous iron ($Fe^{+2}$) in mV (Plumb et al. 2008).

Changes of the observed pH and ORP over time have been used in this study to drive polynomial equations, which were then incorporated into the pH and Nernst equations to obtain the ferric to ferrous iron [$Fe^{+3}/Fe^{+2}$] ratios used to obtain the rates of specific iron utilization (Torma, 1977). Modeling the kinetics of biodesulfurization was accomplished by applying the rate of biodesulfurization into the Michaelis-Menten equation. The ratio of dissolved sulfur to total sulfur ($S_d/S_t$) was determined at the end of the experiments.

## MATERIALS AND METHODS

The microbial cultures and substrate mixtures were prepared according to the symbiotic relationship between *S. cerevisiae* and *A. aceti*. Both strains were isolated and incubated according to the isolation and incubation method. The main purpose of the experimental set up was to allow *S. cerevisiae* to produce alcohol from glucose and to allow *A. aceti* to naturally produce acetic acid from alcohol. The *S. cerevisiae* strain was isolated from commercial baker's yeast and the *A. aceti* strain was isolated from vinegar. A sample of pyrite ash with high iron (61.08%) and low total sulphur (0.59%) content was supplied from the calcinated waste ashes of the Eti Mining Bandırma Borax Plant in Turkey. Five percent pulp solutions were used in the incubation trials which were performed at different temperatures (25ºC, 30ºC and 35ºC) for eight weeks. Non sulphur containing media was used during the incubation of the biodesulfurization experiments. Glucose, ethyl alcohol and acetic acid were used as substrates for incubation in batch-type liquid-state bioprocesses. The pH and ORP data were recorded at 25ºC, 30ºC and 35ºC. Hanna instruments were used for pH and ORP measurements and sulphate readings were taken using a Shimadzu spectrophotometer. MATLAB software was used for calculations and plotting.

The experimental set up for pyrite ash biodesulfurization processes was designed as following mixtures:

a) Pyrite ash + Glucose + *S. cerevisiae*

b) Pyrite ash + Glucose + *S. cerevisiae* + *A. aceti*

c) Pyrite ash + Glucose + Alcohol + *S. cerevisiae* + *A. aceti*

d) Pyrite ash + Alcohol + A. acid + *S. cerevisiae* + *A. aceti*

e) Pyrite ash + Glucose + Alcohol + A. acid + *S. cerevisiae* + *A. aceti*

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## RESULTS AND DISCUSSION

Biooxidation of iron and sulphur in pyrite ash involves protonolysis and redoxolysis reactions in which ferric iron is produced by the microbial oxidation of ferrous iron via protonation ($H^+$). A pH equation was derived using the iron oxidation reaction, which contains the ferric to ferrous iron ratio as a variable.

$$4Fe^{+2}+O_2+4H^+ \xrightarrow{biooxidation} 4Fe^{+3}+2H_2O$$

[**Equation 1**]

The equilibrium constant of the reaction gives the following equation for pH:

$$K = \frac{\left[Fe^{+3}\right]^4}{\left[Fe^{+2}\right]^4\left[H^+\right]^4}$$

[**Equation 2**]

$$-4\log[H^+] = -4\log\frac{[Fe^{+3}]}{[Fe^{+2}]} + \log K_{Fe^{+2}}$$

[**Equation 3**]

$$pH = 1/4\log K - \log\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$$

[**Equation 4**]

$$\left(\frac{dpH}{dt}\right) = \frac{d}{dt}\left(-\log\left[\frac{Fe^{+3}}{Fe^{+2}}\right]\right)$$

[**Equation 5**]

The biooxidation of ferrous iron to ferric iron generates an ORP that is calculated using the Nernst equation as follows:

$$Fe^{+2} \longrightarrow Fe^{+3}+e^-$$

[**Equation 6**]

$$E = E^{o} + \frac{2.303RT}{nF}\log\left[\frac{Fe^{+3}}{Fe^{+2}}\right]$$

[**Equation 7**]

$$\left(\frac{dE}{dt}\right) = \frac{2.303RT}{nF}\frac{d}{dt}\left(\log\left[\frac{Fe^{+3}}{Fe^{+2}}\right]\right)$$

