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Development of a biosensor telemetry system for monitoring fermentation in craft breweries

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ABSTRACT

The development and applications of biosensors in the food industry has had a rapid grown due to their sensitivity, specificity and simplicity of use with respect to classical analytical methods. In this study, glucose and ethanol amperometric biosensors integrated with a wireless telemetry system were developed and used for the monitoring of top and bottom fermentations in beer wort samples. The collected data were in good agreement with those obtained by reference methods. The simplicity of construction, the low cost and the short time of analysis, combined with easy interpretation of the results, suggest that these devices could be a valuable alternative to conventional methods for monitoring fermentation processes in the food industry.

1. Introduction

Beer is the most consumed alcoholic beverage in the world and global beer production has had a rapid growth and achieved great success also in countries that are not traditional beer producers.

The final beer composition is the result of various biochemical phenomena involving the interaction of raw materials, as malt, water, hops and yeast (Cortacero-Ramírez, Hernáinz-Bermúdez De Castro, Segura-Carretero, Cruces-Blanco, & Fernández-Gutiérrez, 2003; Sanna & Pretti, 2015).

Commonly two different systems of fermentation are used in brewing, known as "top fermentation" (TF) and "bottom fermentation" (BF), involving different kind of yeasts and fermentation temperatures (between 8 and 12 °C in BF and between 15 and 25 °C in TF). These differences in temperature lead to differences in kinetics and sugar uptake by yeast cells (Boulton & Quain, 2006).

In order to get the desired quality of beer, it is important to control the critical parameters during the brewing process (Monošík, Magdolen, Stred'anský, & Šturdík, 2013). The main parameter measured by brewers is the sugar content in wort expressed in Plato degrees (°P). Plato degree, developed by Karl Balling in 1843 and later improved by Fritz Plato, refers to the specific gravity of wort and beer and is an indicator of the extract content (equivalent to the grams of sucrose in 100*g* of wort). The hydrometer is the most widely used device and is also a cheap tool to determine the extract content in wort and beer in °P. This device has a low resolution and requires sample pre-treatment (filtration) (Giovenzana, Beghi, & Guidetti, 2014). Hydrometers are calibrated to measure the sugar content of a solution of water from its density. Finished beer, however, contains ethanol which lowers the liquid density interfering with the hydrometer measurement. Therefore, a hydrometer measurement taken on finished beer will show lower values (less extract content) and for this reason this measure is also called Apparent Extract (AE). The measure of sugar accounting for the actual alcohol content is called Real Extract (RE), and is calculated from the Original Extract (OE, the amount of sugar in wort before fermentation) and AE according to Balling's formula (Balling, 1865; Cutaia, 2009).

Another essential parameter for brewers is ethanol concentration expressed as %v/v ($EtOH_{v/v}$), which is often calculated from

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OE and AE by the Dave Miller equation (Miller, 1988), instead of being measured directly.

Spectrophotometric and chromatographic analytical techniques are also widely used to monitor the changes that occur during manufacturing process. These techniques are expensive, time-consuming, laborious and a pre-treatment of the sample is often required (Pérez & Fàbregas, 2012).

Methods based on biosensors, coupled with telemetric systems, could be a good alternative to these classical methods. They offer fast analysis, easy and direct analytes detection at low cost.

Biosensors are made up of a transducer and a sensitive biological element (tissues, microorganisms, organelles, enzymes, etc.). Most of the commercially successful devices are based on enzymatic amperometric biosensors (Kuswandi, Irmawati, Hidayat, Jayus, & Ahmad, 2014).

The biosensor selectivity is guaranteed by the presence of the enzyme, needed for the recognition of the target analyte (Lowinsohn & Bertotti, 2008), and the deposition of a permselective film for rendering negligible the current of the oxidizable interfering species (Calia et al., 2015).

The goal of this study was to monitor glucose and ethanol concentrations in fermenting worts by means of a low-cost wireless amperometric system (Fig. 1). This allows the start of the fermentation to be evaluated by glucose consumption, trend of ethanol production and specific gravity (in °P) decrease in beer fermentation.

Glucose and ethanol biosensors can be obtained by using either dehydrogenase (glucose and alcohol dehydrogenase respectively) (Manso et al., 2008) or oxidase enzymes (glucose and alcohol oxidase) (Azevedo, Prazeres, Cabral, & Fonseca, 2005; Rocchitta et al., 2012, 2013; Wang, 2008) on suitable transducers.

Biosensors based on dehydrogenases are more sophisticated and expensive since they require the presence of NAD^+, a cofactor which must be close to the surface where the enzyme is deposited without becoming irreversibly entrapped (Karyakin, Karyakina, Schuhmann, Schmidt, & Varfolomeyev, 1994; Leca & Marty, 1997; Lobo, Miranda, Tunon, & Fisica, 1996a). This feature can lead to a decrease in sensitivity (Kulys, Bilitewski, & Schmid, 1991). When cofactor incorporation is not possible, it has to be added in large amounts to the analysis cell, thus increasing costs (Pérez & Fàbregas, 2012).

