Short communication

Spectrophotometric flow injection monitoring of sulfide during sugar fermentation

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A B S T R A C T

A spectrophotometric flow injection procedure involving N,N-dimethyl-p-phenylenediamine (DMPD) is applied to the sulfide monitoring of a sugar fermentation by Saccharomyces cerevisiae under laboratory conditions. The gaseous chemical species evolving from the fermentative process, mainly CO2, are trapped allowing a cleaned sample aliquot to be collected and introduced into the flow injection analyzer. Measurement rate, signal repeatability, detection limit and reagent consumption per measurement were estimated as 150 h−1, 0.36% (n = 20), 0.014 mg L−1 S and 120 /H9262 g DMPD, respectively. The main characteristics of the monitoring record are discussed. The strategy is worthwhile for selecting yeast strain, increasing the industrial ethanol production and improving the quality of wines.

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1. Introduction

During sugar fermentation by Saccharomyces cerevisiae aiming at industrial ethanol or wine production, several volatile compounds are generated [1], and sulfide plays a relevant role in the context [1,2]. Excess of sulfide may alter the yeast metabolism thus affecting the fermentative process [2,3]; moreover, this excess may impart undesirable aromas associated with off-odors of beverages as e.g. wines [4].

The presence of sulfide in the yeast cells is primarily due to the enzymatic reduction of sulfate or sulfite in excess of cell requirements; sulfide is routinely formed in controlled amounts as an intermediate for the synthesis of yeast essential sulfur amino acids such as cysteine and methionine [5,6]. Nevertheless, the sulfide production is influenced by yeast strains and fermentation conditions, such as the sulfur precursor compounds, the culture growth rate and yeast assimilable nitrogen content in the fermented medium [4]. Depending on the relative rates of the sulfate/sulfite reduction, amino acid synthesis and release towards the environment, sulfide can be accumulated in the yeast cells, thus affecting the fermentation [3].

Influence of the main parameters associated with the sulfide formation and accumulation should be investigated for an efficient control of the sugar fermentation by specific yeast strains under different conditions. Moreover, efforts aiming at a better sensory quality of fermented beverages [7] are recommended.

To this end, analytical procedures yielding reliable results in a fast manner are needed for on-line sulfide monitoring in order to permit an improved industrial ethanol production, a better-quality wine production and the selection of yeasts leading to lower sulfate formation.

Due to its favorable characteristics of versatility, efficiency, ruggedness, easy implementation and low cost, the flow injection analyzer has been widely accepted for industrial process monitoring [8]. Regarding sulfide, a flow-based analytical procedures involving the oxidative coupling of sulfide with N,N-dimethyl-p-phenylenediamine (DMPD) in the presence of ferric ions yielding the colored methylene blue was already applied to water analysis [9]. The aim of the present work was then to develop a flow injection procedure for sulfide monitoring during the sugar fermentation under laboratory conditions.

2. Experimental

2.1. Reagent, standard and sample solutions

The solutions were prepared with chemicals of analytical grade quality and distilled-deionized water.

The chromogenic reagent (R1, Fig. 1) was a weekly prepared 5.0 mmol L−1 DMPD plus 1.0 mol L−1 HCl solution. The R2 reagent was a 50 mmol L−1 Fe3+ (as FeCl3·6H2O) plus 1.0 mol L−1 HCl.
solution. The C chemically inert sample carrier stream was a 0.025 mol L\(^{-1}\) NaOH solution.

The T\(_1\) trapping solution (Fig. 2) was a 0.10 mol L\(^{-1}\) Ca (as CaCl\(_2\)) plus 0.01 mol L\(^{-1}\) Na\(_2\)B\(_4\)O\(_7\) solution (pH = 11), and the T\(_2\) trapping solution was a 0.10 mol L\(^{-1}\) NaOH solution.

The sulfide stock solution (1000 mg L\(^{-1}\) S, also 0.1 mol L\(^{-1}\) NaOH) was based on Na\(_2\)S·9H\(_2\)O The working standard solutions (0.10–1.0 mg L\(^{-1}\) S, also 0.025 mol L\(^{-1}\) NaOH) were daily prepared.

The fermentation medium was established by combining 5.0 g of yeast (S. cerevisiae), 30 mL of wort and 15 mL of water (20% total reducing sugars), the same proportions as used in a typical industrial fermentation [1].

### 2.2. The flow injection system

The flow set-up comprised a model 482 Femto UV–vis spectrophotometer provided with a glass flow-cell (80–μL inner volume, 10-mm optical path), a model 111 Kipp & Zonen strip-chart recorder, a model 7618–40 Ismatec peristaltic pump, a manually operated injector [10], Perspex connectors and accessories. The manifold was built up with 0.8-mm i.d. polyethylene tubing of the non-collapsible type. Wavelength was set as 668 nm. The gas diffusion unit used in the preliminary experiments was similar to that already described [11] and included a commercial strip of Teflon thread sealing as the semi-permeable membrane.

The flow injection system in Fig. 1 was operated as follows. The sample aliquot was introduced into the chemically inert carrier stream and the established sample zone converged with the previously mixed R\(_1\) and R\(_2\) reagents at the x confluence point, allowing the methylene blue formation inside the following coiled reactor. Passage of the sample zone through the detector resulted in a transient modification in absorbance, which was recorded as a peak. Height of this recorded peak constituted itself as the measurement basis.

