Anal Bioanal Chem (2013) 405:3791–3799
DOI 10.1007/s00216-012-6598-y

ORIGINAL PAPER

At-line measurement of lactose in dairy-processing plants

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Received: 12 September 2012 / Revised: 6 November 2012 / Accepted: 21 November 2012 / Published online: 15 December 2012

Abstract Environmental and process control applications have needs for sensors that operate continuously or repeatedly, making them applicable to batch measurement and flowing product stream measurement. Additionally, for lactose monitoring in dairy-processing plants, the sensors must have sufficient flexibility to handle a wide range of substrate concentration and be resilient to withstand wide pH excursions brought about by frequent exposure to clean-in-place chemicals that happen without any warning. This paper describes the development and trialling of an at-line lactose biosensor that meets the needs of the dairy industry for loss monitoring of lactose in dairy-processing plants by the combination of a third-generation enzyme biosensor with a sequential injection analyser. Results, both from grab sample analysis and an at-line factory prototype, are shown from their operation when installed at a Fonterra dairy factory (New Zealand) during the 2011–2012 season. Previous sensor fabrication methods were converted to a single-step process, and the flow-through cell was adapted to bubble-free operation. The lactose concentration in wastewater-processing streams was successfully monitored by taking and analysing samples every 2–3 min, semi-continuously, for 3 months by an unskilled operator. The Fonterra site flushes approximately 100–300,000 L of wastewater per hour from its lactose plant. In the 2011–2012 season, the daily mean lactose content of this wastewater varied significantly, from 0.0 to 8.0 % *w*/*v* (0–233,712 μM) and equated to substantial total losses of lactose over a 6-month period. These lactose losses represent lost saleable or useable product.

Keywords At-line process analysis · Lactose measurement · Screen-printed electrodes · CDH-based biosensors · Flow injection analysis

Published in the topical collection *Bioelectroanalysis* with guest editors Nicolas Plumeré, Magdalena Gebala, and Wolfgang Schuhmann.

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Introduction

Globally, milk is a big business. Figures published in 2012 by the Food and Agriculture Organisation report global milk production to be 750,000,000 metric tons per annum (p.a.). The largest milk-producing area is the European Union, with more than 153,000,000 metric tons p.a. By country, the largest producer is India (55 % from buffalo milk), the largest cow milk exporter is New Zealand, and the largest importer is China [1]. Bovine milk typically contains around 13 % *w*/*v* milk solids comprising lactose (4.8 % *w*/*v*), fat (3.9 % *w*/*v*) and protein (3.3 % *w*/*v*). Milk and its components are now used to produce a vast array of highly nutritious foods that include cheeses, ice creams, yogurts, milk powders, whey protein products, butter and other cultured products. Significant increases in volume and product array have led to highly automated handling and processing of milk, with the industry becoming increasingly reliant on sensor measurement of process variables in order to maintain final product quality within defined specifications [2]. There is a need for more rapid correction of deviations from processing and quality specifications so that valuable ingredients are not lost to the environment. This paper describes

the development of an automated at-line lactose biosensor for monitoring wastewater streams in order to increase plant efficiency, maximise saleable product and reduce effluent discharge from dairy-processing plants.

A number of reviews on commercialising biosensor technologies highlight that, in spite of a significant increase in the output of publications and patents, there has not been a corresponding surge in successful commercial biosensor products available in the market [3]. In particular, this applies to biosensors in the bioprocessing, environmental and industrial sectors [3]. The success of disposable single-use enzyme-based sensors in medical applications, e.g. the glucose sensor with consumables changed with every use, is inappropriate for these sectors. They need sensors that operate continually or repeatedly, to facilitate batch measurement and flowing stream measurement. Unfortunately, biosensor devices designed for the bioprocessing, environmental and industrial sectors, to date, have failed to meet expectations with few examples of biosensors being successfully deployed in these sectors [4]. Other factors contributing to their lack of success [3] include the need for extended biocomponent viability, measurement speed, matrix effects, selectivity, dynamic signal range and integration between the biosensor and a fluidic sample handling system. Absence of, or weakness in, any of these factors is likely to compromise technology transfer to a commercial end user.

Previously, attempts to deliver lactose sensors to the dairy industry have used multi-enzyme cascades involving β-galactosidase and galactose oxidase [5], and sometimes glucose oxidase [6] or peroxidase [7]; such devices report a useful range for estimating lactose to be 10–60 gL^{-1}. Ferreira [8, 9] demonstrated that combining a flow injection analyser (FIA) and a lactose biosensor facilitated the experimental monitoring of lactose concentrations during real fermentations. Ferreira et al. [9] concluded that their linear range (1–30 gL^{-1}) and response times (3 min) were better than reported literature values but that more attention should be given to the oxygen electrode's membrane, avoidance of air bubbles and compensation for temperature effects.

