


RESEARCH ARTICLE

Inline noninvasive Raman monitoring and feedback control of glucose concentration during ethanol fermentation

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Abstract

Raman spectroscopy as a process analytical technology tool was implemented for the monitoring and control of ethanol fermentation carried out with *Saccharomyces cerevisiae*. The need for the optimization of bioprocesses such as ethanol production, to increase product yield, enhanced the development of control strategies. The control system developed by the authors utilized noninvasive Raman measurements to avoid possible sterilization problems. Real-time data analysis was applied using partial least squares regression (PLS) method. With the aid of spectral pretreatment and multivariate data analysis, the monitoring of glucose and ethanol concentration was successful during yeast fermentation with the prediction error of 4.42 g/L for glucose and 2.40 g/L for ethanol. By Raman spectroscopy-based feedback control, the glucose concentration was maintained at 100 g/L by the automatic feeding of concentrated glucose solution. The control of glucose concentration during fed-batch fermentation resulted in increased ethanol production. Ethanol yield of 86% was achieved compared to the batch fermentation when 75% yield was obtained. The results show that the use of Raman spectroscopy for the monitoring and control of yeast fermentation is a promising way to enhance process understanding and achieve consistently high production yield.

KEYWORDS

bioprocess control, closed-loop feedback control, ethanol fermentation, industry 4.0, inline noninvasive Raman spectroscopy, PAT

1 | INTRODUCTION

The inevitable depletion of fossil fuel sources, the rapidly increasing worldwide energy consumption accompanied by environmental pollution and global warming are all driving forces for finding alternative sources of energy.^{1–4} During the last decade, numerous research and development have been focused on biomass-based energy production, as a potential

renewable and environmentally sustainable energy source.^{5–7} One of the most promising alternative biofuel is bioethanol, considered as the cleanest liquid fuel mostly used in the transportation sector.⁸ Bioethanol can be produced from feedstock with a wide range of high sugar (e.g., sucrose, fructose, or glucose) content through biotechnological process.^{9,10}

The most commonly employed microorganism for ethanol fermentation is *Saccharomyces cerevisiae*, due to its robust and highly

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efficient ethanol production capability and high tolerance to the end-product ethanol.^{11–14} Although bioethanol production has been greatly improved over the past decades, yeast fermentation technology still has challenges. The most important factor is focusing on the optimization of the technology to increase product yield and to ensure high-quality products. The use of process analytical technology (PAT) outlined by the U.S food and drug administration (FDA) which is about identifying, monitoring, and controlling critical process parameters, can increase efficiency, improve quality, and reduce costs.^{15,16}

Generally, the *S cerevisiae* ethanol production is characterized as a complex system, exhibiting nonlinear and dynamically changing processes, furthermore, it is affected by several critical process parameters such as temperature, pH, dissolved oxygen, cell density, the concentration of the substrate (sugar), or the end-product (ethanol).¹⁷ However, receiving information about these key cultivation parameters, conventionally manual sampling and traditional offline analysis are used. Unfortunately, sampling is always a critical point of the process due to the risk of contamination. Offline analysis is time-consuming, requires sample preparation, and specific analytical skills (e.g., HPLC). In addition, the feedback to the process is hard to perform, which makes difficult to meet the demand for automation and continuous process control.

Therefore, there is a growing need in the fermentation process to find suitable sensors to monitor and control critical process parameters in real-time. Over the last years, many techniques have been studied and developed for real-time bioprocess monitoring by using spectroscopic methods, such as UV-visible, mid- and near-infrared (MIR/NIR), fluorescence, and Raman spectroscopy.^{18–21} Among these spectroscopic technologies, Raman spectroscopy is a challenging, but a very promising technique for applications in bioprocess monitoring. Main advantages are weak interference of water, not destructive, simultaneous determination of various components and no sample pretreatment requirements. However, Raman measurements are disturbed by light-scattering on solid particles as well as bubbles, or cells in a turbid system. The removal of particulate matter by filtration in a by-pass allowed Shaw et al.²² the use of Raman spectroscopy for the online monitoring of glucose and ethanol concentration during a fermentation process. Wang et al.²³ used a filter tube around the inline probe to prevent the scattering on the particles in the turbid system during wine fermentation.

When using an immersion probe without filtration, cells and solid particles increase the noise in the spectra by not desired light scattering, which can be decreased with the use of an internal standard. Normalization of the spectra using the water stretching band at 1627 cm^{-1} ²⁴ or sulfate S—O stretching band at 980 cm^{-1} ²⁵ allowed the quantitative monitoring of ethanol production by *S cerevisiae* using simple data analysis methods (e.g., linear regression). Another method is to use multivariate data analysis such as partial least squares (PLS) regression for the determination of glucose and ethanol concentration in the presence of cells.^{26,27}

The development of specific probes made new possibilities in the monitoring of bioprocesses. The immersion probes coupled with optic cable allowed the monitoring of fermentation process inline.^{24,28}

Noninvasive glucose measurements in biological systems are generally of high interest. Demand has emerged for noninvasive monitoring of yeast fermentation to avoid possible sterilization problems and biofilm formation on the probe tip. A large-aperture Raman probe was developed by Shalk et al.²⁹ and the design of the probe enabled noninvasive inline measurements of glucose and ethanol through the bioreactor glass wall.