[**Equation 8**]

The integrations of the derivative equations were used to obtain the predictive ferric to ferrous iron ratios and the specific iron utilization rate. The processes of the microbial oxidation of ferrous iron were modeled by introducing the specific iron utilization rate and ferric to ferrous iron ratio into the Michaelis-Menten model (Boon and Heijnen, 1998) as follows:

$$q_{Fe^{+2}} = \frac{q_{Fe^{+2}}^{\max}}{1 + K_{Fe^{+2}}\left[\frac{Fe^{+3}}{Fe^{+2}}\right]}$$

[**Equation 9**]

$$\frac{d}{dt}\left(-\log q_{Fe^{+2}}\right) = \frac{d}{dt}\left(\log\left[\frac{Fe^{+3}}{Fe^{+2}}\right]\right)$$

[**Equation 10**]

$\frac{d}{dt}\left(-\log q_{Fe^{+2}}\right)$was equated to the derivative of the polynomial equations extracted from mV vs t plots.

The immobilization of microorganisms on the mineral surfaces is an important observation (Rodriguez et al. 2003) and in this study, it has been shown that *S. cerevisiae* was fairly immobilized on the surface of the pyrite ash particles; however, *A. aceti* showed less attaching behaviour throughout the experiments. Figure 1 shows a photo of the mixed cultures of *S. cerevisiae* + *A. aceti* under light microscopy and displays the attachment of the microorganisms to the surfaces of the pyrite ash particles via biofilms. The plots of the variation of $[q_{Fe}^{+2}]_{pH}$ and $[q_{Fe}^{+2}]_{mV}$ derived from the measurements of pH and mV are displayed in Figure 2, Figure 3 and Figure 4. The maximum values of specific growth rates $[q_{Fe}^{+2}]_{pH}$ and $[q_{Fe}^{+2}]_{mV}$, and correlation coefficients are given in Table 1, in which the highest rates were found at 30ºC.

The initial pH of the bioprocess medium was decreased to approximately 4 in the presence of either glucose or acetic acid or both, even though acetic acid was supposed to be generated via glucose consumption by *A. aceti*. The initial redox potential of the bioprocess medium was above 200 mV and

was then increased to approximately 240 mV during the bioleaching. The insignificant changes in pH and mV predict few mobilizations of iron ions and thus, the dissolution of sulphur elements. Obviously the experimentally observed pH 5-4 and mV (200-240) data of the pyrite ash biodesulfurization have shown different results from numerous other common pyrite bioleaching processes that primarily result in very acidity with high redox potential.

**Table 1. The maximum values of specific growth rates $[q_{Fe}^{+2}]_{pH}$ and $[q_{Fe}^{+2}]_{mV}$ with correlation coefficients.**

