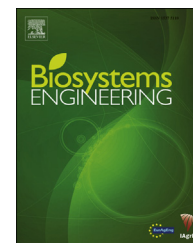


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## Research Note

# Tracking oxygen and temperature dynamics in maize silage—novel application of a Clark oxygen electrode



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Making silage involves a complicated biochemical process where oxygen (O<sub>2</sub>) is rapidly consumed within the sealed environment leading to fermentation and stable storage of the biomass. Reintroduction of the oxygen from a leak or the feed-out process results in silage degradation. Monitoring silage O<sub>2</sub> concentration and temperature (T<sub>si</sub>) can provide critical insight regarding silage quality. Thus, an in situ biosensor for simultaneous monitoring of O<sub>2</sub> and T<sub>si</sub> to track silage aerobic deterioration has long been needed but was unavailable. Although the Clark oxygen electrode (COE) is traditionally applied for O<sub>2</sub> concentration dissolved in liquids, we extended its use to the gaseous phase of silage based on Henry's law. This study tested COE's using two trials, where trial-1 explored the initial-aerobic/ensiling phase and trial-2 examined the silage feed-out phase. The experimental results of both trials demonstrated that the COE can be an important monitoring tool for assessing the O<sub>2</sub> dynamics throughout the silage lifecycle.

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## 1. Introduction

Silage is one of main feedstuffs for livestock worldwide. Silage making process is based on the anaerobic fermentation of lactic acid bacteria with an inherent risk of aerobic deterioration during silage storage and feed-out process (Pitt & Muck, 1993; Wilkinson & Davies, 2012). When a silo is opened for

feeding to livestock, the face of the silo is exposed to air, resulting in aerobic deterioration as O<sub>2</sub> diffuses into the silage. The process of aerobic deterioration is essentially microbial (Williams, Hoxey, & Lowe, 1997). The entrained O<sub>2</sub> is consumed by microbial respiration in the silage. Then heat released by the microbial activity causes T<sub>si</sub> rise, which plays an accelerating role in the microbial growth rate until an

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optimum  $T_{si}$  is reached. Above the optimum  $T_{si}$ , the growth rate will decline, and the thermally induced death rate will increase (Membré et al., 2005; Muck & Pitt, 1994; Van Derlinden & Van Impe, 2012; Williams et al., 1997). One of deleterious results is that glucose ( $C_6H_{12}O_6$ ) in the silage is decomposed into  $CO_2$  and  $H_2O$  leading to nutrient loss (i.e.,  $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ ) (Williams et al., 1997).

Aerobic deterioration of silage is normally monitored by measuring changes in temperature or pH, but these approaches can become problematic when comparing treatments that differ in water content or buffering capacity, respectively. Therefore,  $O_2$  or  $CO_2$  production may be the most sensitive indices monitored. As  $O_2$  and  $T_{si}$  are regarded as critical paired factors governing the microbial activity of the silage, simultaneous in situ monitoring of these is of high importance for silage research. However, successful on-line measurement of  $O_2$  in silage is rare and challenging. The similar situation in food engineering along with the technical limitations of available instruments has been reviewed recently by Pénicaud, Peyron, Gontard, and Guillard (2012). To our knowledge, Muck and Pitt (1994), Williams et al. (1997) and McEniry, Forristal, and O'Kiely (2011) have measured  $O_2$  in silage to data. Muck and Pitt (1994) and McEniry et al. (2011) extracted gas samples from silage and then analysed them using a gas chromatograph. The resulting  $O_2$  measurements of both studies were restricted to a daily measurement. Williams et al. (1997) installed a pair of tubes that linked each sampling site in the silage to an  $O_2$  meter placed outside the silage but the technical information of the  $O_2$  meter was lacking. According to their description, before measuring  $O_2$ , a pump had to run for 1 h to re-circulate gas around the sampling loop to reach equilibrium. Similarly, their measurements were also restricted to being made on a daily basis. However, the modelled results from Pitt and Muck (1993) showed that  $O_2$  concentration in the silage could change substantially throughout a daily interval.