Besides the real-time monitoring of ethanol production, the growing demand for ethanol leads to the need for the development of advanced control strategies. The method developed by Ávila et al.,²⁷ the multivariate control chart analysis was efficient for detection of possible faults caused due to variation in the temperature, contamination, and modification in the substrate of the process. Raman spectroscopy was capable of providing information about the state of fermentation processes in real-time. However, there is no example found in the literature for the control of carbon source (sugar, for example, glucose) concentration, which is a critical parameter during yeast fermentation. High glucose concentrations may inhibit glucose utilization and decrease ethanol fermentation efficiency.³⁰ Nevertheless, with the control of glucose concentration, there is a possibility to minimize substrate inhibition and improve the production of ethanol.

In this study, the advantages of noninvasive inline Raman spectroscopic measurements were utilized to develop feedback control system of glucose concentration to improve ethanol production. For this purpose, a PLS calibration model was developed, which enabled real-time quantitative analysis of glucose and ethanol during *S cerevisiae* fermentation. However, to decrease the scattering effect of cells (not desired) and the intensity change caused by the noninvasive measurements, the pretreatment of the spectra were necessary using different normalization methods.

2 | EXPERIMENTAL

2.1 | Cultivation parameters

Commercial baker's yeast (Lesaffre, Hungary) of species *S cerevisiae* was used as the inoculum for the fermentation process. A fermentation volume of ~100 mL was used and 0.28 g compressed yeast in an active form was added to the reactor. The culture medium contained 0.4 g/L $(\text{NH}_4)_2\text{HPO}_4$, 2 and 1 g/L MgSO_4 . The initial glucose concentration was 160 g/L. Using a control system described in the next section, the process conditions were set to 30°C and 150 rpm. The pH was kept constant at 4.0 through the automatic addition of 1 M NaOH. The feed solution contained glucose at high concentration (300 g/L) and the same salt concentrations as the culture medium.

2.2 | Bioreactor and control system

Experiments were performed in a computer-controlled laboratory reactor system (see Figure 1) developed earlier.³¹ The control of temperature, stirrer, and the dosing system of the 150 mL jacketed glass reactor were performed by a Stardom FCN-type programmable logic controller (PLC) manufactured by Yokogawa Electric Corporation

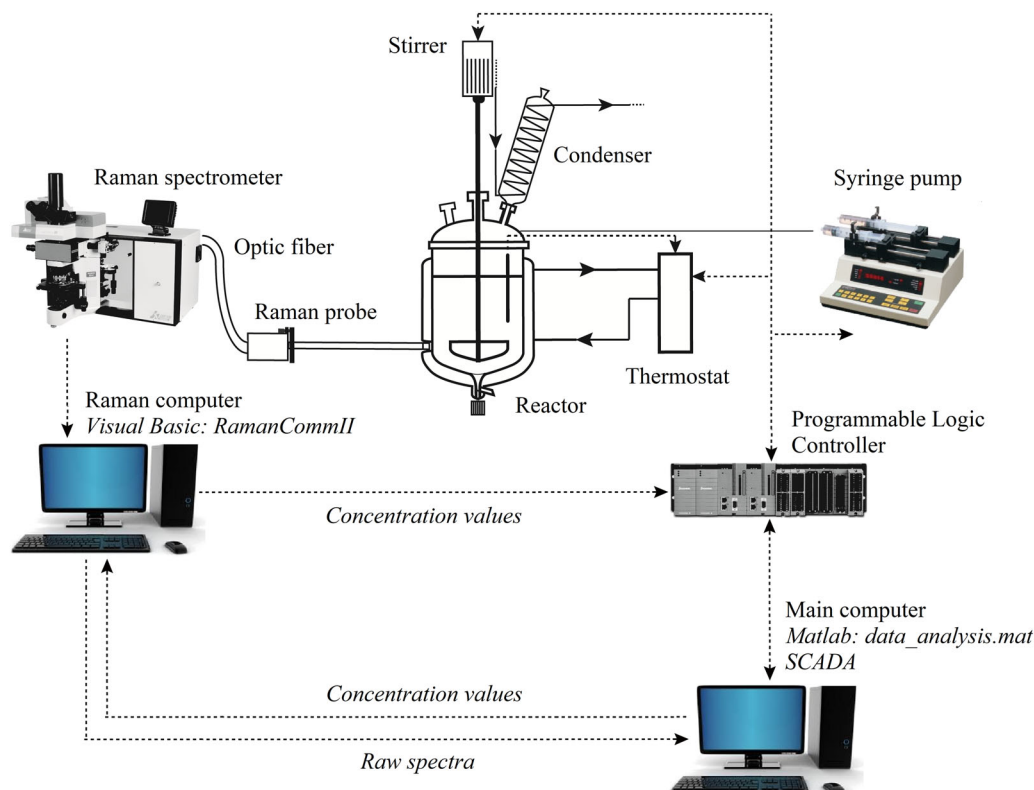


FIGURE 1 Hardware, software, and communication network of the control system based on real-time Raman spectrometry

(Tokyo, Japan). The temperature of the reactor was controlled by a PID controller manipulating the temperature of the circulating oil in the jacket of the reactor. To maintain the homogeneity of the reactor an Eurostar power control-visc type stirrer (IKA-Werke GmbH & Co. KG, Staufen, Germany) was used. The dosing system contained a Harvard 33 type syringe pump (Instech Laboratories, Inc., Plymouth Meeting, PA), furthermore, the pH was monitored by a combined glass electrode (DO 9403 T-R1 made by Delta Ohm S.r.L., Padua, Italy) and controlled using 1 M NaOH solution.