| | | | | |
|---|---|---|---|---|
| **Pyrite ash + Glucose + *S. cerevisiae*** | | | | |
| $[q_{Fe}^{+2}]_{pH\ max}$ | 25ºC | 0.7656 | $R^2$ | 0.9999 |
| | 30ºC | 1.119 | $R^2$ | 0.9227 |
| | 35ºC | 0.7980 | $R^2$ | 0.9870 |
| $[q_{Fe}^{+2}]_{mV\ max}$ | 25ºC | 0.7601 | $R^2$ | 0.9999 |
| | 30ºC | 0.9073 | $R^2$ | 0.9227 |
| | 35ºC | 0.8010 | $R^2$ | 0.9870 |
| **Pyrite. ash + Glucose + *S. cerevisiae* + *A. aceti*** | | | | |
| $[q_{Fe}^{+2}]_{pH\ max}$ | 25ºC | 0.8017 | $R^2$ | 0.9922 |
| | 30ºC | 0.7176 | $R^2$ | 0.9570 |
| | 35ºC | 0.755 | $R^2$ | 0.9997 |
| $[q_{Fe}^{+2}]_{mV\ max}$ | 25ºC | 0.8425 | $R^2$ | 0.9922 |
| | 30ºC | 0.8382 | $R^2$ | 0.9570 |
| | 35ºC | 0.7968 | $R^2$ | 0.9997 |
| **Pyrite. ash + Glucose + Alcohol +*S. cerevisiae* + *A. aceti*** | | | | |
| $[q_{Fe}^{+2}]_{pH\ max}$ | 25ºC | 0.7005 | $R^2$ | 0.9866 |
| | 30ºC | 0.6745 | $R^2$ | 0.8122 |
| | 35ºC | 0.6907 | $R^2$ | 0.8834 |
| $[q_{Fe}^{+2}]_{mV\ max}$ | 25ºC | 0.8754 | $R^2$ | 0.9866 |
| | 30ºC | 0.9752 | $R^2$ | 0.8122 |
| | 35ºC | 0.9532 | $R^2$ | 0.8834 |
| **Pyrite ash + Alcohol + A. acid + *S. cerevisiae* + *A. aceti*** | | | | |
| $[q_{Fe}^{+2}]_{pH\ max}$ | 25ºC | 0.7663 | $R^2$ | 0.9958 |
| | 30ºC | 0.8424 | $R^2$ | 0.9906 |
| | 35ºC | 0.7992 | $R^2$ | 0.9876 |
| $[q_{Fe}^{+2}]_{mV\ max}$ | 25ºC | 0.8184 | $R^2$ | 0.9958 |
| | 30ºC | 0.8601 | $R^2$ | 0.9906 |
| | 35ºC | 0.8612 | $R^2$ | 0.9876 |
| **Pyrite ash + Glucose + Alcohol + A. acid + *S. cerevisiae* + *A. aceti*** | | | | |
| $[q_{Fe}^{+2}]_{pH\ max}$ | 25ºC | 0.8426 | $R^2$ | 0.9901 |
| | 30ºC | 0.7768 | $R^2$ | 0.9049 |
| | 35ºC | 0.7933 | $R^2$ | 0.9721 |
| $[q_{Fe}^{+2}]_{mV\ max}$ | 25ºC | 0.925 | $R^2$ | 0.9901 |
| | 30ºC | 1.009 | $R^2$ | 0.9049 |
| | 35ºC | 0.8733 | $R^2$ | 0.9721 |

Bioprocess of the mixed cultures of *S. cerevisiae* and *A. aceti* displayed less effective in ferrous iron biooxidation possibly due to affinity of the microorganisms to the iron and limited available oxygen in the medium despite the fact that the bioreactors had been shaken during the experimental observations. Variation in the glucose levels between biobatches did not excessively affect iron oxidation results. The selected time parameters of the bioleaching process may mediate the slight changes that occurred in pH and mV measurements.

The pH and mV originated predictive ferric to the ferrous iron ratios were found to be numerically comparable by the variation of the log constant 2.303 in the mV derived oxidation ratio. The ratios of the predictive specific growth rates $[q_{Fe}^{+2}]_{pH}$ vs $[q_{Fe}^{+2}]_{mV}$ that originated from the pH and mV observations consequently have introduced a slight amount of oxidation of ferrous iron ions in the microbial environment. Figure 2, Figure 3 and Figure 4 show the plots of changes of the predictive specific growth rates $[q_{Fe}^{+2}]_{pH}$ vs $[q_{Fe}^{+2}]_{mV}$ at 25ºC, 30ºC and 35ºC respectively. The amount of dissolved sulphur measured as sulphates at the end of the 8 weeks of incubation experiments. The bioconversion of the dimensionless sulphur ratio is shown in Figure 5 and the highest ratio of dissolved sulphur to total sulphur ($S_d/S_t$) was found to be 0.5 for the chemical and microbial mixture of pyrite ash + glucose + alcohol + acetic acid + *S. cerevisiae* + *A. aceti.*

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## CONCLUDING REMARKS

Pyrite ash biodesulfurization processes using *S. cerevisiae* and *A. aceti* have shown that the selected parameters for experimental conditions have achieved slight iron biooxidation. It has been experimentally shown that the changes in pH and the ORP of the bioprocesses present trivial decreases in pH and some increase in redox potential. The model provides predictions of ferric to ferrous iron rates and specific growth rates $[q_{Fe^{+2}}]_{pH}$ and $[q_{Fe^{+2}}]_{mV}$, and can be used to determine oxidized and reduced ions. The ratios of dissolved sulphur to total sulphur ($S_d/S_t$) have shown some promising results for *S. cerevisiae* to be used as a biodesulfurization and refining microorganism for pyrite ash and the other sulphide minerals.