In this work, biosensors were based on glucose oxidase and alcohol-oxidase where the cofactor ($FAD/FADH_2$) is actually bound to the enzyme (Boujtita, Hart, & Pittson, 2000).

Glucose oxidase (GOx) catalyzes the oxidation of β-D-glucose to form D-glucono-1,5-lactone and hydrogen peroxide, according to the reactions 1 and 2:

$$\beta\text{-d-glucose} + GOx/FAD \rightarrow \text{d-glucono-}\delta\text{-lactone} + GOx/FADH_2 \quad (1)$$

$$Gox/FADH_2 + O_2 \rightarrow Gox/FAD + H_2O_2 \quad (2)$$

Alcohol oxidase (AOx) is able to catalyze the oxidation of primary, aliphatic short-chain alcohols (such as ethanol or methanol) to the respective aldehydes as shown in the reactions (3) and (4).

$$RCH_2OH + AOx/FAD \rightarrow RCHO + AOx/FADH_2 \quad (3)$$

$$AOx/FADH_2 + O_2 \rightarrow AOx/FAD + H_2O_2 \quad (4)$$

The hydrogen peroxide produced by reactions 2 and 4 can be amperometrically detected on a Pt surface by applying a fixed anodic potential of +700 mV versus Ag/AgCl (reaction 5):

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (5)$$

In this study the results obtained with the biosensors were compared with those obtained with an enzymatic spectrophotometric kit for glucose detection and with a NIR modular measurement system for ethanol detection.

2. Materials and methods

2.1. Reagents and materials

D-(+)-Glucose, absolute ethanol, hydrogen peroxide (H_2O_2), *o*-phenylenediamine (oPD), polyethylenimine (PEI), glycerol (GLYC), polyurethane (PU), tetrahydrofuran (THF), glucose oxidase from *Aspergillus niger* (GOx, EC 1.1.3.4), alcohol oxidase (AOx, 1.1.3.13)

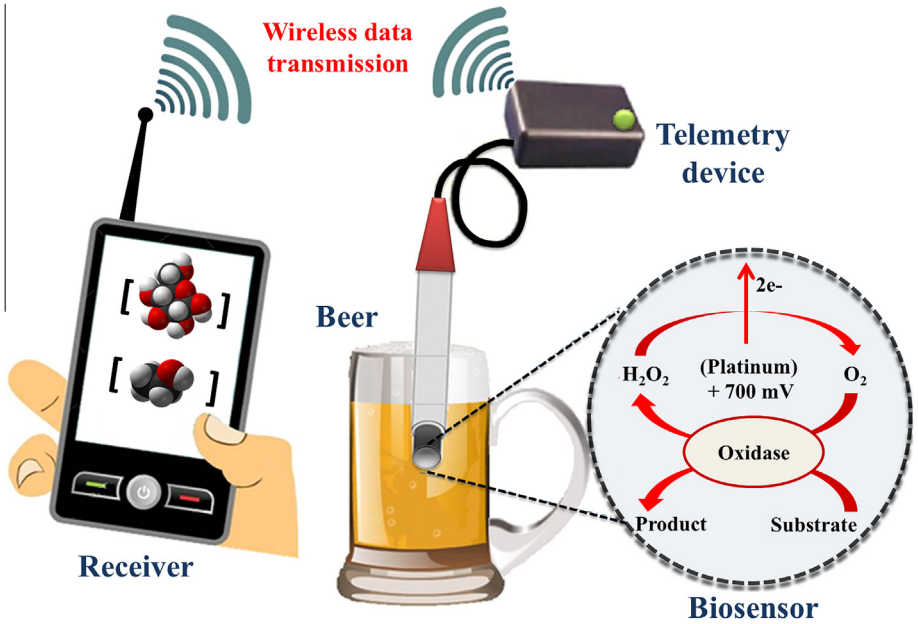

Fig. 1. Schematic representation of biosensor-coupled telemetry device used in this study.

solution from *Pichia pastoris* were purchased from Sigma–Aldrich (Milano, Italy).

Teflon®-coated platinum (90% Pt, 10% Ir; Ø = 125 μm) and silver wires (Ø = 250 μm) were purchased from Advent Research Materials (Eynsham, UK). Biosensor measurements were performed using the four channel equipment (eDAQ QuadStat, e-Corder 410, eDAQ, Australia).

Wort was provided by the Sardinian craft brewery "*P*3 Brewing Company" (Sassari, Italy).

Yeast strains Safale US-05 and Saflager W34-70 were purchased from Fermentis-Lesaffre.

Spectrophotometric kit for D-Glucose detection (K-GLUC, GOPOD FORMAT) was purchased from Megazyme International (Bray, Ireland).