The reactor, containing an inspection window inserted in the jacket of the reactor, is suitable for inline noninvasive Raman measurements. Spectra were collected during the fermentation through the reactor wall by a Labram type Raman spectrometer manufactured

by Horiba Jobin Yvon (France) connected with optic fiber and a long focus Raman probe. The focal point of the Raman probe was adjusted manually immediately behind the inner side of the reactor wall, minimizing the influence of scattering due to the solid particles (cells). Real-time Raman spectra were acquired between $1,438\text{--}162\text{ cm}^{-1}$. A 300 mW diode laser (785 nm) was used for excitation and the scattered light was detected by an air-cooled CCD. Exposure time was 60 s per spectrum with four accumulations. A computer program (*RamanCommII*), developed by the authors³¹ written in Visual Basic with a two-way communication function, was used to trigger the Raman measurements and exchange data between the PLC and the MATLAB evaluation software using a TCP/IP based connection (see Figure 1). The PLC controlled the concentration in the reactor by

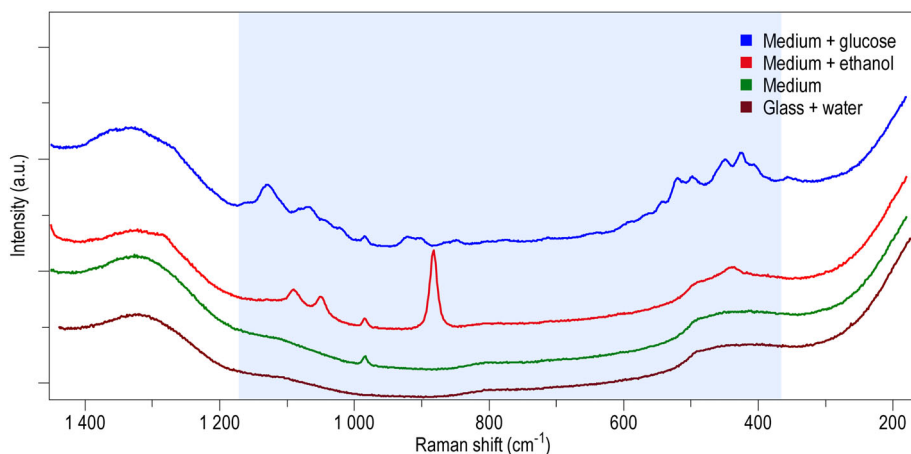


FIGURE 2 Reference Raman spectra of water, medium (containing only salts), medium with 160 g/L glucose and medium with 81.6 g/L ethanol acquired from the reactor (through the glass). The spectra are shown without any preprocessing

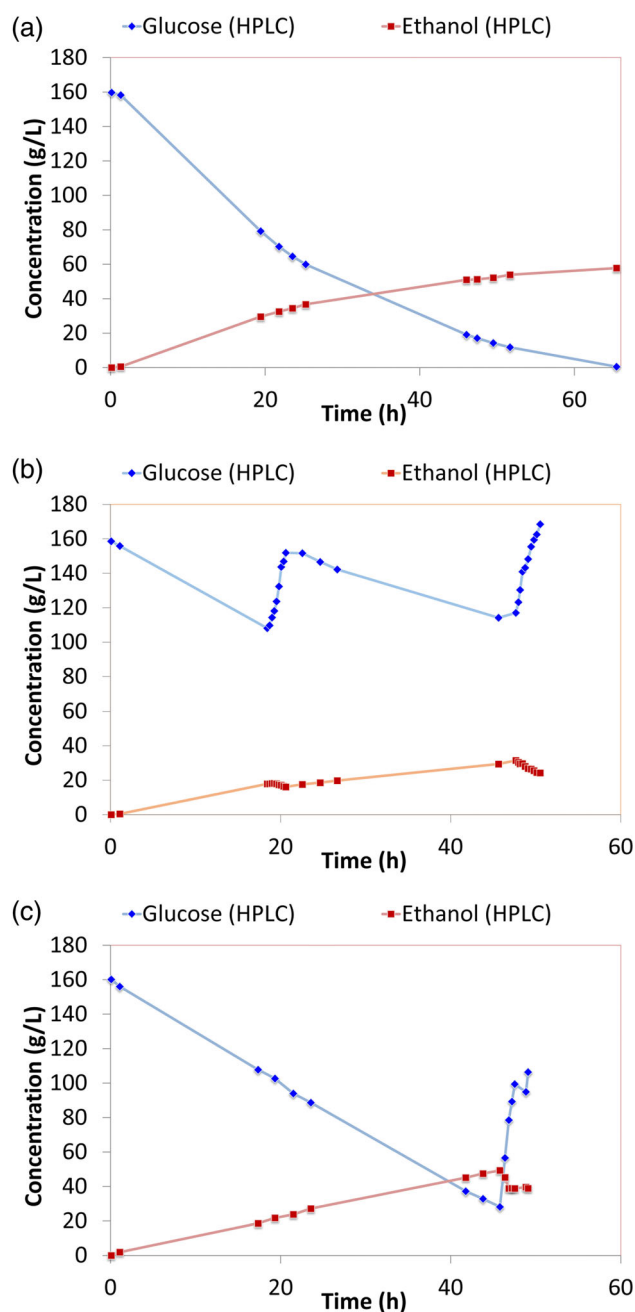


FIGURE 3 Concentration profiles of one batch (a) (data for other batch fermentation is not shown) and two fed-batch experiments (b, c). The feed solution was added to the reactor in small volumes (5–10 mL) and samples were taken after each feeding. The designed setting of glucose concentration was performed to increase the variability of the reference points to obtain a robust calibration model for Raman measurements