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# Figures

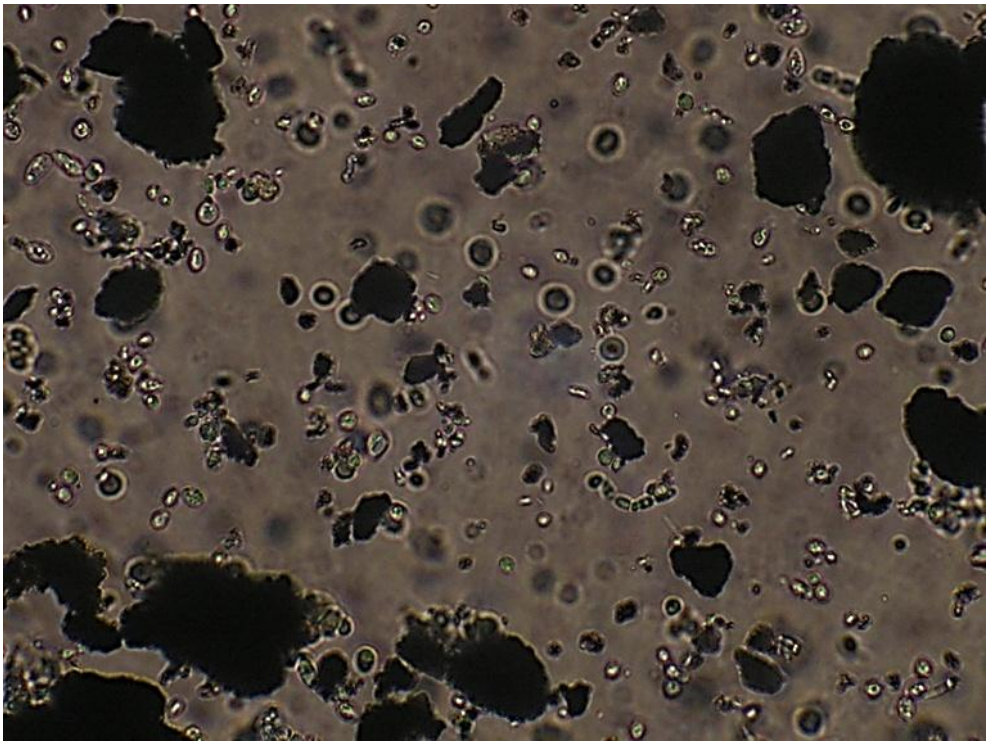

**Fig. 1** Mixed culture of *S. cerevisiae* + *A. aceti* in a pyrite ash bioleaching experiment.

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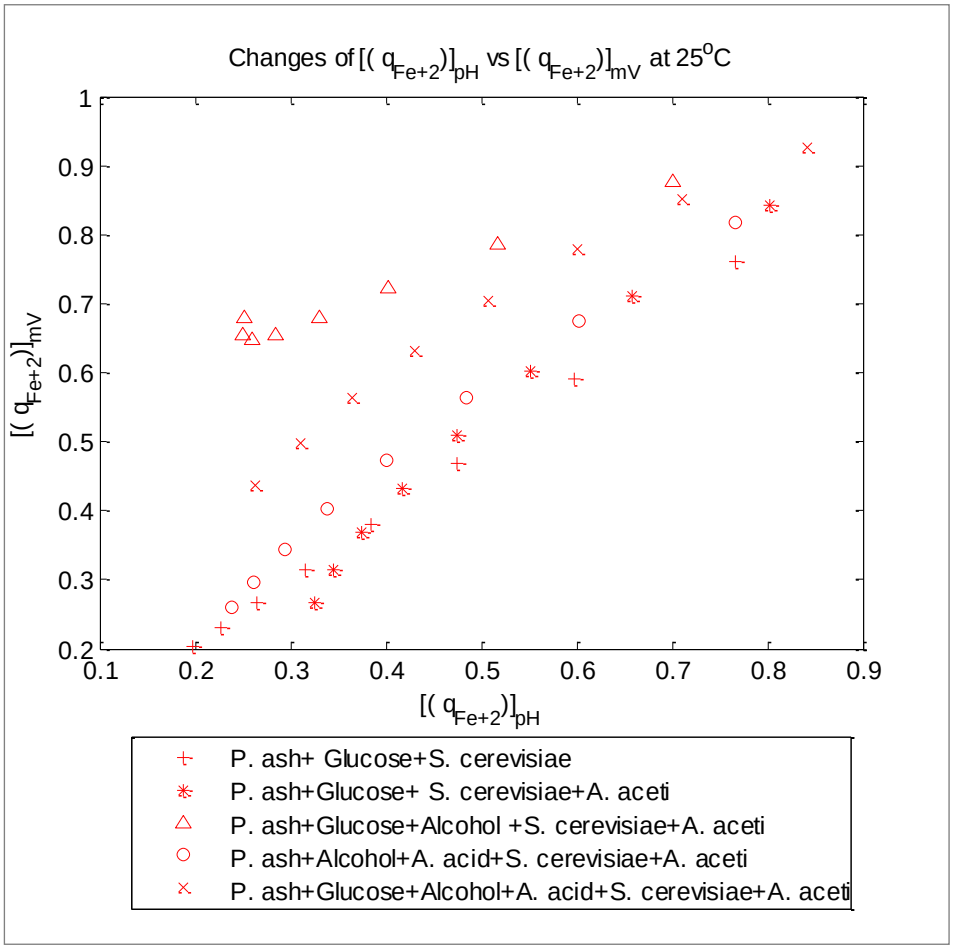