The Clark oxygen electrode (COE) is well-known as an effective sensor for measuring dissolved oxygen concentration in various solutions, including blood (Clark, Wold, Granger, & Taylor, 1953; Koley et al., 2009; Pénicaud et al., 2012; Wu, Yasukawa, Shiku, & Matsue, 2005). Actually, the COE also facilitates measuring oxygen ( $O_2$ ) concentration in tissue/cell (Diepart et al., 2010; von Heimburg, Hemmrich, Zachariah, Staiger, & Pallua, 2005) or gaseous  $O_2$  in porous materials such as soil (Sexstone, Revsbech, Parkin, & Tiedje, 1985). The latter is equally important but seems to have received less attention in the applications of the COE. This study proposed to simultaneously measure  $O_2$  and  $T_{si}$  dynamics within silage by testing a commercial COE with three considerations: (i) Silage is also known as a porous material like soil. Thus, according to the study measuring  $O_2$  in soil using the COE technique (Sexstone et al., 1985),  $O_2$  in silage should also be measurable; (ii) The COE has adequate response at the minute-scale to track dynamics of  $O_2$ , which may be more important than the absolute measurement for some silage research; (iii) Because the concentration of dissolved  $O_2$  is a function of the temperature and air pressure, all types of commercial COE include an internal temperature sensor so that one can measure  $O_2$  and  $T_{si}$  simultaneously without any additional cost.

The silage making process is generally divided into four phases: (i) the ensiling phase, (ii) the fermentation phase, (iii) the stable-storage phase, and (iv) the feed-out phase/unloading phase/aerobic spoilage phase (Pahlow, Muck, Driehuis, Oude Elferink, & Spoelstra, 2003; Wilkinson & Davies, 2012). The ensiling phase relates to an  $O_2$  depletion process, whereas the feed-out phase to an  $O_2$  penetrating process in silage. The primary objective of this study was to test one type of COE for observing  $O_2$  and  $T_{si}$  dynamics associated with ensiling- (trial-1) and feed-out-phases (trial-2) of silage making process. Because density is a critical factor governing  $O_2$  dynamics in silage, both trials were carried out with two levels of silage density.

## 2. Materials and methods

### 2.1. Measuring principle and the tested sensor

As shown in Fig. 1a, a typical COE consists of an anode (Ag/AgCl electrode) and a cathode (platinum electrode), which are immersed in saturated KCl electrolyte and covered with a membrane permeable only by gases (Roy, Emilia Abraham, Abhijith, Sujith Kumar, & Thakur, 2005). Regardless of whether the  $O_2$  molecules are from air or dissolved in solution, when the  $O_2$  diffuses through the gas permeable membrane to the cathode, it is reduced to hydroxide ions resulting in an amperometric signal that has a linear relationship with the  $O_2$  concentration (Diepart et al., 2010; Ramamoorthy, Dutta, & Akbar, 2003). The concentration of dissolved oxygen ( $C_{DO}$ ,  $mg L^{-1}$ ) in solution is directly proportional to the partial pressure of  $O_2$  in the air ( $p$ , kPa) based on the Henry's law of physical chemistry

$$p = \frac{k_H C_{DO}}{10} \quad (1)$$

where  $k_H$  is the Henry constant with the dimension of pressure divided by concentration and equal to  $2400 L Pa mg^{-1}$  at 298 K for  $O_2$ . On the other hand, the Henry constant is temperature dependent, and thus has to be corrected using the van't Hoff equation

$$k_H(T) = k_H \exp \left[ -C \left( \frac{1}{T} - \frac{1}{298} \right) \right] \quad (2)$$

where  $C$  is 1700 K for  $O_2$ . Therefore, the  $O_2$  in porous materials such as silage can be calculated as the partial pressure of  $O_2$  when the readings of COE and the temperature sensor are brought together into Eqs. (1) and (2).

For this study, commercial COE's (DO-10, range: 0–20  $mg L^{-1}$ , output of ampere-to-voltage converter: 0–2.5 V, accuracy:  $\pm 3\%$  FSC, Beijing UNISM Technologies Inc., China) were tested, each included an intergraded thermistor (MF5, range: 0–50 °C, resolution 0.1 °C). The linear characteristic of the COE output has been confirmed by previous studies (Klimant, Ruckruh, Liebsch, Stangelmayer, & Wolfbeis, 1997; Lee, Lima, Seo, Bishop, & Papautsky, 2007; Sosna, Denuault, Pascal, Prien, & Mowlem, 2007; Trettnak, Gruber, Reininger, Leary, & Klimant, 1996; Wittkamp, Chemnitius, Cammann, Rospert, & Mokw, 1997), and allows calibration using two points, one in  $O_2$ -free 5% sulfite solution and one in 21% air

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