using the results of real-time Raman data processing (*data_analysis.mat*) based on the PLS regression model. The in-house MATLAB program developed by the authors³¹ was used for real-time spectrum evaluation resulting in ethanol and glucose concentration values simultaneously during yeast fermentation.

The feedback control of glucose concentration was achieved with PID control using only proportional gain. The proportional gain was tuned

manually and there was no need to adapt the gain over the course of the fermentation. When the concentration of glucose decreased below the setpoint (100 g/L), the 300 g/L glucose feed solution was dosed into the reactor using a syringe pump. Yokogawa Fast/Tools supervisory control and data acquisition (SCADA) software package were applied to display, store and export the data of the process.

2.3 | Raman analysis

PLS regression method was used during the real-time analysis of Raman spectra. The calibration dataset was prepared using MATLAB PLS Toolbox. Calibration points were obtained from five batch and two fed-batch experiments. The fed-batch experiments were performed to extend the applicability of the calibration data set for the control of glucose concentration. Samples were taken from the reactor for offline HPLC measurements resulting in 100 reference data points. The acquired spectra contained 1,000 variables in the range $1,438\text{--}162\text{ cm}^{-1}$. To reduce the effect of the noninvasive measurements (signals of the glass wall of the reactor, Figure 2) on data analysis 622 variables between $1,173\text{--}373\text{ cm}^{-1}$ were included in the PLS model. The spectra were preprocessed using baseline correction (Automatic Whittaker Filter, $\lambda = 10,000$, $p = 0.005$), standard normal variate (SNV) transformation, normalization (area = 1, range: $992\text{--}972\text{ cm}^{-1}$) and mean centering. To correct the intensity change of the spectra, there was a need for the use of an internal standard. For this purpose, the S–O stretching peak at 980 cm^{-1} was used for the normalization of the spectra.

2.4 | Reference method

Offline high-performance liquid chromatography (HPLC) measurements provided reference data for the validation of real-time Raman analysis. Throughout the fermentation process, samples of 2 mL were taken and centrifuged at 4500 rpm (Microcentrifuge 320a, Mechanika Precyzyjna, Poland) for 10 min at room temperature. The supernatant was filtered through a $0.45\text{ }\mu\text{m}$ syringe filter (Millipore® Millex® LCR HPLC Syringe Filters, Merck KGaA, Germany) and stored immediately at -18°C . The ethanol and glucose concentrations in the samples were determined using HPLC equipped with Shimadzu RID-10A differential refractive index detector and BIO-RAD (Bio-Rad, Hercules, CA) Aminex HPX-87H ($300 \times 7.8\text{ mm}$) cation-exchange column. The column temperature was 65°C . Sulfuric acid (5 mM) was used as eluent with 0.5 mL/min flow rate. The thawed supernatants of the centrifuged and filtered fermentation samples were diluted to 1:20 with the distilled water before injection. The sample volume was $40\text{ }\mu\text{L}$.

3 | RESULTS AND DISCUSSION

3.1 | Noninvasive Raman analysis and spectral pretreatment

The feasibility of noninvasive Raman measurements was evaluated using the reference spectra of water, medium, medium containing 160 g/L glucose, and medium containing 81.6 g/L ethanol, which

