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A NEW APPROACH TO DETERMINE OXYGEN SOLUBILITY IN COMPLEX CULTURE MEDIA

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ABSTRACT

Dissolved oxygen concentration is a key parameter in aerobic processes due to the low solubility of oxygen in culture media, which are usually aqueous solutions. To better evaluate oxygen mass transfer and its uptake rate in bioprocesses, the solubility (expressed in terms of Henry's constant) of oxygen in the liquid phase must be known, by measuring the dissolved oxygen saturation. One of the challenges faced through oxygen transfer and consumption is the determination of Henry's law constant, since it depends on many physicochemical parameters. In this work, we determined the oxygen solubility, as well as Henry's constant, in culture broths containing sugarcane molasses and concentrated malt extract as carbon sources. The results show that sugarcane molasses-based medium has a lower capacity to dissolve oxygen than malt extract-based medium at the same sugar content.

Keywords: Aerobic bioprocess. Dissolved oxygen. Henry's constant. Sugarcane molasses. Malt extract.

1 INTRODUCTION

In bioprocess industry, the majority of cultivation processes are aerobic, which means that cells must consume oxygen to complete cellular respiration.[1] The concentration of dissolved oxygen is a critical cultivation parameter, since good cell growth and many products formation requires cellular respiration.[2] Its solubility in distilled water is only about 7 mg.L^{-1} at 30 ºC, which can be quickly consumed in aerobic cultures.[3] In a typical cultivation broth, oxygen solubility is 5-25% lower than in pure water.[4] Due to its low solubility in culture media, usually aqueous solutions, oxygen must be continuously supplied from a gas to the liquid phase, by introducing air or another gas containing oxygen into the system.[1,2] This mechanism is governed by the liquid phase mass transfer resistance. Therefore, to evaluate oxygen mass transfer and its uptake rate in bioprocesses, it is essential to know about concentration of dissolved oxygen, particularly at the saturation point.[5]

One of the biggest challenges faced in measuring oxygen transfer and consumption is the determination of Henry's law constant, since it depends on various physicochemical parameters, such as temperature, pressure and solute concentration.[5] The scarcity of experimental data and the complexity of culture media has incited the development of several methods to estimate and predict oxygen solubility in these solutions.[6] Galvanic and polarographic probes can measure the partial pressure of dissolved oxygen or oxygen tension in the medium during bioreactor cultivations. Still, to convert this to dissolved oxygen concentration, the solubility in culture broth at specific conditions of temperature and pressure must be known.[3] There are methods that provide a direct relation between the oxygen partial pressure measured by the probe and the concentration of dissolved oxygen.[5] However, their application in culture media may have some limitations, since the enzyme could be inhibited by substrates.

The present work offers an alternative method for determining oxygen solubility, as well as Henry's constant, in complex culture media, such as sugarcane molasses-based and malt extract-based, starting from the value calculated for distilled water.

2 MATERIAL & METHODS

A methodology used to measure dissolved oxygen in complex culture media was adapted,[5] based on the application of Henry's Law (1), which states that oxygen solubility (S_{O2}) is directly proportional to the partial pressure of oxygen (p_{O2}) above the liquid surface. The proportionality factor is called Henry's constant (H).

$$S_{O2} = H \times p_{O2} \quad (1)$$

A dissolved oxygen (DO) saturation curve was plotted by adding specific amounts of hydrogen peroxide (0.94 M, standardized with 0.10 M $KMnO_4$)[7] into distilled water (250 mL), previously saturated with nitrogen gas (to remove O_2), in the presence of catalase (Catazyme 25 L, Novozymes) in excess. Using O_2 polarographic sensor responses (InPro 6000 Series, Mettler Toledo) coupled to a bioreactor controller (BioFlo 110, New Brunswick), the oxygen solubility in distilled water (at 30 °C, 1 atm) was determined through stoichiometry (2), given the molar mass (*MM*) of substances (3).

$$H_2O_2 \xrightarrow{catalase} ½\ O_2 + H_2O \quad (2)$$

$$S_{H2O}^{O2}\left[\frac{mg_{O2}}{L}\right] = \frac{1}{2} \times \frac{C_{H2O2}\,[M] \times V_{H2O2}\,[L]}{V_{H2O}\,[L]} \times MM_{O2}[\frac{mg}{mol}] \quad (3)$$

For sugarcane molasses and malt extract culture media (generally used for yeast culture), enriched with yeast extract (5 $g.L^{-1}$), the O_2 sensor was calibrated assuming 100% DO saturation for distilled water (at 30°C, 1 atm). DO measurements (in %) were made in media saturated with air (21% O_2), under the same conditions of temperature and pressure. The oxygen solubility in culture media was determined as a relative value (in $mg_{O2}.L^{-1}$) of oxygen solubility in distilled water (4).

$$S_{medium}^{O2}\left[\frac{mg_{O2}}{L}\right] = DO\ [\%] \times S_{H2O}^{O2}\left[\frac{mg_{O2}}{L}\right] \quad (4)$$

Figure 1. Instrumental apparatus.