**Fig. 2 Changes of specific growth rates $[q_{Fe}^{+2}]_{pH}$ and $[q_{Fe}^{+2}]_{mV}$ at 25ºC.**

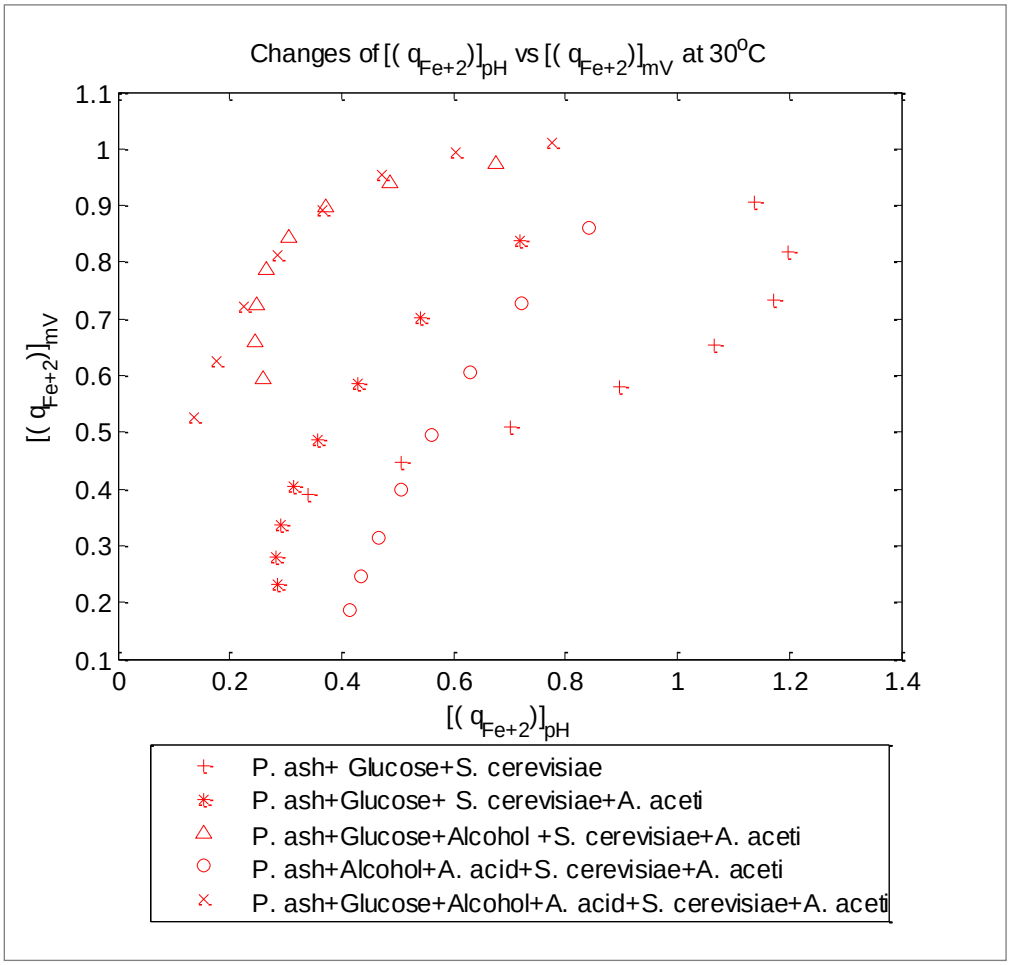


**Fig. 3 Changes of specific