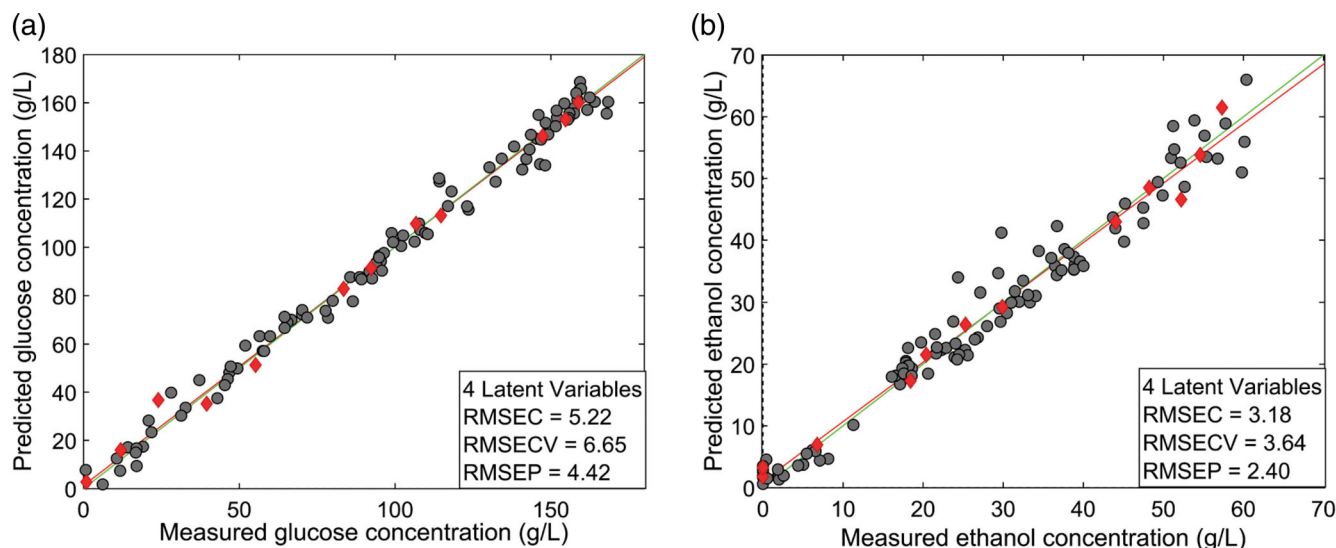


FIGURE 4 Correlation between predicted and measured data during PLS regression for glucose (a) and ethanol (b) concentration. Calibration data set is indicated with grey circles and the test data set is indicated with red diamonds. Test data are acquired from monitoring of an independent fermentation process

corresponds to 100% relative yield, presumed 0.51 g ethanol is produced from 1 g glucose. The characteristic peaks of glucose and ethanol can be distinguished using an acquisition time of 60 s and four accumulations. The water and the reactor glass had large interference on the spectra shown in Figure 2. Thus, the wavenumber region below 373 cm^{-1} and above $1,173\text{ cm}^{-1}$ were removed before data analysis. The excluded part of the spectra contains no relevant information regarding ethanol and glucose concentration.

The intensity of Raman spectra was sensitive to the position of the probe (distance and the detection angle in relation to the inspection window), thus the Raman signal intensity originating from the reactor glass changed between each fermentation. Therefore, a spectral treatment was necessary to correct the intensity change. After baseline correction, SNV was performed, which is a path-length variation correction method like normalization, to limit the spectral intensity variation. Besides the reactor glass the light scattering on the cells and particles caused intensity change during the fermentation, thus normalization using an internal standard was chosen. Internal standard of the S—O stretching mode at 980 cm^{-1} was selected for the correction of intensity change from light scattering. Sulfate is always added to growth media for the synthesis of sulfur proteins. However, the amount of sulfate utilized by the cells is negligible compared to the initial concentration (5 g/L). Baseline correction, SNV, and normalization were followed by mean centering to complete the pretreatment of spectra.

3.2 | Calibration of the PLS model

During the development of the PLS model for quantification of glucose and ethanol, noninvasive inline monitoring of five batch and two fed-batch fermentation were performed. An example of glucose and ethanol concentration during batch fermentation is shown in Figure 3a. The fed-batch experiments were used to increase the

variability (glucose concentration and matrix) of the reference points, which ensured good predicting capabilities without built-in correlations. The feeding of the first reactor (Figure 3b) was performed at 18 and 48 hr maintaining the glucose concentration between 108 and 168 g/L. To increase the number of the reference data under 100 g/L glucose concentration, the second reactor (Figure 3c) was fed only once at 46 hr. The feeding was accomplished with the dosing of ~50 mL feed solution into the reactor in several installments. Samples were taken from the reactor after each section for offline HPLC analysis. A total of 100 data points were used in model development from the batch (59 data points) and fed-batch experiments (41 data points).

In the model development, the ideal number of latent variables (LV) was selected considering the values of root-mean-square error of cross-validation (RMSECV) obtained by cross-validation of the calibration data. Venetian blinds cross-validation was applied with eight splits and four LVs were chosen based on the results. The measured and predicted data shown in Figure 4, correlated highly for glucose and ethanol concentration. The root-mean-square error of calibration (RMSEC) (glucose: 5.21 g/L; ethanol: 3.18 g/L) and the RMSECV (glucose: 6.65 g/L; ethanol: 3.64 g/L) were also calculated.

3.3 | Monitoring of glucose and ethanol concentration

The PLS model was proved to be well applicable to the inline monitoring of yeast fermentation. For the real-time analysis of the spectra, a MATLAB program (*data_analysis.mat*) was developed. The concentrations of glucose and ethanol calculated from the Raman spectra overlapped with the HPLC reference values shown in Figure 5. The model's predictive performance was quantified using root-mean-square error of prediction (RMSEP), which proved to be 4.42 g/L for glucose and 2.40 g/L for ethanol. After 60 hr, the glucose depleted in