3 RESULTS & DISCUSSION

Linear regression was done with the obtained saturation data (Figure 2), where 100% of DO saturation corresponded to 132.74 µL of H_2O_2 (at 0.94 M). The linear coefficient of saturation curve represents the method's experimental error, which can be related to solution titration procedures or due to the commercial enzyme (not analytical standard). Considering the stoichiometric ratio of hydrogen peroxide and oxygen (2:1) and the volume of distilled water (250 mL), the oxygen solubility was 7.97 $mg_{O2}.L^{-1}$ at 30°C and 1 atm. This atmospheric pressure, at sea level, corresponds to the location where the experiments were done (Florianopolis/Brazil). Literature data shows that the oxygen solubility in distilled water, at sea level, are 8 $mg_{O2}.L^{-1}$ (25 ºC, 1 atm)[4] and 7.68 $mg_{O2}.L^{-1}$ (30º C, 1 atm)[3]. Other authors determined experimental values for distilled water at 30 ºC, in locations above sea level, where atmospheric pressure is lower (7.49 $mg_{O2}.L^{-1}$ at 685 m altitude[8]; 7.77 $mg_{O2}.L^{-1}$ at 524 m altitude[9]).

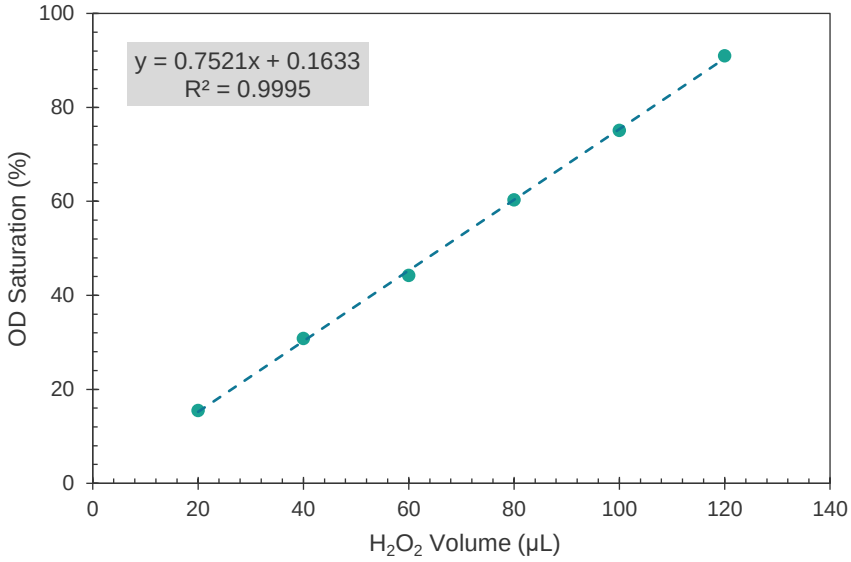

Figure 2. Polarographic sensor responses of dissolved oxygen (%) in distilled water at 30 ºC and 1 atm, by adding specific volumes of standardized hydrogen peroxide (0.94 M) in the presence of catalase in excess.

The same method[5] was applied to culture media composed of sugarcane molasses and concentrated malt extract as carbon sources. The pH of the media was adjusted to 5.0, which is within the optimal pH range of catalase (4 to 8). Despite this, the catalase action did not have the same efficiency to release oxygen as in distilled water. Given the complex nature of both substrates, intrinsic compounds, such as iron and cupper ions in molasses or proteins in malt extract, may cause interference in the enzymatic method.[9] Therefore, a new method was proposed to quantify the DO concentration at saturation in complex media, using as reference the absolute value of oxygen solubility determined for distilled water, under the same operating conditions. Henry's constant was also calculated from the solubility values (Table 1), which expresses the capacity of dissolving oxygen in each culture medium. H is an empirical parameter that depends on physical-chemical characteristics of the solution, such as

composition and temperature. Since the temperature of media and the partial pressure of oxygen were both constant, the difference between H values is only related to the composition of substrates. In addition of sugar content, the concentration of other elements such as salts, proteins and insoluble solids may have contributed to the difference in oxygen solubility between the culture media.

Table 1. Empirical parameters of oxygen solubility (at 30 ºC and 1 atm).

Culture medium	Dissolved oxygen (%)	Oxygen solubility ($mg_{O2}.L^{-1}$)	Henry's constant* ($mg_{O2}.L^{-1}.atm^{-1}$)	Sugar content ($g.L^{-1}$)
Distilled water	100.0	7.97	38.14	0.00
Malt extract (at 7 ºBrix)	98.6	7.86	37.60	58.20
Sugarcane molasses (at 5 ºBrix)	89.8	7.16	34.25	29.03
Sugarcane molasses (at 7 ºBrix)	84.6	6.74	32.26	56.03

*For O_2 partial pressure equal to 0.209 atm.[4]

4 CONCLUSION

This work proposed an adaptation of the classical method[5] to determine oxygen solubility in complex culture media, which may contain elements that interfere with the catalase action. The results show that is possible to quantify the dissolved oxygen concentration at saturation, as well as Henry's constant, in culture media composed of sugarcane molasses and concentrated malt extract as carbon sources. At the same sugar content, sugarcane molasses-based medium has a lower capacity to dissolve oxygen than malt extract-based medium.

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