Two recent papers by Gorton and coworkers [10, 11] and a review [12] describe a rapid and much simpler approach to lactose analysis based on advantages gained through third-generation amperometric biosensors, whereby direct electron transfer (DET) from the enzyme's active site to the surface of an electrode occurs without requiring a co-substrate (first generation) or a mediator (second generation). The third-generation biosensors used in the above studies were fabricated by immobilising cellobiose dehydrogenase (CDH) from two different white rot fungi, *Trametes villosa* or *Phanerochaete sordida*, onto carbon surfaces (spectrographic graphite or screen-printed carbon electrodes (SPCE)). In comparison to the enzyme cascade biosensors (response time, 40 s; limit of detection, 45–340 μM [7]), the CDH-modified electrodes have much higher sensitivities and faster response times (response time, 4 s; limit of detection, 0.25 μM [11]).

CDH is an extracellular glycosylated monomeric two-domain redox enzyme, where the two domains are connected through a flexible polypeptide linker region [13]. Only recently, its physiological redox partner protein was discovered explaining its physiological role in lignocellulose degradation [12, 14–18]. The sugar substrate is oxidized in the catalytically active flavine adenine dinucleotide (FAD) containing dehydrogenase domain (DH_{CDH}) yielding the fully reduced flavin ($FADH_2$) and a lactone. Reoxidation of the reduced DH_{CDH} can occur through different routes [12]. In the absence of a soluble electron acceptor, the electrons from the reduced DH_{CDH} are sequentially transferred to the heme b containing cytochrome domain (CYT_{CDH}) through an intramolecular electron transfer pathway from the $FADH_2$ in the DH_{CDH} to the surface-exposed heme b of the CYT_{CDH}. From there, the electrons are directly transferred to the electrode surface acting as the final electron acceptor. CDH is produced by both basidiomycete (white rot, class I CDH) and ascomycete fungi (brown rot and plant pathogens, class II and III CDH) [15]. The white rot class I CDHs are acidic with a pH optimum around pH4–5, and they only catalyse cellodextrins and lactose (connected through 1,4-β-linkages) with high turnover rates and have a high discrimination against, e.g. monosaccharides and oligosaccharides connected through 1,4-α-linkages [13]. In contrast, class II and III CDHs may have a much broader substrate spectrum, including glucose, and they may also have pH optima in more neutral pH [19]. As milk and other dairy products do not contain any other sugar substrate with a high turnover rate with class I CDHs in any appreciable concentration other than lactose, a biosensor based on such a class I CDH founds a perfect choice for making a lactose biosensor for the dairy industry.

Cow's milk contains between 4 and 5 % *w/v* lactose and can vary between 0 and 8 % *w/v* in processing effluent, depending on the efficiency of the processing plant. Loss monitoring in New Zealand dairy-processing plants relies on a laboratory measurement of a composite 12- or 24-h sample using near infrared reflectrometry (NIR) to provide a daily average lactose concentration, as well as using on-line measurements of turbidity in the wastewater flume. Measurements performed after a 12- or 24-h delay can only provide a historical snapshot of plant performance and give no opportunity for trace-back or intervention by plant operators. NIR is also used by all New Zealand dairy plants at the beginning of processing to standardise milk composition. However, NIR cannot be used where the background matrix is variable, as found in wastewater effluent streams. Likewise, lactose's solubility in water suggests that turbidity is unlikely to be a good indicator of lactose concentration.

Currently, the limitations for lactose monitoring in dairy-processing plants are twofold: (1) there is no automatic at-line analyser available for measuring lactose because of limited performance of the sensor and (2) automatic dilution is required to adjust samples to a suitable linear range and optimal pH. The attributes of the CDH-modified electrodes for lactose detection could resolve this conundrum.