2.1.1. Solutions

Glucose (1.0 M), ethanol (1.0 M), hydrogen peroxide (100 mM), polyethylenimine (1%) and glycerol (1%) solutions were prepared with MilliQ water (Millipore, Inc.; Ω = 18 MΩ/cm). Phosphate-buffered saline solution (PBS) was prepared by dissolving NaCl (8.9 g), NaOH (1.76 g), and NaH_2PO_4 (6.89 g) in 1 L of MilliQ water and then adjusted to pH 7.4.*ortho*-Phenylenediamine monomer solution (oPD, 300 mM) was prepared in PBS. Polyurethane solution (PU, 1.0% w/v) was obtained dissolving PU beads in THF.

GOx solution was prepared by dissolving 180 units of enzyme in 10 μL of PBS. Alcohol oxidase solution from *Pichia pastoris* was 1130 units/mL in PBS. All enzyme solutions were stored at −20 °C.

2.2. Methods

2.2.1. Wort samples and microfermentations

Experiments were set up in order to monitor top and bottom fermentation, using two different yeast strains and temperatures: Safale US-05 at 22 °C for top and Saflager W34-70 at 10 °C for bottom fermentation.

Yeast precultures were grown in wort for 24 h at 22 °C and then centrifuged and washed twice in sterile distilled water. The density of yeast was determined spectrophotometrically by measuring its optical density at 600 nm (OD600) and a calibration curve by Thoma chamber was made for each yeast strain. 10×10^6 cell/ml and 20×10^6 cell/ml were inoculated for top and bottom fermentation respectively.

Top and bottom fermentations were carried out in 800 ml of wort. Fermentation parameters were checked every 2 h, for the first 8 h in TF and for the first 12 h in BF, then every 24 h until the end of fermentation.

Each experiment was conducted in duplicate.

2.2.2. Extract and Ethanol quantification

The OE, AE and $EtOH_{v/v}$ were measured with a density meter DMA 4500 and Alcolyzer plus (model PBA-B Generation M, Anton Paar, Graz, Austria).

In order to calculate the AE from ethanol biosensor the equations 3b of Cutaia (2009) was adapted to obtain the following equation:

$$AE = OE - (EtOH_{w/w}/(3.72 \times 10^{-1} + 3.57 \times 10^{-3\times} OE)) \quad (6)$$

2.2.3. Biosensors fabrication

Biosensors were obtained by exposing a cylindrical platinum/iridium electrode (1 mm in length and 125 μm in diameter) by cutting away the Teflon insulation as shown in Fig. 2 (Calia et al., 2009; Rocchitta et al., 2013; Serra et al., 2007).

All designs had in common the same strategy for blocking oxidizable interferents in beer (polyphenols, AA, citric acid): the electro-deposition of a poly-*ortho*-phenylenediamine (pOPD) nanometer-thick membrane. The pOPD membrane was obtained by electropolymerization of a oPD monomer solutions. The polymerization was carried out amperometrically at +700 mV versus the Ag/AgCl reference electrode for 30 min.

Glucose biosensor (Fig. 2A) was prepared depositing the enzyme layers on the p-OPD covered Pt/Ir by mean of five quick dips into GOx solution. Ethanol biosensor was made in the same manner by using the AOx enzyme, and by dipping biosensors in a PEI and GLYC solution (Fig. 2B). The use of PEI as biosensor enhancer (it reduces the apparent K_M and increases the I_{MAX}) and GLYC as long-time stabilizer are enzyme-dependent and have been characterized in a previous study for ethanol detection (Rocchitta et al., 2012). PEI and GLYC are not necessary for safeguarding the analytical performance of glucose biosensors (Rocchitta et al., 2013).

Finally, all the biosensors were quickly dipped in a PU 1% solution (Fig. 2A and B) in order to immobilize the enzyme layers on the transducer surface (Rocchitta et al., 2016). The completed biosensors were kept at room temperature for 30 min and finally stored in fridge and used 24 h after.

Sentinel sensors were also prepared following the same procedure of biosensors preparations but omitting the enzyme deposition.

The usefulness of the materials used for construction of the glucose and ethanol biosensors has been discussed in detail in a recent review (Rocchitta et al., 2016).

2.2.4. Amperometric measurements

The electrochemical studies were performed at room temperature in an open electrochemical cell consisting of the working electrodes, the biosensor and the sentinel sensor (made as the biosensor but enzyme free), the reference electrode (Ag/AgCl in NaCl, 3.0 M) and the auxiliary electrode (a high surface Pt wire).