growth rates [$q_{Fe}^{+2}$]$_{pH}$ and [$q_{Fe}^{+2}$]$_{mV}$ at 30°C.**

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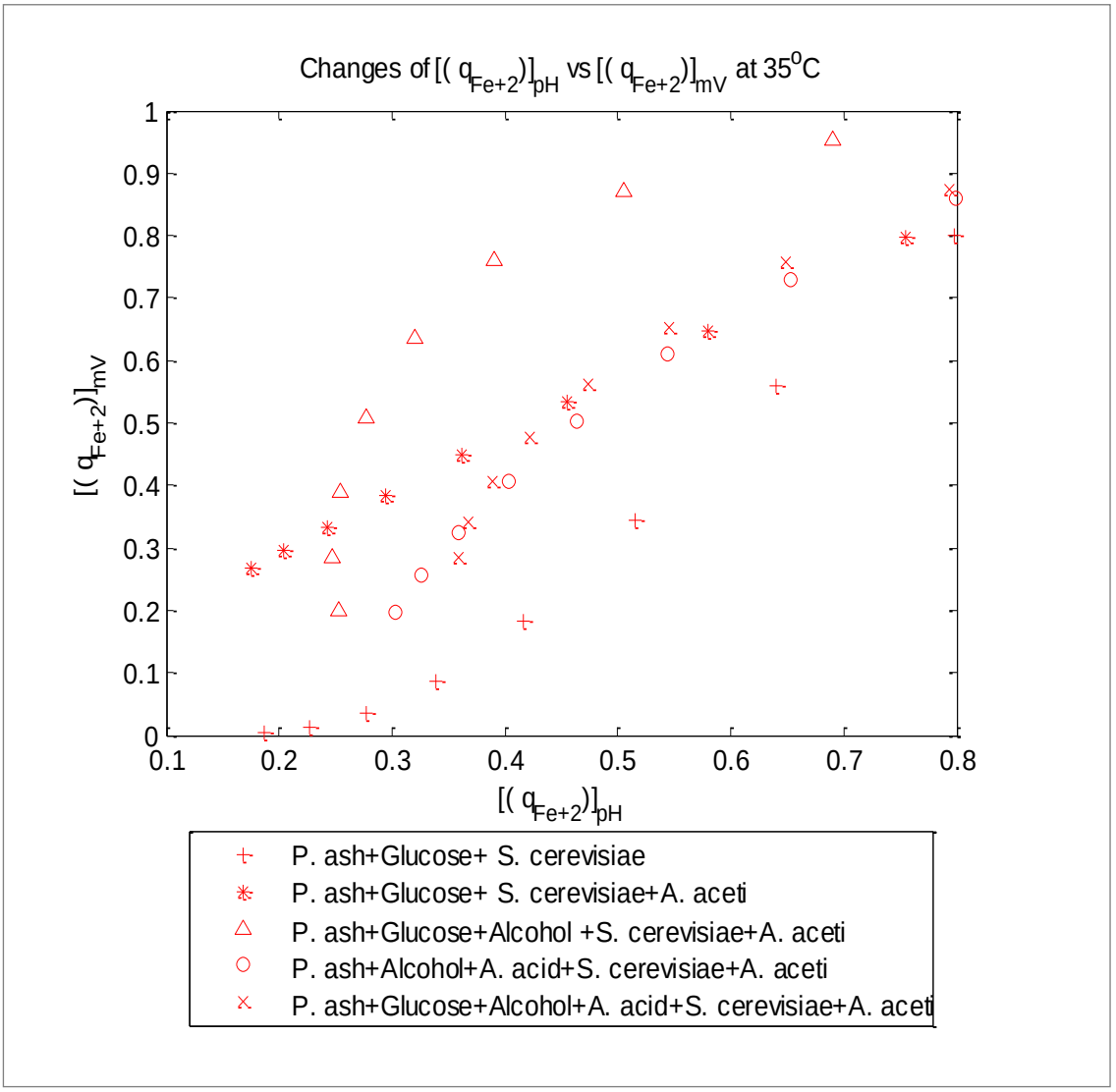