Experimental

Reagents

CDH (cellobiose: (acceptor) 1-oxidoreductase; EC 1.1.99.18) from *P. sordida* (volumetric activity, 190 U mL^{-1}; specific activity, 23.9 Umg^{-1}) was supplied by the University of Natural Resources and Applied Life Sciences, Vienna, and used for electrode modification. Fresh stock solutions of β-lactose (Fisher Scientific AR, Auckland, New Zealand) were held overnight at 4 °C, allowing for mutarotational equilibrium prior to use. Two different media, citric acid (CA) buffer and synthetic dairy processing wastewater, were used to make the lactose stock solutions. The CA buffer comprised 0.02 M citric acid and 0.1 M KCl adjusted to pH4.5 by NaOH additions. The synthetic dairy processing wastewater media were made to a formulation specified by Fonterra Co-Operative Group Ltd and contained: casein hydrolysate (Merck, Auckland, New Zealand) (1,300 mgL^{-1}), KNO_3 (BDH AR, Auckland, New Zealand) (50 mgL^{-1}), $CaCl_2{\cdot}2H_2O$ (400 mgL^{-1}), $MgSO_4{\cdot}7H_2O$ (BDH AR, Auckland, New Zealand) (200 mgL^{-1}), NaCl (BDH AR, Auckland, New Zealand) (250 mgL^{-1}), KH_2PO_4 (Fisher Scientific AR, Auckland, New Zealand) (650 mgL^{-1}) and β-lactose at concentrations 5,236, 10,526 and 42,104 mgL^{-1}, corresponding to 0.5, 1 and 4 % *w*/*v*, respectively. This synthetic wastewater formulation reports a pH of 4.1 and has a chemical oxygen demand of 6,400 mgL^{-1} and total Kjeldahl nitrogen of 100 mgL^{-1}. All subsequent dilutions of the two lactose stock solutions were made with the CA buffer. Unless otherwise specified, all chemicals were purchased from LabServ, Auckland, New Zealand.

Sensor fabrication

SPCE modified with carboxyl-functionalized multiwalled carbon nanotubes (DropSens 110CNT), manufactured by DropSens (Oviedo, Spain), were used for sensor fabrication. Each electrode comprises a 4-mm-diameter carboxyl-functionalized multiwalled carbon nanotube working electrode (SPCE-MWCNT), a silver reference electrode and a graphite auxiliary electrode printed onto a ceramic support. Previous studies by Safina et al. on immobilising *P. sordida* CDH on screen-printed electrodes [11] established that the SPCE-MWCNTs gave better electrochemical responses than counterpart SPCEs without carbon nanotubes. Similar results were also shown for a class II CDH [20] as well as for a haemoglobin [21] supporting the belief that at the more nanostructured CNT-based sensors, a higher percentage of the immobilised enzyme/protein molecules are orientated for DET on the electrode surface. Glutaraldehyde (Sigma-Aldrich, grade I, 5 % *w*/*v*) and Triton X-100 (Ajax 1552, 0.1 % *w*/*v*) were mixed with the *P. sordida* enzyme to aid immobilising the enzyme onto the SPCE-MWCNT working electrode. The final CDH enzyme-modified biosensors were fabricated by depositing a 2-μL aliquot of the *P. sordida*/Triton/glutaraldehyde mixture onto the MWCNT working electrode surface.

Flow system and measurement cycle

The performance of the CDH enzyme-modified biosensors was characterised and tested using two flow systems, a FIA and a sequential injection analyser (SIA). FIA was the preferred method for characterising the response of the modified CDH biosensors in terms of their sensitivity, linear range and susceptibility to matrix changes. The FIA combined a Shimadzu LC-20AT solvent delivery unit with a Shimadzu SIL-10ADVP sample processor/injector for delivering samples to the detector. Two electrochemical flow cells were used as the detector, either a methacrylate wall-jet flow cell (DropSens FLWCL) or an acrylic, custom-built laminar flow cell. The electrode was connected to a three-electrode potentiostat (EDAQ Quadstat 164 and e-corder 410), and the resulting current signal recorded with EDAQ Chart software. All measurements were performed at ambient temperature. The laminar flow cell was designed to have a vertical upwards flow path so that the buoyant tendency of a gas bubble and flow were working in the same direction.

The SIA was supplied by Sensolytics Gmbh, Germany, and is designed to automatically deliver precisely metered amounts of sample or standard using a reversible high-precision pump (HPLH20 Zipperer Gmbh) and a multi-position selection manifold (Sensolytics), thereby providing a robust methodology for at-line chemical analysis [22]. At-line monitoring at the dairy-processing plant was performed exclusively with the SIA in conjunction with the custom-built laminar flow cell connected to a customized BASi potentiostat (BAS LC-Petite Ampere) for current measurement.

At-line sampling from the lactose flume

The lactose flume at a New Zealand dairy-processing plant was sampled using a Watson-Marlow (120S/DM3) 3-channel peristaltic pump. Each channel of the DM3 pump