### 2.3. Procedure

The flow injection system was dimensioned by the univariate method: the working standard solutions were run in triplicate after each parameter variation. Table 1 indicates the parameter variation ranges and the selected values. The main analytical figures of merit were then evaluated. Repeatability was expressed as the relative standard deviation of the results estimated after twenty successive measurements of a typical sample aliquot (0.63 mg L\(^{-1}\) S and sampling rate was calculated as the inverse of the washing time [12].

### 3. Results and discussion

#### 3.1. Monitoring strategies

Two strategies for sulfide monitoring were investigated namely: (i) the direct analysis of the fermentate with a flow injection system involving gas diffusion similar to that already described [11]; and (ii) the analysis of sample aliquots with the flow injection system in Fig. 1 after a sample clean-up with the trapping system in Fig. 2.

Sample aliquots directly taken from the fermentate medium could not be analyzed by the flow injection system due to the excessive gas release, mainly the CO\(_2\) formed in large amounts during the fermentative process. This aspect resulted in the formation of gas bubbles inside the analytical path which affected the sample dispersion and impaired detection [13]. It should be recalled that CO\(_2\) was able to cross the semi-permeable membrane. It was then decided to trap the released CO\(_2\) and to analyze the cleaned sampled aliquot.

In order to investigate the feasibility of this strategy, the fermentation was accomplished inside a 500-mL Erlenmeyer as the process reactor, which was connected to two trapping flasks (Fig. 2). The first one contained 250 mL of the T\(_1\) solution and retained the evolved CO\(_2\) as the slightly soluble calcium carbonate, whereas the second one contained 70 mL of the T\(_2\) solution and retained the H\(_2\)S as the HS\(^-\). The sample aliquots (about 2 mL) were then taken from the second flask. Sulfide trapping inside the T\(_1\) solution was minimized due to combined influence of several phenomena: the large excess of formed CO\(_2\) relatively to H\(_2\)S (mol L\(^{-1}\), mmol L\(^{-1}\)), the higher CO\(_2\) solubility in water relatively to H\(_2\)S (3.8 and 1.7 g kg\(^{-1}\) in water at 20 °C [14]); the high solubility of calcium hydrogen carbonate in the T\(_1\) solution; the H\(_2\)S carrying by CO\(_2\), the higher alkalinity of T\(_2\) relatively to T\(_1\) and the kinetic aspects involved. The pH of T\(_1\) solution decreased during fermentation monitoring and this is another favorable aspect in relation to the H\(_2\)S release towards T\(_2\) solution.

As the alkalinity of the T\(_2\) solution also underwent a slight decrease during the fermentation, and the working standard solutions and sample carrier stream of the flow injection system were prepared in 0.025 mol L\(^{-1}\) NaOH and the flow injection system in Fig. 1 was dimensioned accordingly. The alkalinity variations in the sample zone were not relevant: preliminary experiments revealed no modifications in recorded peak height when the alkalinity of the

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**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variation range</th>
<th>Selected value</th>
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<tr>
<td>Reaction coil length (cm)</td>
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<td>Sample volume (μL)</td>
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<td>Fe(III) concentration (mmol L(^{-1}))</td>
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<tr>
<td>DMPD concentration (mmol L(^{-1}))</td>
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<tr>
<td>R(_1) acidity (mol L(^{-1}) HCl)</td>
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<td>1.0</td>
</tr>
<tr>
<td>R(_2) acidity (mol L(^{-1}) HCl)</td>
<td>0.20–1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Flow diagram. S = sample; C = sample carrier stream (0.025 mol L\(^{-1}\) NaOH at 3.9 mL min\(^{-1}\)); x = confluence site; R\(_1\) and R\(_2\) = DMPD and Fe(III) reagent streams (0.42 mL min\(^{-1}\)); R\(_C\) = coiled reactor; D = detector.

**Fig. 2.** The trapping system. M\(_F\), T\(_1\), and T\(_2\) = fermentation medium, first trapping solution and second trapping solution. Tube at T\(_2\) stopper needed for gas outlet and sample aliquot collection. For details, see text.
Regarding sulfide monitoring during fermentation, analysis of Fig. 3 permits one to infer that sulfide production is not evident during the first 50 min, probably because of the solubility of hydrogen sulfide [14] in the fermentation medium and its consumption by the cells for amino acid synthesis [5]. Thereafter, sulfide formation becomes evident, and increases during the next 3 h, approaching the maximum value after about 5 h. The ordinate in Fig. 1 refers to sulfide content in the sample aliquot taken from the T2 solution. The instant sulfide concentration in the fermentation medium is then not straightforward evident. It could be determined by adding a known sulfide amount to the fermentation medium which would act as an external standard. This strategy was not however implemented, as analysis of Fig. 3 permits to evaluate the speed of the fermentation as well as the relative production of sulfite at the end of several fermentative processes.

4. Conclusions

The proposed strategy demonstrated to be useful for evaluating the speed of the fermentation process and the total accumulated sulfide, and this information is worthwhile for controlling the fermentation process and selecting the yeast strain. With the designed trapping system for sample clean-up, cumbersome steps of gas diffusion are not required, thus simplifying the design of the flow injection system, and this aspect is relevant for the system applicability in routine analysis.

Investigations on the feasibility a similar flow-based strategy for micro-scale (~ few mL) monitoring of a fermentative process is presently in progress.

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References