The theoretical framework of agent based monitoring for use in the production of gellan gum in a microbial fermentation system

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ABSTRACT

This paper introduces the application of an agent-based software system for monitoring the process of gellan gum production. Gellan gum (biopolymer) is produced in industrial scale in bioreactors (sealed vessels) where the microbial culture is grown in a liquid fermentation medium under controlled environmental conditions (temperature, pH, aeration and agitation). The multi agent system will view the monitoring problem as the interaction of simple independent software entities, for effective use of the available data. The outcome of this agent - based solution will include the automatic on-line data acquisition and correlation of the most important parameters. Within such a dynamic process, like the gellan gum production, certan parameters (such as biomass, gellan and glucose concentration) change continuously and have to be measured and controlled. Also automatic knowledge derivation from past cases through the multi agent software system can be of future benefit.

Keywords: agent-based monitoring, polysaccharides production

1. INTRODUCTION

Biopolymers and particularly microbial polysaccharides are polymerised macromolecules produced during the natural metabolism of bacteria, yeast, or fungi. They are commercially important, multifunctional products with several uses in the food, pharmaceutical and other industries. The demand for new (food) products with improved physicochemical characteristics has necessitated the addition of polysaccharides in order to achieve the desirable texture, gel strength, elasticity and physical stability. In addition, the need to replace traditional plant polysaccharides such as pectins, guar gum or carageenan (which are characterised by variable quality and availability, as well as high production cost) with microbial ones (which are economical and their production and quality can be easily controlled) has led to increased industrial production of several biopolymers of microbial origin, such as xanthan gum, pullulan, gellan gum, scleroglucan, alginate and others [1, 2, 3].

Gellan gum, in particular, is a versatile polysaccharide produced by the bacterium *Sphingomonas paucimobilis*, formerly called *Pseudomonas elodea*, in industrial scale in bioreactors. During this fermentation there have been identified the most important parameters: biomass (mass of microbial culture), gellan and glucose concentration, which are commonly estimated by laborious, costly and timeconsuming analytical (chemical, physical and enzymatic) methods, which are carried out on the laboratory bench after a sample is taken from the bioreactor.

The data acquisition and analysis has brought about the development of several techniques for on-line, real time monitoring and control of bioprocesses, which usually involve an equipment performing some biochemical, physicochemical and/or spectroscopic (involving emission and absorbance of light) analysis on-line [4, 5, 6, 7, 8, 9]. When referring to "on-line" methods we mean that they are performed continuously, parallel to the process, without any samples being withdrawn from the bioreactor vessel for analysis.

Despite ongoing research into the on-line control of fermentations, there is little investigation so far into the use of Agent based monitoring of such bioprocesses. However, the limited current research into the application of different Artificial Intelligence (AI) [10, 11] and computational techniques [12, 13] for bioprocess monitoring has shown

encouraging results. Agent based solutions for microbial fermentations could utilise on-line numerical data automatically acquired and displayed by the bioreactor throughout the process, in order to correlate the data to unknown parameters that need to be estimated, such as biomass, gellan and glucose concentration. This paper presents the theoretical framework of a multi-agent system, which will enable on-line data monitoring from the bioreactor and displaying useful information to the user, based on the three layered approach of the already developed condition monitoring multi-agent system (COMMAS) [14].

2. PROBLEM DOMAIN

Gellan is currently being manufactured under patent (by Kelco Ltd.) in two different forms: Kelcogel and Gelrite. The former is used as a thickener and gelling agent mainly in food products (desserts and confectionery products, yoghurt and ice cream, restructured meat, jams) [15], and the latter is used as a solidifying agent, replacing agar in solid media for microbial growth [16], as well as for plant tissue cultivation [17]. Gellan can also be used in pharmaceutical products to control drug release, bioavailability and absorption [18], as well as in dental and personal care products [19].