head was fitted with Watson-Marlow Marprene tubing of different internal diameters: channel 1, 2.79 mm; channel 2, 1.65 mm; channel 3, 1.52 mm. In order to access the lactose flume, channel 1 was extended with silicon tubing, and a wire mesh was fitted to exclude influx of particles exceeding 0.5 mm^2. The channel 1 pump, when operated at 10 rpm, delivered a continuous flow at 3.6 $mL\,min^{-1}$ through an on-line automatic filtration (OLAF) unit (Sensolytics), which fed a 20-cm serpentine pattern across a 0.2-μm-pore nitrocellulose filter disc. The retentate of OLAF, comprising larger material and excess liquids, was pumped by channel 1 to waste, whereas the diffusate, which passed through a 0.2-μm filter, was pumped continuously by channel 3 at 1.3 $mL\,min^{-1}$ into the bubble trap. The bubble trap was a polycarbonate block with a 2-cm^3 vertical cavity. The cavity had a conical base, and its top was exposed to the environment. Sample filtrate from OLAF entered the cavity midway, and any entrained air bubbles immediately moved to the surface and exited to the environment. The sample to be analysed was taken from the bubble trap cavity base from below the entry point by the on-line general analyser (OLGA) unit (Sensolytics), as required. The channel 2 pump emptied the bubble trap from a waste outlet at a flow rate of 1.5 $mL\,min^{-1}$, greater than that of the flow into the bubble trap, in order to prevent overflow and remove it to waste.

Results and discussion

Operating environment

Lactose concentrations in dairy processing effluent are characterised by rapid fluctuations spanning 0–8 % *w*/*v* due to short-lived periods of high loss interspersed with periods of clear discharge. Also, plants conduct regular clean-in-place (CIP) operations, which produce significant pH swings in the processing effluent due to the presence of CIP chemicals.

These fluctuations are illustrated in Fig. 1, where grab samples of processing effluent were taken over a 12-h period and analysed for their lactose concentration and pH. For much of the time, the lactose concentration tracks below 1 % *w*/*v* except for two digressions: one where the lactose concentration surges to 5.52 % *w*/*v* but with negligible pH shift, and in the other, the lactose concentration and pH have significant shifts, the lactose from 3 0 to 8.43 % *w*/*v* and the pH from 2 0 to 5.9. An earlier pH swing from pH 2.4 to pH12.7 appears not to be linked to a lactose concentration perturbation. Clearly, dairy processing effluent provides a challenging environment for an environmental sensor, particularly so for biosensors, which are not well renowned for their stability. Also, the fluctuations in lactose

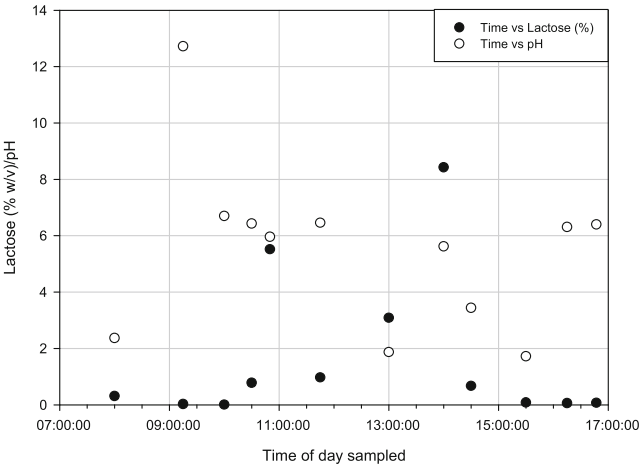

Fig. 1 Grab sample analysis of operating environment using a CDH sensor

concentration between 0 and 8 % *w*/*v* (corresponding to 0–222,025 μM) means a programmable auto-dilution device is required to maintain the sensor's optimal performance [22] and for regular re-calibration of the sensor.

Sensor characteristics: calibration, linear range, reproducibility

We measured the current response of a CDH-modified lactose biosensor to a series of known lactose concentrations, where the samples were made up in two different media, CA buffer (pH4.5) and synthetic dairy processing wastewater (pH4.1). The lactose samples in both media spanned a concentration range of 29,214 to 2 μM (equating to 1.0 to 0.00007 % *w*/*v*). This range was selected because its midpoint, 0.5 % *w*/*v* lactose, is typical of dairy processing wastewater. We also sought to demonstrate that the sensor's sensitivity (2 μM limit of detection) and linear range (0–200 μM) were not compromised when confronted with a matrix that reflects dairy processing wastewater. Fortuitously, the pH optimal for the CDH-modified lactose biosensor, pH4.6, was not far removed from the pH of the synthetic dairy processing wastewater (pH4.1)

Duplicate measurements were taken at each concentration, first in the CA buffer and then in the synthetic dairy processing wastewater. The resulting current signals were recorded using the FIA in combination with the wall-jet flow-through cell (Fig. 2). The injection volume and carrier stream flow were 50 μL and 0.57 $mL\,min^{-1}$, respectively.