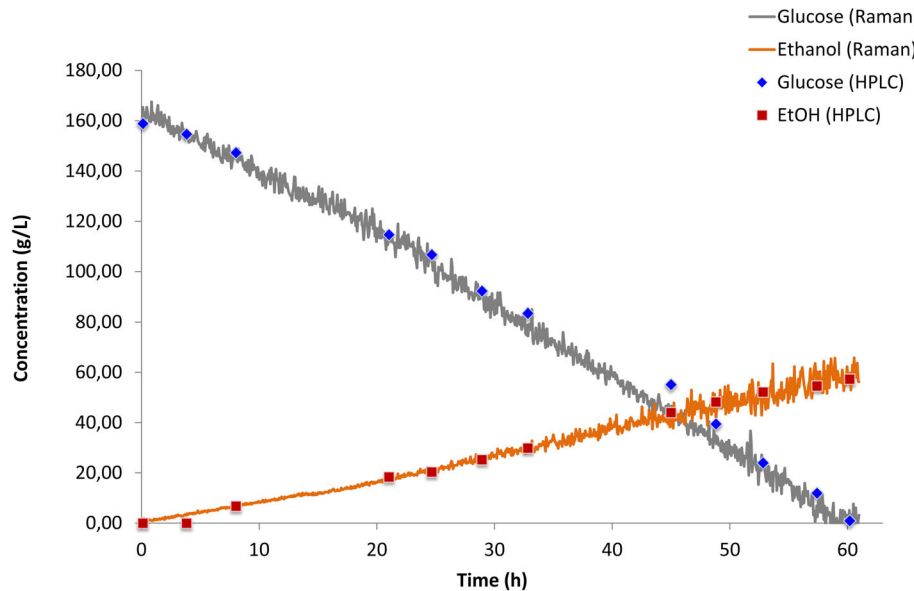


FIGURE 5 Monitoring of yeast fermentation using noninvasive inline Raman analysis. Glucose and ethanol concentrations were calculated from Raman spectra in real-time and reference data were obtained from HPLC measurements. HPLC, high-performance liquid chromatography

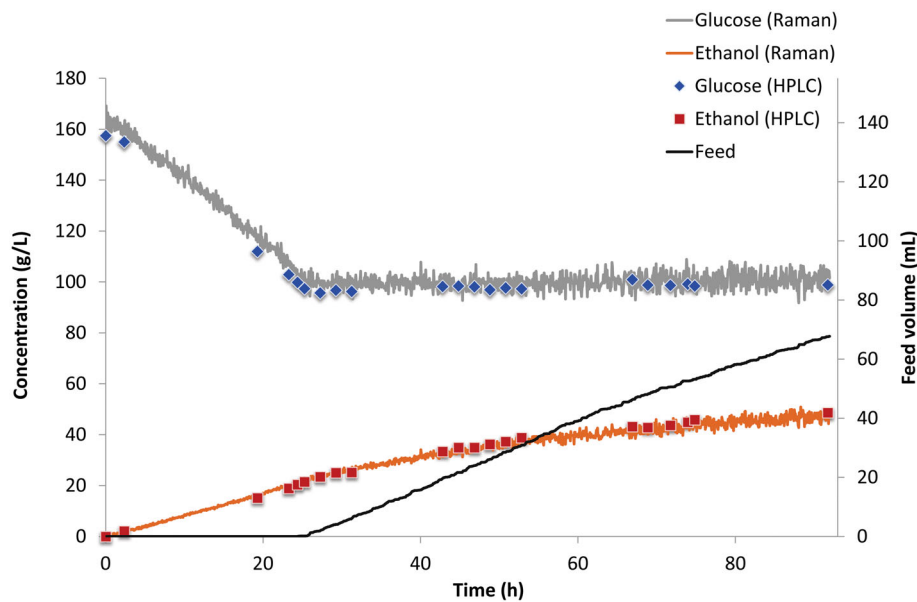


FIGURE 6 Feedback control of glucose concentration based on real-time Raman analysis. Glucose and ethanol concentrations were calculated from Raman spectra in real-time and reference data were obtained from HPLC measurements. Dosing of the feed solution started at 24.4 hr to maintain the glucose concentration at 100 g/L. HPLC, high-performance liquid chromatography

the cell culture indicating the end of the fermentation and the ethanol concentration was 57.33 g/L.

3.4 | Control of glucose concentration

The aim of the closed-loop feedback control of yeast fermentation was to maintain the glucose concentration at 100 g/L (set point) by automatic feeding a solution containing 300 g/L glucose. For the control of glucose concentration based on Raman analysis, a control system (described before, see Figure 1) with proportional setting was applied. The concentration of glucose calculated from the Raman spectra was the input of the controller, meanwhile, the output of the controller was the feeding rate of the syringe pump. If the glucose concentration decreased below the set point (100 g/L), the PLC directed the syringe pump to increase the dose with a calculated

speed. The actual volume and feeding rate were calculated by the PLC using initial parameters (medium volume, concentration of feed solution, syringe diameter) and control parameters (proportional setting).

The glucose and ethanol concentration calculated from the Raman spectra followed the values of offline measurements accurately within the whole feeding period. The Raman-based glucose concentration shows greater deviation after 60 hr, because of the (not desired) light scattering on the cells and solid particles. Nonetheless, the control of the glucose concentration was accurate and the glucose concentration was controlled successfully in the range of 95.69–100.95 g/L with an average of 98.08 g/L for 67.6 hr (from 24.4 until 92 hr), which shows the feasibility of Raman-based glucose control during yeast fermentation (Figure 6). Lower ethanol concentration was achieved during the glucose control (40.10 g/L), due to the dilution effect of the feeding.

