Diabetic Glucose Meter for the Determination of Glucose in Microbial Cultures

Raphael Flavigny

PII: S0167-7012(14)00074-8
DOI: doi: 10.1016/j.mimet.2014.03.002
Reference: MIMET 4340

To appear in: *Journal of Microbiological Methods*

Received date: 25 February 2014
Revised date: 5 March 2014
Accepted date: 5 March 2014

Please cite this article as: Flavigny, Raphael, Diabetic Glucose Meter for the Determination of Glucose in Microbial Cultures, *Journal of Microbiological Methods* (2014), doi: 10.1016/j.mimet.2014.03.002

Diabetic Glucose Meter for the Determination of Glucose in Microbial Cultures

Raphael Flavigny,
School of Engineering and Information Technology, Murdoch University, 90 South Street, Murdoch, WA, 6150, Australia

ABSTRACT:

In wastewater, biological phosphate removal can fail because of the presence of glycogen accumulating organism (GAO), therefore measuring glycogen stored in microbial cultures provides information on the bacterial population type. Once glycogen is hydrolysed to glucose it was accurately measured using a human glucose meter. The standard curves demonstrate linearity regardless of the pre-treatment of the glucose solution at neutral pH.

Keywords: Novel Method, PHA, Glucose, Glycogen Accumulating Organisms, Diabetic Glucose Meter

Enhanced biological phosphate removal (EBPR) sludge uses phosphate accumulating organisms (PAO) to store poly-phosphate under anaerobic conditions. In aerobic conditions poly-phosphate is lysed, resulting in an ATP source for soluble carbon uptake in the form of poly-hydroxy-alkanoate (PHA)[1]. However PAO can be outcompeted by glycogen accumulating organisms (GAO) resulting in poor phosphate removal in wastewater treatment plants[2]. GAO use glycogen anaerobically as an ATP source to store organic carbon, producing PHA[3]. Measuring glycogen in microbial cultures reveals the presence of the GAO. Measuring glycogen requires it to be split into two glucose molecules. This paper

demonstrates that a diabetic glucose meter can be used to measure the glucose issued from GAO glycogen.

Glycogen quantification relies on its separation, by a strong acid or base [4, 5], to glucose molecules which can be measured. Glucose is measured using three different methods. Once glucose is turned into a volatile compound, it is quantified with high pressure liquid chromatography (HPLC) [4, 6] or gas chromatography (GC)[7]. Alternatively enzymatic assays are used for their reliability, but require the purchase of expensive chemicals which expires rapidly [7].

Blood glucose analysis is required for 347 million diabetic people [8] in the world. Glucose meters were developed in 1962, and are now cheap and readily available [9]. The glucose meter uses an enzyme immobilised onto a strip, which produces a current proportional to the blood glucose concentration. Strips containing the enzymes have been developed for accuracy and extended duration. Wang [9] extensively reviewed each components required for accurate blood glucose measurements. The proposed method uses the widely available diabetic glucose meter.

In this paper, glucose was analysed, in duplicate, using an AccuCheck Active (Roche) glucose meter with the corresponding test strips. The linear trends line were fitted using Excel™ 2010 and based on the average of the duplicate readings. Note that the solution without glucose did not produce a usable reading and therefore were not included in the results.

The initial test was to ensure that a glucose solution could be detected by the glucose meter. The following concentration of 0, 2, 3, 4, 5, 6, 7, 8, 9, 10 mmol/L glucose (AR grade, Merck) were prepared and 10 µL of solution applied on a strip. The linear relationship until 10 mmol/L of the standard solution demonstrates that glucose can be accurately read (□ in

Figure 1). The effect of volume applied to the strip (5, 10, 20 μL) and solution temperature did not affect the readings linear relationship (R^2 value > 0.98).

The glucose extracted from biomass would be in acidic solution, therefore the glucose solution was digested with 0.9 mol/L HCl. Given it is a enzymatic method, the pH was neutralised using two aliquots: one of 0.350 mL of 10 mol/L NaOH, and the second of 0.5 mL of 0.9 mol/L KH_2PO_4. The effect of acidity on the linearity was negligible (○ in Figure 1). Separately, the effect of boiling was tested by putting the acid solution in a water bath at 100 ^oC for 5 h (Δ in Figure 1). The linearity of the standard curve demonstrates that the both effects of acid and boiling are negligible (Figure 1).