Figure 2 shows minimal change in signal due to the different background matrices over the lactose concentration range of 0 to 29,214 μM. Transformation of the two sets of data using a Hanes-Woolf plot estimated the Michaelis constant (K_m) and maximum reaction rate (V_{max}) for lactose to be 1,191 μM and 1,548 nA, respectively, in CA buffer

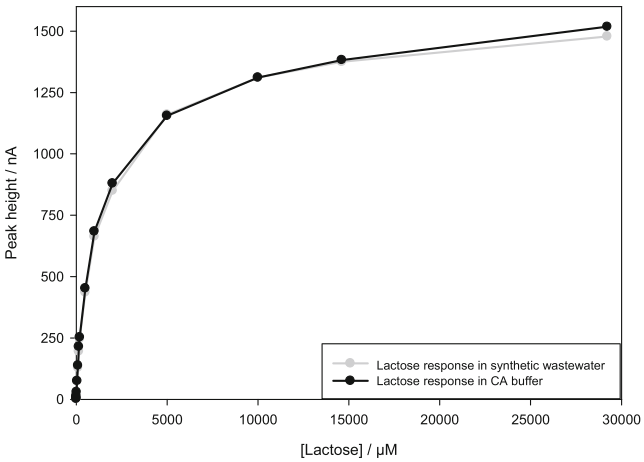

Fig. 2 Matrix dependency of CDH sensor's lactose response

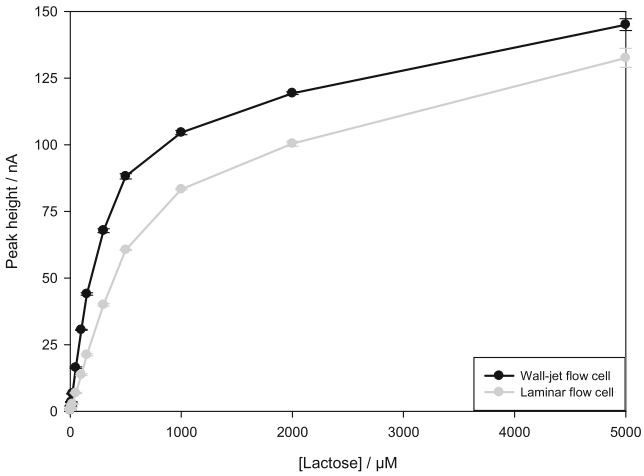

Fig. 3 Comparison of flow-through cells: wall-jet and laminar flow

and to be 1,193 μM and 1,520 nA, respectively, in synthetic wastewater. These values for lactose using CDH enzyme derived from *P. sordida* are similar to values reported for *Phanerochaete chrysosporium* CDH enzyme [12].

Development of a flow-through cell

FIA and SIA both rely on the injection of a well-defined volume of sample solution into a carrier flow stream, with subsequent detection of the target analyte in a flow-through cell. While measuring the matrix dependency (Fig. 2) using the FIA in combination with the wall-jet flow-through cell as the detector, we found the flow cell to be susceptible to trapping air bubbles and leakage of the carrier fluid. More often than not, the system had to be stopped to clear a bubble, while carrier fluid leakage accelerated corrosion of the connector plug attached to the SPCE-MWCNT electrode. These problems were eliminated by re-designing the flow cell to have a vertically directed flow path and the SPCE-MWCNT electrode connector fittings sited above the flow path, thus isolating them from the carrier fluids, leading to trouble-free operation of the detector. Comparative signal responses recorded using the laminar flow and wall-jet flow-through cells and a lactose concentration series from 0 to 5000 μM are shown in Fig. 3. Triplicate measurements were made, and the error bars are shown for each data point. The injection volume and carrier stream flow were 50 μL and 0.57 mLmin^{-1}, respectively. A linear regression of the data in Fig. 3 between 0 and 500 μM showed the custom-built laminar flow cell to have an extended linear range (R^2=0.995), compared to the wall-jet flow-through cell (R^2=0.936). Subsequently, the rest of our trials were performed exclusively with the custom-built laminar flow cell.

Lactosenz TM—prototype design

Meeting the analytical needs of the bioprocessing, environmental and industrial sectors requires sensor devices to combine the necessary performance characteristics with liquid handling systems that can automatically regulate incoming samples to be within optimal limits for the sensor. Figure 4 depicts the measurement cycle, beginning with sample acquisition from a dairy processing wastewater drain; the sample was continuously removed at 3.6 mL min^{-1} from the drain by a peristaltic pump. Particles larger than 0.2 μm were removed by a cross-flow nitrocellulose filter disc (OLAF), and the diffusate from the filter was pumped at 1.3 mLmin^{-1} into a bubble trap to ensure the sample stream was purged of particulates and bubbles prior to delivery to the auto-dilution device (OLGA).