The mentioned electrodes are used in a typical three electrode configuration, which consist of a working electrode (WE), a reference electrode (REF) and a counter electrode (AUX) (Rocchitta et al., 2007; Serra et al., 2007). For each biosensor a corresponding sentinel sensor is present. Although both biosensor and sentinel sensor are covered with the pOPD permselective film to exclude interferences from electroactive molecules, it could be possible that some unexpected oxidizable compounds pass through the film and can be detected at the platinum surface. The presence of the sentinel sensor is then necessary because it records the currents derived from all the residual electroactive compounds present in the matrix (crossing the polymer), except the hydrogen peroxide generated by the enzymatic reaction occurring at the biosensor surface. Thus, the subtraction of the signal recorded by the sentinel from that obtained from the biosensor, allows obtaining an interference-free glucose or ethanol signal.

All biosensors were calibrated 24 h after preparation (day 1), connecting them to the potentiostat and then immersing them in a phosphate buffer solution at pH 7.4. After a stable baseline was reached, a 10-point calibration was made by adding known amounts of glucose or ethanol stock solution, ranging from 0 to 140 mM and 0–100 mM respectively.

The detection of the H_2O_2 produced by the enzymatic reactions (reactions 2 and 4) was performed by means of constant potential amperometry by fixing the oxidation potential for the H_2O_2 to +700 mV versus an Ag/AgCl reference electrode (reaction 5). Glucose and ethanol biosensor were characterized in terms of Michaelis–Menten kinetics (I_{MAX} and apparent K_M), sensitivity (linear region slope (LRS), limit of detection (LOD), and limit of quantification (LOQ)) (Table 1). In the biosensor used for wort or beer analysis, low-range calibrations (0–1 mM for glucose and 0–10 mM for ethanol) were performed in PBS (10 mL) by connecting

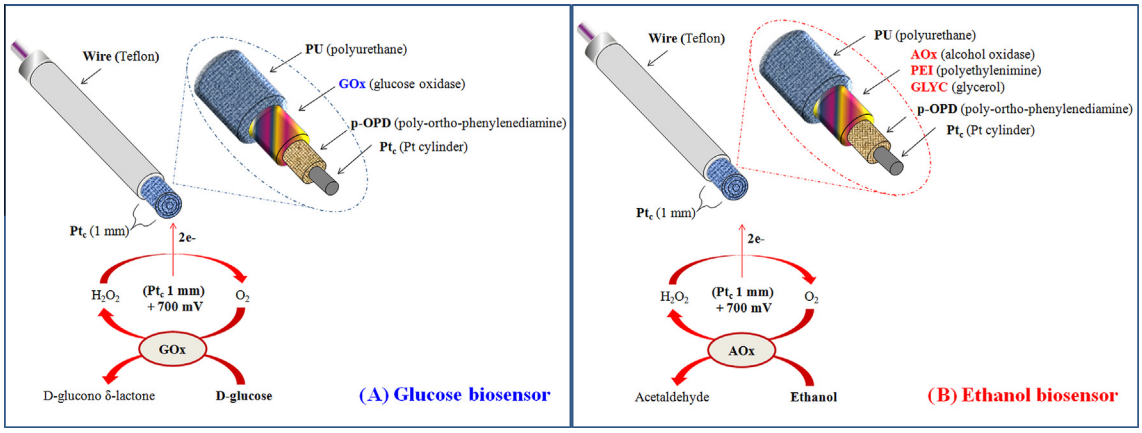

Fig. 2. Details of glucose (A) and ethanol (B) biosensors developed for this study and reaction schemes.

Table 1
In vitro characterization of biosensors at Day 1 (n = 6 for each group) in terms of Michaelis-Menten kinetic parameters (I_{MAX} and apparent K_M), Linear Region Slope (LRS) and LOD and LOQ calculated as 3.3σ/LRS and 10 σ/LRS, respectively.

Biosensor design (Day 1)	I_{MAX} (nA)	Apparent K_M (mM)	LRS (nA/mM)	R^2	LOD (μM)	LOQ (μM)
Glucose: Ptc/pOPD/$[(GOX)]_5$/$(PU\ 1\%)_1$	203.9 ± 2.9	2.1 ± 0.1	63.2 ± 0.0	0.998	2.6 ± 0.1	8.1 ± 4.2
Ethanol: Ptc/pOPD/$[(PEI\ 1\%) + (Glyc\ 1\%)/(AOX)]_5$/$(PU\ 1\%)_1$	300.0 ± 8.0	8.9 ± 1.0	25.4 ± 0.9	0.996	48.8 ± 12.1	148.2 ± 0.5

the biosensors (a biosensor-sentinel couple) to the telemetry device described in the following paragraph.

Analyte detection in real matrices was performed by adding 10 μL of untreated wort or beer to the calibration solutions (glucose, ethanol).

2.2.5. Telemetric device

The two-channel telemetry system used in this study was based on previous designs (Barberis et al., 2010, 2014; Rocchitta et al., 2007, 2013; Serra et al., 2007). Biosensor signals were acquired every second, averaged, and transmitted to a notebook or a smartphone in real time. A detailed description of the telemetric device is provided in the Supplementary information.