Like other biopolymers, gellan gum is produced in industrial scale in bioreactors (sealed vessels) where the microbial culture is grown in a liquid fermentation medium under controlled environmental conditions (of temperature, pH, aeration and agitation) (Fig. 1). The fermentation medium consists of water, some type carbon source (e.g. glucose, corn syrup, starch hydrolysates), and several minerals and salts [20]. Like every bioprocess, this fermentation is dynamic, which means that process parameters change continuously and have to be measured and controlled, during the 40-60 hours that this process can last. Three of the most important process parameters are the biomass (mass of microbial culture), gellan and glucose concentration. A typical simplified profile of the concentration of these parameters during the gellan process is shown in Fig. 2. These parameters are commonly estimated by laborious, costly and time-consuming analytical (chemical, physical and enzymatic) methods, which are carried out on the laboratory bench after a sample is taken from the bioreactor. However, in an industrial environment more rapid, simpler, economical and automated ways of analysis and data acquisition are required.

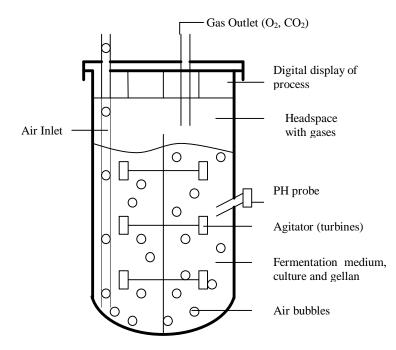


Figure 1: Schematical representation of the fermentor used for gellan production.

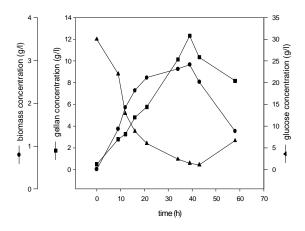


Figure 2: A general representation of the profiles of biomass, gellan and glucose concentration during the gellan process. (Based on data from [21]).

3. AGENT-BASED SOLUTION

"An intelligent agent is an encapsulated computer system that is situated in some environment, and that is capable of flexible and autonomous action in that environment in order to meet its design objectives" Wooldridge, 1995

Within this paper the term 'agent' corresponds to the software problem-solving entities, which are situated in a particular environment, with specified functions, in order to process the inputs received related to the problem domain. The dynamic multi-agent software system will employ communication skills, with decision-making functions for data interpretation in monitoring the production of gellan gum. Currently, the important parameters are estimated by analytical methods (chemical, physical, enzymatic), carried out after samples taken from the bioreactor, as shown in Fig. 3. However the information environment is extremely complex. Describing the level of biomass, gellan and glucose and how these can be better interpreted to derive meaningful conclusions, are very complex tasks but very important. In order to automate this process and assist online the operator, a theoretical framework is being proposed as shown in Fig. 4. Each software agent is a different specialist, able to accomplish certain tasks. This work, will illustrate how certain parameters can be monitored (i.e. dissolved oxygen, acid, alkali, carbon evolution rate etc.) from a multi-agent software system, utilising computational techniques and decision-making functions for data analysis and interpretation to extract useful information from received data.

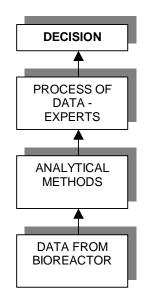


Figure 3: Current State

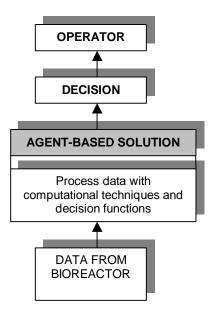


Figure 4. Proposed Solution

The agents have the ability to control their internal state and their behaviour, to exhibit flexible problem-solving techniques in pursuit of their design objectives. The intelligent agent software system must be designed to fulfil the specific purpose of analysing the data received from the bioreactor.

In a more general way as in [22], the term 'agent' can be used, as a software-based computer system, which has the properties:

- *Autonomy:* an agent is a computer system, which is situated in some environment, and it is able to act without the intervention of humans (or other agents), and should have control over its own actions and internal state and have some kind of control over their actions and internal state.
- *Social ability*: Agents can interact with other agents (or humans) via agent communication language (ACL) as described from Finin [23].
- *Reactivity*: agents perceive their environment and respond in a timely fashion to changes that occur in it.
- *Pro-activeness*: Agents do not simply act in their environment, but they can also take initiative. The application domain of applying agent technology is crucial, as we always have to balance between risk and trust when working with software-based systems.