TABLE 1 Ethanol yield of the batch fermentation and using a glucose control system

	Volume (mL)	Glucose concentration (g/L)	Consumed glucose (g)	Ethanol concentration (g/L)	Produced ethanol (g)	Theoretical yield (g)	Relative yield (%)
Batch	~100	0.98	14.90	57.33	5.73	7.60	75%
Controlled fed-batch	~139	100.40	12.74	40.10	5.57	6.50	86%

Note: Initially, 15 g glucose was present in the reactor and 39 mL 300 g/L glucose solution was used for glucose control. All data were given and the yield was calculated at 60 hr. The volume of the samples was neglected.

There was no significant ethanol production after 60 hr due to the inhibition effect of ethanol on yeast cell culture, thus the ethanol yield was calculated at this time (Table 1). The control of glucose concentration and the continuous feeding of the concentrated feed solution resulted in 86% ethanol yield, which is higher compared to the batch fermentation (75%). With the use of Raman spectroscopy, there is a possibility for better process optimization to increase ethanol production during yeast fermentation.

4 | CONCLUSION

This work demonstrates the use of noninvasive Raman spectroscopy as a useful tool for the inline monitoring and control of ethanol fermentation using *S. cerevisiae*. Raman measurement using a noninvasive probe was used to decrease the risk of contamination, furthermore, there was no need to autoclave or disinfect the probe.

Reference data was obtained from five batch and two fed-batch fermentations. The designed setting of glucose concentration during fed-batch experiments resulted in a good calibration data set for acquiring a robust model. The pretreatment of the spectra before analysis proved to be essential in case of noninvasive measurements. The normalization using standard normal variate and an internal standard decreased the effect of intensity changes due to noninvasive measurements (through inspection window) and light scattering on cells. PLS regression was successfully applied for the monitoring of glucose and ethanol concentration. For the validation of the PLS model, batch fermentation was performed with prediction error of 4.42 g/L for glucose and 2.40 g/L for ethanol. The real-time determination of glucose concentration with good accuracy made it possible to implement a control strategy to improve production yield.

Thus, Raman spectroscopy-based closed-loop feedback control of glucose concentration was accomplished and the control of glucose concentration at 100 g/L was successful dosing a 300 g/L feed solution into the reactor with a control system developed by the authors. The glucose concentration was maintained in the range of 95.69–100.95 g/L for 67.6 hr. The control of glucose concentration at 100 g/L resulted in higher ethanol yield (86%) compared to batch fermentation (75%). The implementation of Raman spectroscopy for the monitoring and control of ethanol fermentation could improve ethanol production by understanding the process and maintaining optimal parameters. The Raman-based control of the glucose concentration can improve the ethanol production processes by enhancing glucose utilization and increasing ethanol fermentation efficiency.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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REFERENCES

- Greene DL, Hopson JL, Li J. Running out of and into oil: analyzing global oil depletion to 2050. *Transp Res Rec J Transp Res Board*. 2004; 1880:1-9.
- Nigam PS, Singh A. Production of liquid biofuels from renewable resources. *Prog Energy Combust Sci*. 2011;37(1):52-68. <https://doi.org/10.1016/j.pecs.2010.01.003>.
- Hoekman KS. Biofuels in the U.S. - challenges and opportunities. *Renew Energy*. 2009;34:14-22. <https://doi.org/10.1016/j.renene.2008.04.030>.
- Kiran B, Kumar R, Deshmukh D. Perspectives of microalgal biofuels as a renewable source of energy. *Energy Convers Manag*. 2014;88:1228-1244. <https://doi.org/10.1016/j.enconman.2014.06.022>.
- Demirbas A. Biofuels securing the planet's future energy needs. *Energy Convers Manag*. 2009;50(9):2239-2249. <https://doi.org/10.1016/j.enconman.2009.05.010>.
- John RP, Anisha GS, Nampoothiri MK, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresour Technol*. 2011; 102(1):186-193. <https://doi.org/10.1016/j.biortech.2010.06.139>.
- Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. Biofuels from Microalgae. *Biotechnol Prog*. 2008;24(4):815-820. <https://doi.org/10.1021/bp.070371k>.
- Sarris D, Papanikolaou S. Biotechnological production of ethanol: biochemistry, processes and technologies. *Eng Life Sci*. 2016;16(4):307-329. <https://doi.org/10.1002/elsc.201400199>.