2.3. Statistical analysis

After calibrations, biosensor currents were plotted versus glucose or ethanol concentrations and linear regressions (slope) were calculated at low concentrations (0–1 mM for glucose and 0–10 mM for ethanol). A nonlinear fitting (Michaelis–Menten equation) was performed on the entire concentration range (0–140 mM for glucose and 0–100 mM for ethanol) to evaluate I_{MAX} and apparent K_M. Oxidation currents were expressed in nanoamperes (nA) and given as baseline-subtracted values ± standard error of the mean (ΔnA ± SEM). The LOD and LOQ were determined using a statistical method based on the standard deviation (σ) of the response and the LRS of the calibration curve and then calculated as 3.3 σ/LRS and 10 σ/LRS, respectively. In matrix analysis the glucose and ethanol currents (subtracted from the current registered by the sentinel electrode) were plotted versus the corresponding concentrations obtained in calibration, and the linear regression was calculated. Pearson's correlation coefficient was calculated for glucose/ethanol in the first 8–10 h and real extract/ethanol in the following hours. Statistical analysis was performed by GraphPad Prism 5.02 for Windows software.

3. Results and discussions

Biosensors described above were tested *in vitro* and calibration results at day one are summarized in Table 1. They showed a high sensitivity and a good linearity for the entire duration of the experiments. For enzymes immobilized on amperometric biosensors, I_{MAX} is the maximum biosensor signal recorded under saturation condition (O'Neill, Lowry, Rocchitta, McMahon, & Serra, 2008). Therefore, different values of I_{MAX} determined under the same conditions can reflect differences in the activity of enzyme on the biosensor surface. On the other hand, K_M is the apparent Michaelis constant and is determined by factors such as the binding of substrate to the enzyme and identifies the concentration of substrate that gives half the I_{MAX} response (O'Neill et al., 2008; Rocchitta et al., 2012). The apparent Michaelis constant is also important for determining the LRS which can be approximated by I_{MAX}/K_M (O'Neill et al., 2008). According with the international guidelines (ICH Q2-R1, 2005), LOD and LOQ were determined using a statistical method based on the standard deviation (σ) of the response and the LRS of the calibration curve (Rocchitta et al., 2012). During the development and the characterization of the glucose and ethanol biosensors, the efforts have been oriented in two directions: increasing the number of active enzyme molecules on the biosensor surface (increasing I_{MAX}) and increasing the affinity of the enzyme for its specific substrate (increasing LRS and decreasing K_M, LOD and LOQ).

In recent years the use of electrochemical biosensors has been extensively applied for monitoring food chemistry (Barberis, Fadda, Schirra, Bazzu, & Serra, 2012; Barberis et al., 2010, 2014, 2015), glucose in juices (Gokoglan et al., 2015) and ethanol in alcoholic beverages (Kesik et al., 2014). Gokoglan and coworkers developed a novel biosensor architecture based on a conducting polymer used for glucose detection characterized by a very low apparent K_M (25 μM). In the same study (Gokoglan et al., 2015) a comparison was performed among the apparent K_M of several biosensor designs present in recent literature (the apparent K_M was comprised between 0.025 and 14.90 mM). With a similar approach Kesik and collaborators developed an ethanol biosensor with a low apparent K_M (2.67 mM) and compared their results with other biosensor designs (apparent K_M comprised between 2.4 and 16.95 mM). Although the values of apparent K_M of the biosensors used in this study (2.1 ± 0.14 mM for glucose and 8.9 ± 1.0 mM for ethanol) are not the best in the literature, they result inside