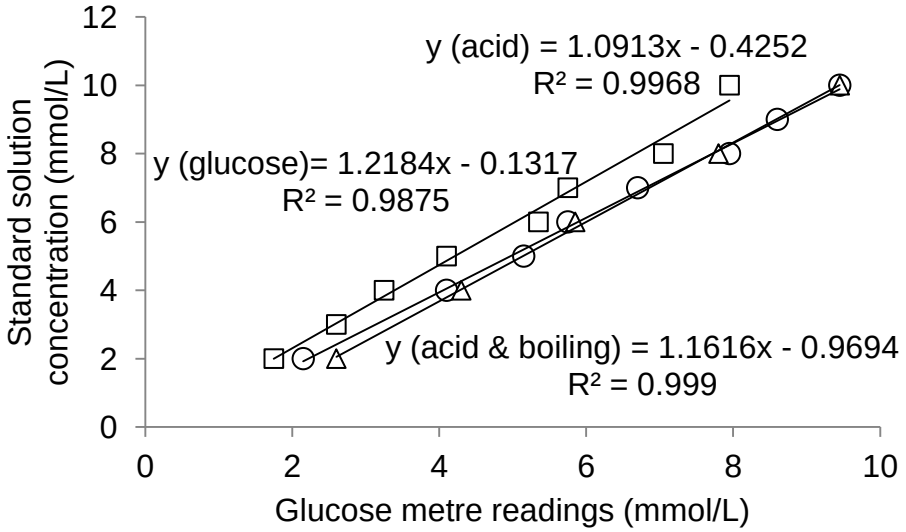

Figure 1: Linear relationship of the glucose meter readings and the standard solutions of glucose solution (□) with acid treatment prior to boiling (○) and after boiling (Δ). All solutions were adjusted to neutral pH (7.5>pH>7.0) before the analysis was conducted.

The previous results were conducted using glucose solution. The method required to be test to detect glucose extracted from GAO. The GAO was obtained from a laboratory reactor from Murdoch University, seeded with activated sludge (Woodman Point, Perth, Western Australia). The reactor biomass was selectively enriched in GAO to store soluble acetate anaerobically. This was achieved by alternating aerobic (1 h) and anaerobic conditions (2 h),

given the low level of phosphate in the aerobic phase, the GAO were grown preferentially over PAO[10].

The reactor was provided with synthetic wastewater consisting of ($mg.L^{-1}$): CH_3COONa 660, NH_4Cl 160, $NaHCO_3$ 125, KH_2PO_4 44, $MgSO_4.7H_2O$ 25, yeast extract 50, and 1.25 $mL.L^{-1}$ of trace element solution, which contained ($g.L^{-1}$): ethylene-diamine-tetra-acetic acid (EDTA) 15, $ZnSO_4.7H_2O$ 0.43, $CoCl_2.6H_2O$ 0.24, $MnCl_2.4H_2O$ 0.99, $CuSO_4.5H_2O$ 0.25, $NaMoO_4.2H_2O$ 0.22, $NiCl_2.6H_2O$ 0.19, $NaSeO_4.10H2O$ 0.21, H_3BO_4 0.014 and $NaWO_4.2H_2O$ 0.050.

Prior to analysis, the GAO was spun at 2500 rpm for 5 minutes and then was freeze-dried (Hetosicc-CD 4) at -50 ^oC for a minimum of 6 hours. The glycogen was extracted and lysed from the two samples of GAO dried biomass (sample 1: 27.1 mg and sample 2: 28.3 mg) using the acid method[7, 11]. An aliquot of 3 mL of 0.9 mol/L HCl was added to the dried biomass in culture tubes. The culture tubes were capped and the content digested at 100 ^oC in a water bath for 5 hours. The solution was then centrifuged at 5000 rpm for 5 minutes to remove the suspended solid resulting from the digestion process, the supernatant contained the glucose to be analysed. The pH was neutralised as explained previously.

To sample 1 an aliquot of glucose solution (2 mmol/L) was added, therefore the readings of both samples are expected to differ by 2 mmol/L. The glucose concentrations obtained from the GAO samples were 4.9 and 3.1 mmol/L respectively (Figure 2). In sample 1, considering the additional glucose the GAO glucose content was calculated to be 2.9 mmol/L, which is close to the glucose concentration in sample 2.