During the gellan process, the concentration of biomass (culture) and especially gellan are two of the most important factors that need to be estimated periodically (every 3-5 hours) so that the operator can follow the evolution of the process and know at which point to interfere. For example, the knowledge of gellan concentration is necessary for deciding when to stop the fermentation and harvest the product (when gellan concentration is maximum). Also for a correct and successful fermentation a certain amount of initial biomass (inoculum) needs to be contained in the bioreactor at the start of the process, and this has to be estimated as well. The measurement of glucose concentration is also significant. In batch processes (when glucose is only added once in the fermentation medium at the start of the process), the process is complete when or soon after glucose is fully utilised (zero concentration). In fed-batch fermentations additional glucose is fed into the process medium during the fermentation, in order to increase glucose concentration and allow more product (gellan) to be synthesised by the culture. This extra glucose is fed into the bioreactor either when glucose becomes depleted (zero concentration), or when it is reduced to a certain level, or when biomass has increased to a certain point. Therefore it is crucial to detect glucose and biomass concentration during this process. The diagram below (Fig.5) shows schematically the main steps for a batch gellan production process and highlight the points where automated, on-line detection and control would be useful.

As mentioned earlier, during the bioprocess for gellan production, numerical data of some process parameters is continuously measured and displayed by the bioreactor. Some of this data could be used to be correlated to unknown values of biomass, gellan and glucose concentration, using an agent-based model. Data that could be used is the following:

- a. Dissolved Oxygen (DO) inside the fermentor, expressed as percentage (%) of air saturation with oxygen.
- b. Oxygen Utilization Rate (OUR), expressed as the amount (in moles) of oxygen (O_2) consumed by the culture, per volume (litres, l) of bioreactor, per hour (moles/litres/hour).
- c. Carbon Evolution Rate (CER), expressed as the amount of carbon dioxide (CO_2) produced by the culture, per volume of bioreactor, per hour (moles/litres/hour).
- d. Alkali (base) consumed, expressed as volume (millilitres, ml) consumed during the process.
- e. Acid consumed, expressed as volume (ml) consumed during the process. (Both base and acid solutions are used in the process for gellan

production, in order to control the pH (acidity) of the fermentation medium to the desirable level. These solutions are pumped automatically into the bioreactor accordingly: when pH is higher than the set value acid is pumped in, when it is lower then some base is consumed.)

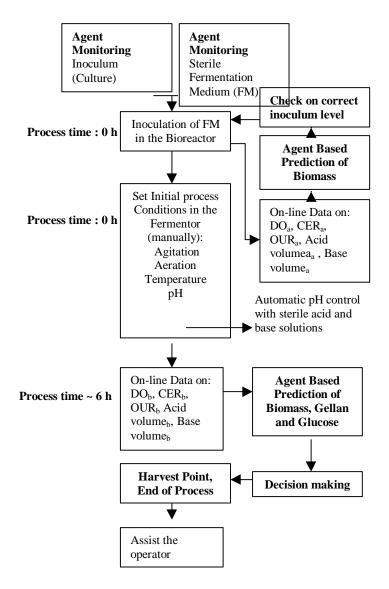


Figure 5: Batch Fermentation: Schematic representation of the main steps for the gellan production process and the process points where on-line monitoring and control could be used.

4. DISCUSSION

The proposed software system overcomes today's problems, as it supports the use of more than one computational intelligence technique through agents' technology. The key function is the application area of interpretation of the available data. Considering a software system responsible for data interpretation, there can be distinguished a number of interesting characteristics:

- The process is distributed. There can be distinguished a number of autonomous processes (data gathering from different sensors for dissolved oxygen, alkali etc.), which interact with each other to end up with a meaningful conclusion.
- The distributed problem domain denotes the development of an hierarchical layered software system, which results in the construction of a structured society of agents, with different groups, each one specialised and with different functionality. Based on COMMAS architecture [24], attribute reasoning agents (ARA) will process the data of specific parameters, corroboration agents (CSCA) will evaluate and corroborate their results and meta-knowledge reasoning agents (MKRA) will apply decision making functions to assist the operator.
- The software system as a whole has to be easily maintained, as new agents might be included, if new parameters are considered as important to be monitored.
- Finally, the requirements of such an automatic system for data interpretation in such a problem domain are high, as it deals within a dynamic environment, where any change is possible, and the system has to be accurate.