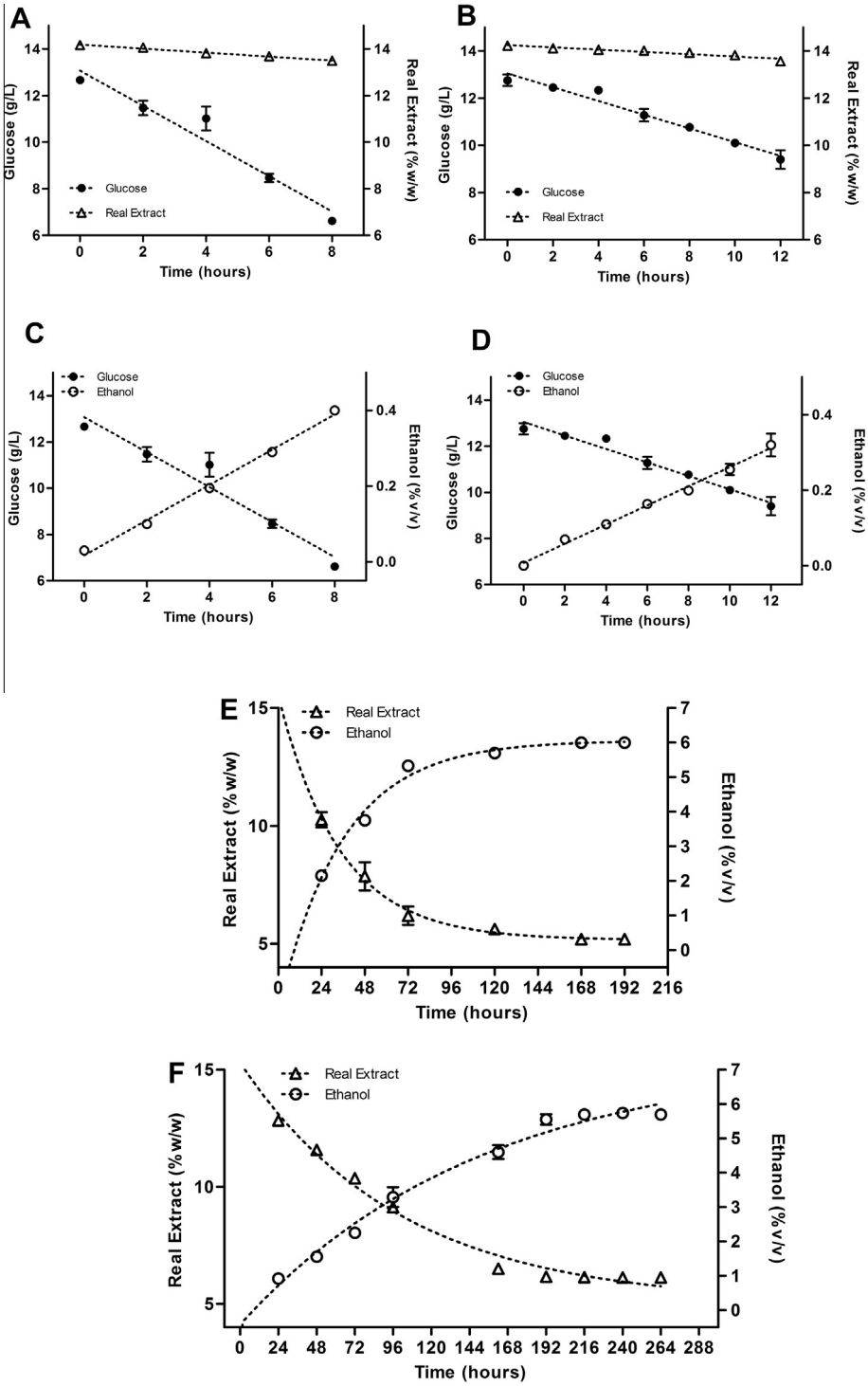

Fig. 3. Glucose, total sugars and ethanol changes in the first 8–12 h (A, B, C, D) and in the following hours of fermentation (E, F). Panels A, C and E represent the analytical trends during top fermentation while panels B, D and F represent the same trends during bottom fermentation. Glucose and ethanol values were measured by biosensors. Real extract was measured by NIR.

the above-indicated ranges confirming that changes in biosensor design influence their analytical performance.

The high sensitivity and stability of biosensors allowed the use of small quantities of untreated sample (10 μl wort or beer in 10 ml of PBS), thus avoiding fouling of the catalytic surfaces.

The electrochemical assay of samples was performed with the standard addition method to avoid any matrix effect. Sample dilution was required in order to fit with the biosensor linear range.

Glucose and ethanol levels were measured in wort starting from yeast inoculation. Brewing yeasts can utilize a wide variety of carbohydrates: glucose, fructose, sucrose, maltose and maltotriose. The patterns of uptake of sugars during fermentation are complex. In beer wort, glucose is metabolized first, and only when approximately 50–60% of it has been consumed by yeast cells the uptake of other sugars (mainly maltose) starts (Boulton & Quain, 2006; D'Amore, Russell, & Stewart, 1989; Stewart & Stewart, 2009).

In Fig. 3 the trend in the first 8–12 h (A, B, C, D) and in the following time until the end of fermentation (E, F) are reported. In the first 8–12 h, as shown in Fig. 3A and B, a strong difference between total sugar (expressed as real extract) and glucose slopes was observed. In top fermentation, the slope values were −0.086 ± 0.004 for the real extract and −0.755 ± 0.038 for glucose, in bottom fermentation slope values were −0.048 ± 0.004 and −0.294 ± 0.023 respectively.

In both fermentations the decrease in total sugars was weak compared to the drop in glucose. In top fermentation 48.7% glucose and 4.7% total sugar reduction was observed, while in bottom fermentation a reduction of 26.3% of glucose and 4.2% of total sugar was measured. For this reason, the linear consumption of glucose (−0.755 ± 0.038 g/L h^{-1} for TF and −0.294 ± 0.023 g/L h^{-1} for BF) can be considered a good marker for the monitoring of the first hours of fermentation.