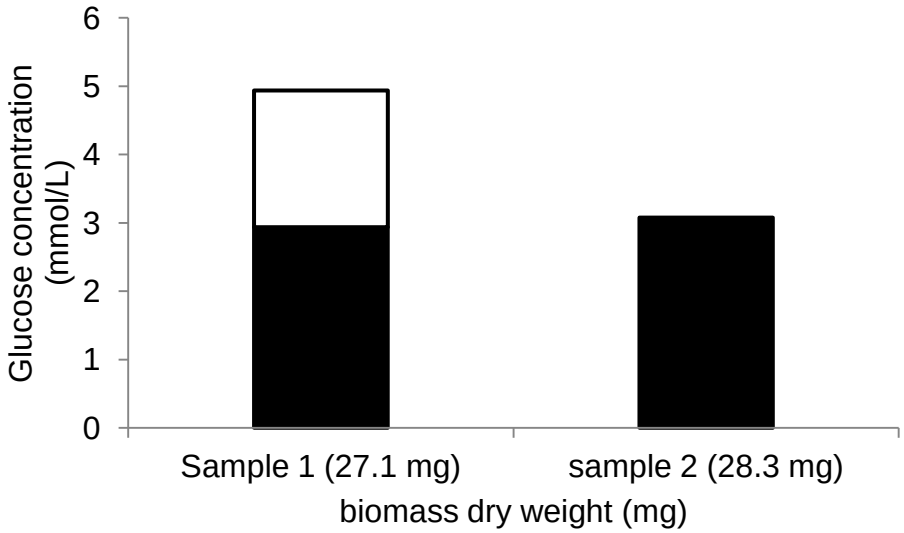

Figure 2: Measurement of glucose concentration in dried biomass sample (black) after acid digestion and boiling. Sample 1 had an additional 2 mmol/L glucose (white) to demonstrate the capacity of the method to measure all glucose.

In conclusion, diabetic glucose measurements of biological glucose were accurate for both laboratory prepared solution and lysed GAO biomass. This method is simple and cheap compared to HPLC, GC. Enzymatic assays are an alternative method, but their lifespan is minimal (< 2weeks) and is expensive. On the other hand the proposed method requires enzymatic strips which last for a year and glucose meters which are readily available at any pharmacy because they are widely used by diabetic people and consequently are significantly cheaper than conventional methods.

ACKNOWLEDGMENT:

The author would like to thank the help and support from Dr Ralf Cord-Ruwisch, and the financial support provided by Murdoch University.

ABBREVIATIONS:

EBRP enhanced biological phosphate removal, PAO phosphate accumulating organisms, GAO glycogen accumulating organism, PHA poly-hydroxy-alkanoate

References

1. Petersen, B.; Temmink, H.; Henze, M.; Isaacs, S., Phosphate Uptake Kinetics in Relation to PHB under Aerobic Conditions. *Water Res* **1998,** *32*, (1), 91-100.
2. Lopez-Vazquez, C. M.; Oehmen, A.; Hooijmans, C. M.; Brdjanovic, D.; Gijzen, H. J.; Yuan, Z.; van Loosdrecht, M. C. M., Modeling the PAO-GAO competition: Effects of carbon source, pH and temperature. *Water Res* **2009,** *43*, (2), 450-462.
3. Dai, Y.; Yuan, Z.; Jack, K.; Keller, J., Production of targeted poly(3-hydroxyalkanoates) copolymers by glycogen accumulating organisms using acetate as sole carbon source. *J Biotechnol* **2007,** *129*, (3), 489-497.
4. Smolders, G. J. F.; van Loosdrecht, M. C. M.; Heijnen, J. J., A metabolic model for the biological phosphorus removal process. *Water Sci Technol* **1995,** *31*, (2), 79-93.
5. Maurer, M.; Gujer, W.; Hany, R.; Bachmann, S., Intracellular carbon flow in phosphorus accumulating organisms from activated sludge systems. *Water Res* **1997,** *31*, (4), 907-917.
6. Bond, P. L.; Erhart, R.; Wagner, M.; Keller, J.; Blackall, L. L., Identification of Some of the Major Groups of Bacteria in Efficient and Nonefficient Biological Phosphorus Removal Activated Sludge Systems. *Appl Environ Microbiol* **1999,** *65*, (9), 4077-4084.
7. Werker, A.; Lind, P.; Bengtsson, S.; Nordström, F., Chlorinated-solvent-free gas chromatographic analysis of biomass containing polyhydroxyalkanoates. *Water Res* **2008,** *42*, (10–11), 2517-2526.
8. Danaei, G.; Finucane, M. M.; Lu, Y.; Singh, G. M.; Cowan, M. J.; Paciorek, C. J.; Lin, J. K.; Farzadfar, F.; Khang, Y.-H.; Stevens, G. A.; Rao, M.; Ali, M. K.; Riley, L. M.; Robinson, C. A.; Ezzati, M., National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *The Lancet* **2011,** *378*, (9785), 31-40.
9. Wang, J., Electrochemical Glucose Biosensors. *Chem Rev* **2007,** *108*, (2), 814-825.
10. Bengtsson, S., The utilization of glycogen accumulating organisms for mixed culture production of polyhydroxyalkanoates. *Biotechnol Bioeng* **2009,** *104*, (4), 698-708.
11. Beun, J. J.; Dircks, K.; Van Loosdrecht, M. C. M.; Heijnen, J. J., Poly-β-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. *Water Res* **2002,** *36*, (5), 1167-1180.

Highlights:

- Glucose from biomass can be accurately measured using a diabetic glucose metre.
- Standard curves demonstrate that digested glucose solution with 0.9 M HCl and boiling could be measured by a diabetic glucose meter without loss of accuracy
- Test on glucose extracted from biomass demonstrate the ability to accurately measure the account for stored glucose in microbial culture, by a diabetic glucose meter