**Fig. 4 Changes of specific growth rates $[q_{Fe}^{+2}]_{pH}$ and $[q_{Fe}^{+2}]_{mV}$ at 35ºC**

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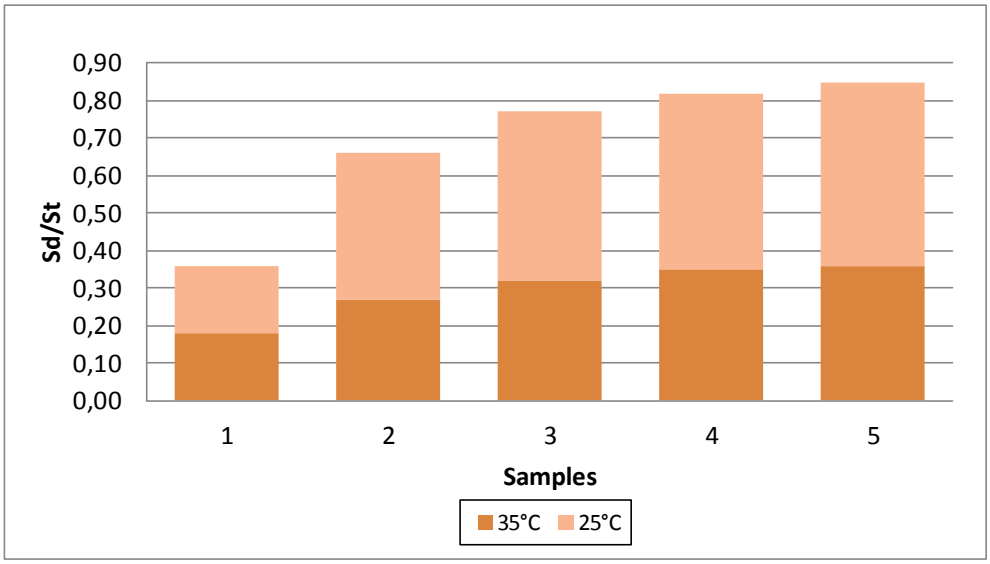


**Fig. 5 Changes in dissolved sulphur to total sulphur ratios over time (8 weeks) at 25ºC and 35ºC.** Samples: (1) Pyrite ash + Glucose + *S. cerevisiae*; (2) Pyrite ash + Glucose + *S. cerevisiae* + *A. aceti*; (3) Pyrite ash + Glucose + Alcohol + *S. cerevisiae* + *A. aceti*; (4) Pyrite ash + Alcohol+ A. acid + *S. cerevisiae* + *A. aceti*; (5) Pyrite ash + Glucose + Alcohol + A. acid + *S. cerevisiae* + *A. aceti.*

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