Furthermore, especially in craft brewery, brewing process (mash and lautering), strongly influences the wort glucose concentration (Boulton & Quain, 2006). The knowledge of this parameter and its comparison to the expected value for each beer recipe, may represent a useful control point for recipe reproducibility.

At 4 h, in both fermentations the glucose decrease has slowed (Fig. 3A and B). As already observed by other authors (Berry & Slaughter, 2003; D'Amore et al., 1989), this phenomenon was probably due to the conversion of the sucrose contained in wort into glucose and fructose by the activity of enzyme invertase secreted by yeasts.

In the first 8 h for top fermentation (Fig. 3C) and 12 h for bottom fermentation (Fig 3D), the ethanol production increased linearly (0.046 ± 0.001%v/v h^{-1} for TF and 0.025 ± 0.001%v/v h^{-1} for BF) and was inversely correlated with glucose consumption (Pearson's correlation: −0.98 for TF and −0.96 for BT; $p < 0.0001$).

After 24 h, glucose levels further decreased down to 0.07 g/L in TF and to 0.16 g/L in BF and then the glucose concentrations reached a plateau (Fig. 4A and B). During the same hours, the ethanol production was a function of the total sugar consumption (Pearson's correlation: −0.99, $p < 0.0001$ for both fermentation) (Fig. 3E and F).

It is very interesting to observe that while in the first 8–12 h (for TF and BF respectively) glucose consumption and ethanol production followed a linear trend, after this starting period the glucose was quickly consumed in TF, decreased in an exponential manner in BF, while the production of ethanol followed a hyperbolic equation in both fermentations but with different time-courses (Fig. 3E and F). These peculiar sequences may be considered as a "biochemical fingerprint" characteristic of the two brewing processes.

In Fig. 4 the whole biosensors monitoring of top (A) and bottom (B) fermentations is reported. AE values (°P) were obtained by the

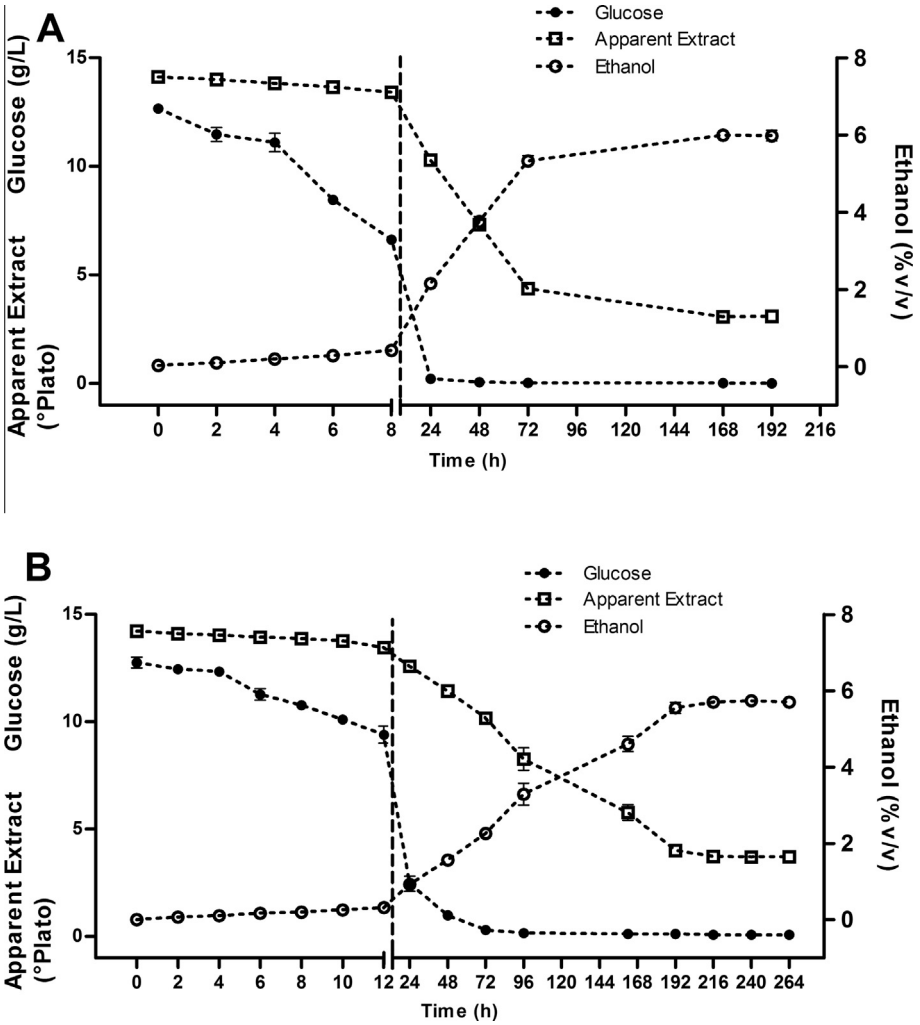

Fig. 4. Summary of the analytical monitoring of glucose, total sugars and ethanol during top (A) and bottom (B) fermentations. Glucose and ethanol values were measured by biosensors. AE values (°P) were obtained by the Eq. (6) reported in Section 2.2.2 from the ethanol values measured with the biosensor (see text).