9. Jayus N, Mayzuroh A, Arindhani S, Caroenchai C. Studies on bioethanol production of commercial Baker's and alcohol yeast under aerated culture using sugarcane molasses as the media. *Agric Agric Sci Procedia*. 2016;9:493-499. <https://doi.org/10.1016/j.aaspro.2016.02.168>.
10. Sánchez ÓJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Technol*. 2008;99:5270-5295. <https://doi.org/10.1016/j.biortech.2007.11.013>.
11. Lin Y, Tanaka S. Ethanol fermentation from biomass resources: current state and prospects. *Appl Microbiol Biotechnol*. 2006;69(6):627-642. <https://doi.org/10.1007/s00253-005-0229-x>.
12. Radecka D, Mukherjee V, Mateo RQ, Stojiljkovic M, Foulquié-Moreno MR, Thevelein JM. Looking beyond *Saccharomyces*: the potential of non-conventional yeast species for desirable traits in bioethanol fermentation. *FEMS Yeast Res*. 2015;15(6):1-13. <https://doi.org/10.1093/femsyr/fov053>.
13. Almeida JRM, Modig T, Petersson A, Hähn-Hägerdal B, Lidén G, Gorwa-Grauslund MF. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. *J Chem Technol Biotechnol*. 2007;82:340-349. <https://doi.org/10.1002/jctb>.
14. Li H, Wu M, Xu L, et al. Evaluation of industrial *Saccharomyces cerevisiae* strains as the chassis cell for second-generation bioethanol production. *Microb Biotechnol*. 2015;8(2):266-274. <https://doi.org/10.1111/1751-7915.12245>.
15. FDA. Guidance for industry pat — a framework for innovative pharmaceutical development, manufacturing, and quality assurance; 2004. doi:<http://www.fda.gov/CDER/guidance/6419fnl.pdf>
16. Käsäkoski M, Kurkinen M, Von Weymarn N, et al. Process analytical technology (PAT) needs and applications in the bioprocess industry; 2006.
17. Beutel S, Henkel S. In situ sensor techniques in modern bioprocess monitoring. *Appl Microbiol Biotechnol*. 2011;91(6):1493-1505. <https://doi.org/10.1007/s00253-011-3470-5>.
18. Claßen J, Aupert F, Reardon KF, Solle D, Scheper T. Spectroscopic sensors for in-line bioprocess monitoring in research and pharmaceutical industrial application. *Anal Bioanal Chem*. 2017;409(3):651-666. <https://doi.org/10.1007/s00216-016-0068-x>.
19. Cozzolino D, Parker M, Damberg RG, Herderich M, Gishen M. Chemometrics and visible-near infrared spectroscopic monitoring of red wine fermentation in a pilot scale. *Biotechnol Bioeng*. 2006;95(6):1102-1107. <https://doi.org/10.1002/bit>.
20. Skibsted E, Lindemann C, Roca C, Olsson L. On-line bioprocess monitoring with a multi-wavelength fluorescence sensor using multivariate calibration. *J Biotechnol*. 2001;88(1):47-57. [https://doi.org/10.1016/S0168-1656\(01\)00257-7](https://doi.org/10.1016/S0168-1656(01)00257-7).
21. Veale EL, Irudayaraj J, Demirci A. An on-line approach to monitor ethanol fermentation using FTIR spectroscopy. *Biotechnol Prog*. 2007;23(2):494-500. <https://doi.org/10.1021/bp060306v>.
22. Shaw AD, Kaderbhai N, Jones A, et al. Noninvasive, on-line monitoring of the biotransformation by yeast of glucose to ethanol using dispersive Raman spectroscopy and Chemometrics. *Appl Spectrosc*. 1999;53(11):1419-1428.
23. Wang Q, Li Z, Ma Z, Liang L. Real time monitoring of multiple components in wine fermentation using an on-line auto-calibration Raman spectroscopy. *Sensors Actuators B*. 2014;202:426-432. <https://doi.org/10.1016/j.snb.2014.05.109>.
24. Iversen JA, Berg RW, Ahring BK. Quantitative monitoring of yeast fermentation using Raman spectroscopy. *Anal Bioanal Chem*. 2014;406(20):4911-4919. <https://doi.org/10.1007/s00216-014-7897-2>.
25. Picard A, Daniel I, Montagnac G, Oger P. In situ monitoring by quantitative Raman spectroscopy of alcoholic fermentation by *Saccharomyces cerevisiae* under high pressure. *Extremophiles*. 2007;11(3):445-452. <https://doi.org/10.1007/s00792-006-0054-x>.
26. Sivakesava S, Irudayaraj J, Demirci A. Monitoring a bioprocess for ethanol production using FT-MIR and FT-Raman spectroscopy. *J Ind Microbiol Biotechnol*. 2001;26(4):185-190. <https://doi.org/10.1038/sj.jim.7000124>.
27. Ávila TC, Poppi RJ, Lunardi I, Tizei PAG, Pereira GAG. Raman spectroscopy and chemometrics for on-line control of glucose fermentation by *Saccharomyces cerevisiae*. *Biotechnol Prog*. 2012;28(6):1598-1604. <https://doi.org/10.1002/btpr.1615>.
28. Gray SR, Peretti SW, Lamb HH. Real-time monitoring of high-gravity corn mash fermentation using in situ raman spectroscopy. *Biotechnol Bioeng*. 2013;110(6):1654-1662. <https://doi.org/10.1002/bit.24849>.
29. Schalk R, Braun F, Frank R, et al. Non-contact Raman spectroscopy for in-line monitoring of glucose and ethanol during yeast fermentations. *Bioprocess Biosyst Eng*. 2017;40(10):1519-1527. <https://doi.org/10.1007/s00449-017-1808-9>.
30. Chang Y-H, Chang K-S, Chen C-Y, Hsu C-L, Chang T-C, Jang H-D. Enhancement of the efficiency of bioethanol production by *Saccharomyces cerevisiae* via gradually batch-wise and fed-batch increasing the glucose concentration. *Fermentation*. 2018;4(2):45. <https://doi.org/10.3390/fermentation4020045>.
31. Hirsch E, Pataki H, Farkas A, et al. Raman-based feedback control of the enzymatic hydrolysis of lactose. *Org Process Res Dev*. 2016;20(10):1721-1727. <https://doi.org/10.1021/acs.oprd.6b00212>.

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