The injection manifold of OLGA comprises four valves working in tandem with a step-motor-driven high-precision pump. In order to prevent contamination, this pump is separated from the injection manifold by a 0.8-mm-diameter Teflon tubing holding coil of 750 μL. The appropriate combination of pumping direction and opened or closed valves determine whether the liquid is sucked in towards the pump or pumped forward to the laminar flow cell containing the CDH-modified electrode or pumped to waste [23]. Measuring the lactose concentration of a sample takes 2–3 min to complete and involves 13 steps that include: (1) 50 μL of sample is taken from the bubble trap, via the manifold, to the dilution vessel; (2) the sample is diluted with buffer to give a total of 1,000 μL in the dilution vessel; (3) a 20-μL sample is taken from the dilution vessel and directed through the flow cell; (4) as the sample passes over the sensor, the peak height is recorded, and this signal is converted to percent *w*/*v* lactose by comparison with a measurement of a sample of known lactose concentration. A primary 1 % *w*/*v* standard lactose solution was used for

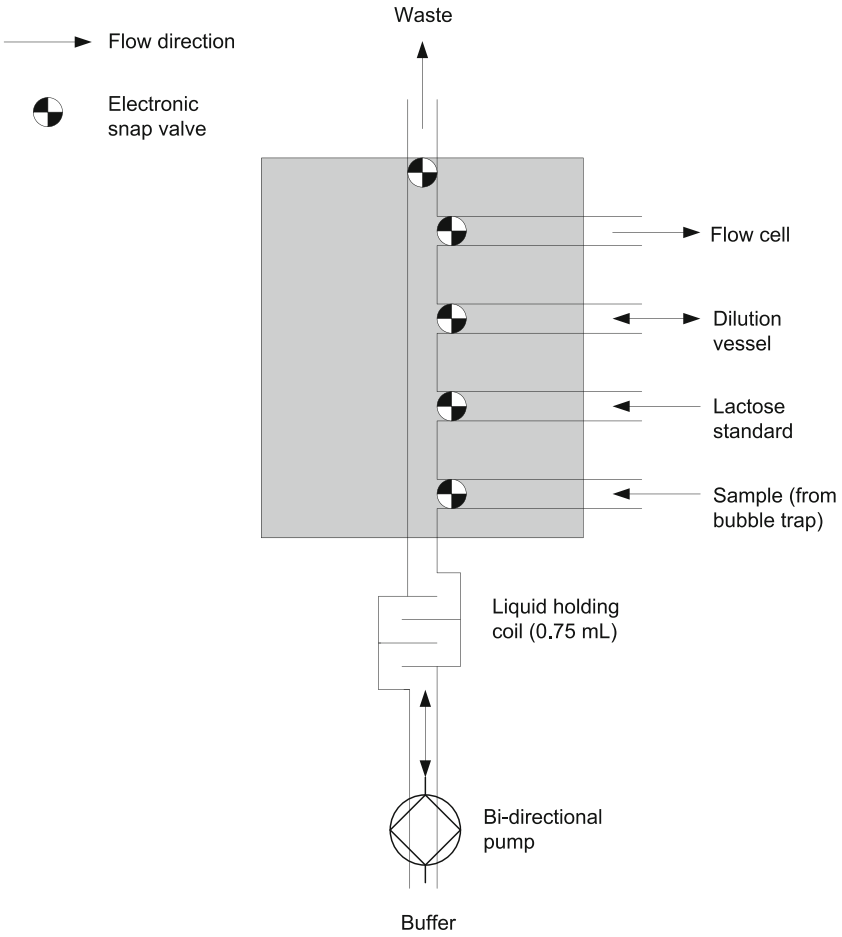

Fig. 4 Schematic of OLGA manifold

this purpose, as well as a 0.5 % *w*/*v* lactose standard—made by dilution in OLGA of the 1 % *w*/*v* standard.

The prototype instrument, called Lactosenz TM, comprises an ensemble of components including pumps, reagents, filters, a liquid handling system, potentiostat, flow cell and computer. When the Lactosenz TM prototype was trialled on-site at a dairy-processing plant, a re-calibration step was performed after every set of five wastewater at-line measurements. This sequence was very conservative given that re-calibration interrupts measuring samples of wastewater and takes around 6 min to complete. Given that most in situ installations have been at external locations, the unit was housed in a weatherproof cabinet, with the only external connections being for electricity, tubing to remove sample from a drain, and communication to the control room.

The CA dilution buffer (4.0 L) was held in a 5-L Tedlar bag and during continuous operation was replaced every 2.5 days. The primary lactose standard (0.5 L) was held in a 1-L Tedlar bag and replaced on a weekly basis. The biosensor chip was replaced every 24 h; the exchange of the biosensor chip takes less than 3 min, and replacing all the consumables takes less than 10 min. A text file on the operation is sent from OLGA to a computer detailing the type and time of each measurement, either calibration or sample. OLGA also generates an Excel file listing the elapsed time and percent *w*/*v* lactose concentration of each measurement cycle since the initial start time.