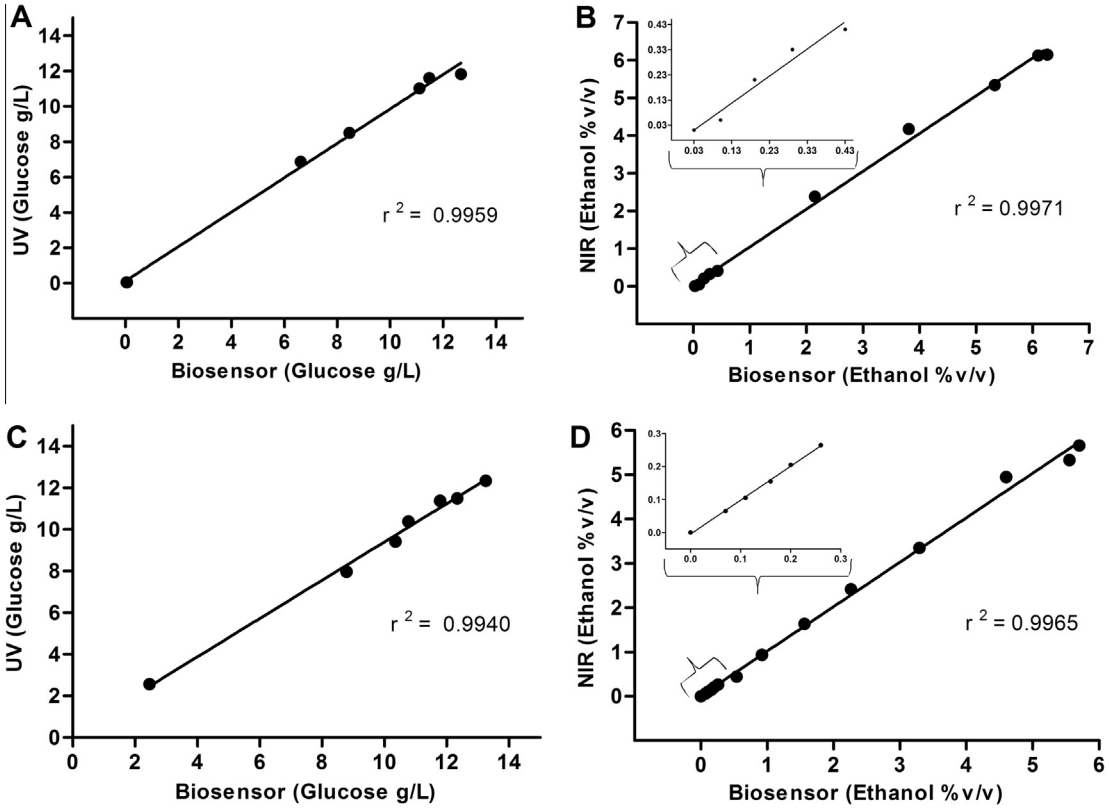

Fig. 5. Correlation between glucose values measured by biosensors and UV (A, C) and ethanol values measured by biosensors and NIR (B, D) in top fermentation (A, B) and bottom fermentation (C, D).

Eq. (6) reported in Section 2.2.2 from the ethanol values measured with the biosensor.

Top fermentation (Fig. 4A) was considered completed after 168 h (no more ethanol was produced) and the experiment was stopped at 192 h while in bottom fermentation (Fig. 4B), in which it was reached the maximum level of ethanol after 216 h, the experiment was stopped at 264 h.

The biosensors had an excellent agreement with the validation methods used, showing a r^2 which varied between 0.994 and 0.997 in the different tests (Fig. 5).

All measurements confirm that the glucose and ethanol biosensors provide a tight control of the fermentation process and are able to detect the beginning and the end of both top and bottom fermentations

4. Conclusions

The progress of fermentation must be monitored throughout, to confirm that performance is as expected, provide a means of early identification of non-standard behavior and verify as rapidly as possible when the end-point has been reached.

A biosensor telemetry system applied to beer production has been investigated. The main advantages of the developed device include the ease and speed of construction. The cheapness of the materials combined with the simplicity and rapidity of the analysis is key another aspect of the work. The developed biosensors were able to detect the main fermentation compounds with high sensitivity and repeatability.

The reliability was held for numerous analysis determining less maintenance and less disposal.

In future applications, a multi-analyte biosensor could be developed and it will require small volumes of sample making it very useful in continuous analysis. The system proposed could represent a new generation of analytical tools useful for brewers, allowing real-time and independent monitoring of fermentation without complex and expensive laboratory equipement.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.09.092.

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