At-line measurements in the dairy factory

At-line processing plant measurements pose numerous challenges, such as 24-h/7-day operation, fluctuating daily temperatures and weather conditions, exposure to CIP chemicals and unattended operation. To meet these challenges, the technology must be robust, flexible and operable by a relatively unskilled person. The Lactosenz TM prototype analyser was installed at a Fonterra Co-Operative Group Ltd. lactose plant, at a flume carrying wastewater and operated over a 3-month period between November 2011 and February 2012. The lactose plant extracts lactose from the by-products generated by other operations in the processing plant prior to sending waste products to wastewater treatment and discharge. The main input received by the lactose plant is permeate from ultra filtration in the whey protein concentrate plant, which in turn has received skim (from milk treatment and cheese milk treatment) and treated whey. The flow rate in the lactose flume typically ranged from 50 to 300 $m^3 h^{-1}$ (50,000 to 300,000 $L h^{-1}$). Figure 5

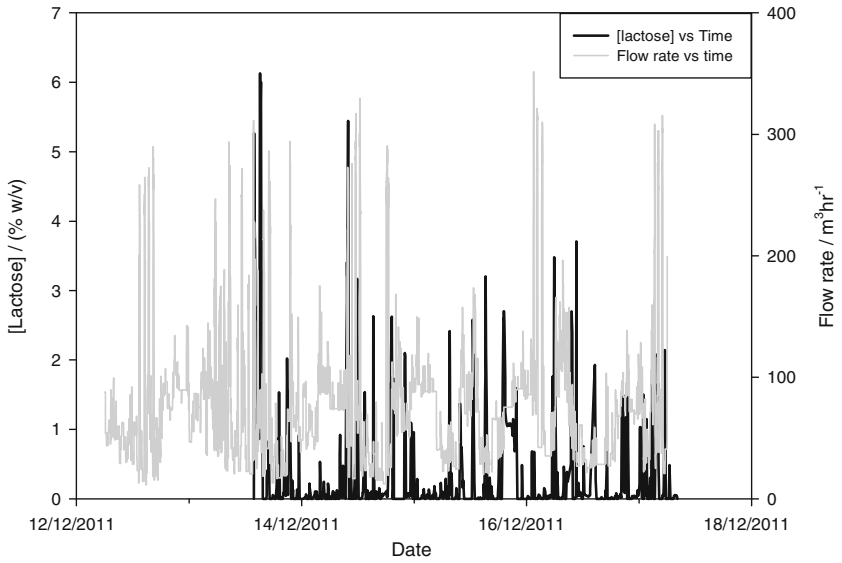

Fig. 5 At-line lactose concentration results gathered by sustained prototype operation over 4 days

reports the percentage of lactose measured by Lactosenz TM, overlaid with flow volume data over a 5-day period (12–17 December 2011).

Over the 4 days, we estimate that Lactosenz TM made 1,300 lactose measurements, compared with four made by the current lactose loss monitoring NIR analysis of composite 24-h samples. The rapid fluctuations of lactose concentration suggest that the true lactose loss is unlikely to be captured by a single 24-h composite sample. Comparing the losses estimated by the two methods—at-line Lactosenz TM and 24-h composite NIR—it is evident there is a significant discrepancy between them. Interestingly, the at-line sensor predicts around 50 % lower losses than the 24-h composite analysis over this period.

A turbidity meter and a flow meter are also located in the lactose flume in close proximity to the lactose sample inlet, and their continuous signals are fed back to the process plant control room. A significant advantage provided by an at-line sensor is the opportunity to trace back, identify and, if necessary, mitigate upstream events that might be responsible for the fluctuations detected by the sensor.

Figure 6 shows the Lactosenz™ data superimposed over the timing of CIP activities from records held at the lactose plant over 24 h. This indicates that the lactose plant CIP cycles are generally associated with wastewater lactose peaks (although not exclusively), suggesting that CIP cycles account for a large amount, but not all, of the lactose losses measured in wastewater effluent.

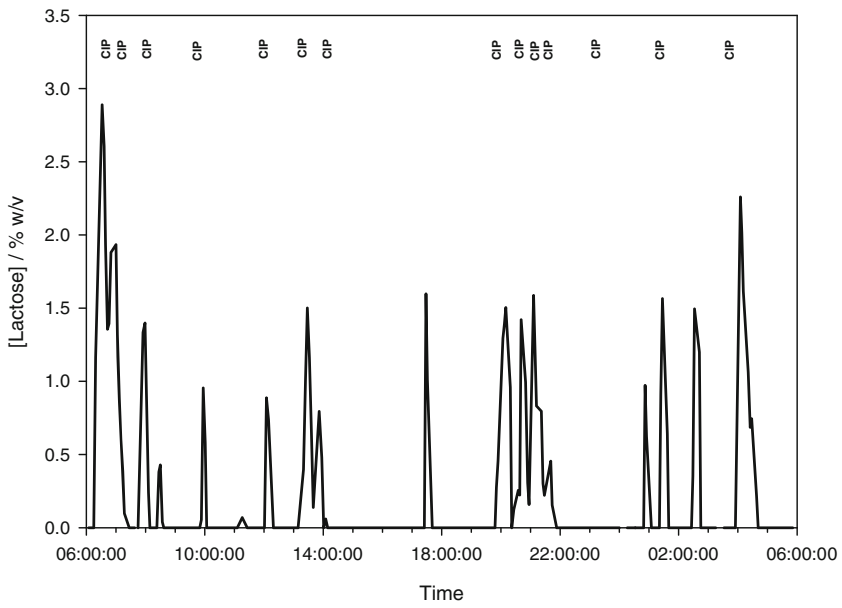

Fig. 6 Comparison of lactose plant CIP activities and lactose losses

Conclusion and future outlook

The development and optimisation of an at-line biosensor to meet the analytical needs of lactose measurement in dairy-processing plant wastewater has been successfully achieved by using CDH-modified enzyme biosensors and a programmable auto-dilution flow-through device, OLGA. Prior to on-site trials, Lactosenz TM was tested to ensure that the sensor met the demands of the bioprocessing sector, namely extended biocomponent viability, suitable measurement speed, absence of matrix effects, selectivity and a dynamic signal range. A 12-h duration viability target was set and surpassed when trialled both in the laboratory and when installed on-site at the wastewater flume. Sample measurements were recorded at 3-min intervals, and the recorded lactose concentration profile (Fig. 5) bears close resemblance to the charted flow data. Despite the 3-min measurement interval, the Lactosenz TM profile was highly spiked, and it has captured the rapidly changing dynamic at the wastewater flume. No doubt, some smoothing of the data does occur due to sample filtration, dilution and the controlled dispersion that occurs within the tubing. The advantage of OLGA is that the system is fully automated, and it can cope with a variety of sample concentrations without re-configuring the manifold; the injection volume, pump rate or pauses between measurements maintain the sample concentration within the sensor's linear range, 0 to 500 μM. Even with an 8 % *w/v* lactose concentration spike (Fig. 5), the dilution factor achieved by a minimal sample injection volume (3–5 μL), dilution vessel and the controlled dispersion delivers a sample at around 200 μM to the flow-through cell. Furthermore, having a fully automated dilution device, such as OLGA, ensures that Lactosenz TM can easily be maintained and operated by a relatively unskilled operator.

Over the 3-month prototype trial period, two problems were highlighted, stalling of OLGA's high-precision pump and diffusion of calibrant into OLGA's manifold. The stalled pump could easily be fixed if an operator was on-site, by switching OLGA off and then back on. However, if the stall occurred when the operator was absent, there could be a lengthy break before data capture was re-activated. A newly installed motor controller for the high-precision pump has now eliminated this problem. The OLGA injection manifold consists of four equivalent inlet/outlet channels connected to a vertical central channel, the bottom of which was connected to the pump and the top connected to the waste outlet. The four inlet/outlet channels were connected, in order from top to bottom, to the flow cell, dilution vessel, calibrant standard and sample. Visual confirmation by adding a dye to the calibrant confirmed that there was ingress of calibrant when OLGA was programmed to inject 0 μL of calibrant. In addition, this ingress was verified by the flow cell detector, which reported a 15.6-nA signal for a 0 % *w/v* calibration. A consequence of having the standards offset by an elevated signal due to an unknown ingress of lactose (but not for the samples) meant that the linear extrapolation of the standards did not go through zero on the *y*-axis. In this particular case, all samples that reported a peak height less than or equal to 15.6 nA were assigned a concentration of 0 % *w/v* by OLGA. This may account for the high number of 0 % *w/v* lactose samples reported in Fig. 5.

Overall, the results obtained during this trial prototype testing period were very encouraging. The Lactosenz TM prototype was operable by a previously unskilled operator; only one sensor failed to operate for more than 12 h, and no stoppages for air bubbles in the flow-through system were encountered. We were able to show that at-line lactose sensing reported lactose losses substantially less than those reported for 24-h composite samples for our trial period. Most significantly, we were able to use the real-time data to trace back and identify the upstream events responsible for these losses, providing operators with the opportunity to enhance the efficiency of their processing systems and to maximise production of saleable product.

Acknowledgments The authors wish to thank the New Zealand Ministry of Innovation, Business and Enterprise (formerly the Ministry of Science and Innovation) for financial support and for funding a Julius von Haast Fellowship to Wolfgang Schuhmann. Also, we wish to thank Fonterra TM for in-kind support, for access to a milk-processing plant and for appointing a technology intern to operate the prototype analyser at a dairy-processing plant for 3 months.

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