5. REFERENCES

- Giavasis I., Harvey L.M. and Brian McNeil. 2002. Scleroglucan. In *Biopolymers*, Vol.8. Edited by Alexander Steinbuchel, Munster, Germany. Published by Wiley-VCH Verlag GmbH, Weinheim, Germany. In press.
- [2] Giavasis I., Harvey L.M. McNeil B. 2000a. Gellan Gum. Critical Reviews in Biotechnology, 20 (3): 177-211.
- [3] Harvey, L.M. and Mc Neil, B. 1998. Thickeners of microbial origin. In: *Microbiology of fermented foods.* 2nd ed. Vol. 1, pp. 150-171. Wood, B.J.B. Blackie Academic & Professional. London.

- [4] Arnold, S.A., Crowley J., Vaidyanathan S., Matheson L., Mohan P., Hall J.W., Harvey L.M. and McNeil B. 2000. At-line monitoring of a submerged filamentous bacterial cultivation using near-infrared spectroscopy. Enz. Microb. Technol. 27: 691-697.
- [5] Hagman A. and Sivertsson P. 1998. The use of NIR spectroscopy in monitoring and controlling bioprocesses. *Process Contr. Quality.* 11(2): 125-128.
- [6] Liden H,. Mandenius C.F., Gorton L., Meinander N.Q., Lundstorm I. and Winquist F. 1998. On-line monitoring of a cultivation using an electronic nose. *Analytica Chim. Acta.* 361(3): 223-231.
- [7] Marose S., Lindemann C. and Scheper T. 1998. Twodimensional fluorescence spectroscopy: a new tool for on-line bioprocess monitoring. *Biotechnol. Prog.* 14 (1): 63-74.
- [8] Von Stockar U., Duboc P., Menoud L. and Marison I.W. 1997. On-line calorimetry as a technique for process monitoring and control in biotechnology. *Thermocimica Acta*. 300: 225-236.
- [9] Van der Pol J.J., de Gooijer C.D., Biselli M., Wandrey C. and Tramper J. 1996. Automation of selective assays for on-line bioprocess monitoring by flow-injection analysis. *Trends Biotechnol*. 14(12): 471-477.
- [10] Simon L and Nazmul K.M. 2001. Probabilistic neural networks using Bayesian decision strategies and a modified Gompertz model for growth phase classification in the batch culture of *Bacillus subtilis*. *Biochem. Eng. J.* 7 (1):41-48.
- [11] Trompetto C., Caponnetto C., Buccolieri A., Marchese R., Abbruzzese G., Warnes M.R., Glassey J., Montague G.A. and Kara B. 1998. Application of radial basis function and feed forward artificial neural networks to the *Escerichia coli* fermentation process-a research tool. *Neurocomputing*. 20 (1): 67-82.
- [12] Mandenius C. F., Hugman A., Dunas F., Sundgren H., Lundstrom I. 1998. A multisensor array for visualising continuous state transitions in biopharmaceutical processes using principal component analysis. *Biosensors and Bioelectronics*, vol. 13 (2): 193-199.
- [13] Chen J. and Liu K.-C. 2002. On-line batch process monitoring using dynamic PCA and dynamic PLS models. Chemical Engineering Science, vol. 57 (1): 63-75.
- [14] Mangina E. E., McArthur S. D. J., and McDonald J. R., Agent-based solution for engineering applications. (invited paper) To appear July 2002., *International Journal of Cybernetics and Systems.*
- [15] Sanderson G.R. and Clark R.C. 1983. Laboratoryproduced microbial polysaccharide has many potential food applications as a gelling, stabilizing and texturizing agent. *Food Technol.* 37: 63-70.

- [16] Pszczola D.E. 1993. Gellan gum wins IFT's Food Technology Industrial Achievement Award. Food Technol. 47: 94-96.
- [17] Shigeta J.I., Sato K., Tanaka S., Nakayama M. and Milli M. 1996. Efficient plant regeneration of asparagus from in vitro multiplied shoot explants using gellan gum and glucose. *Plant Sci.* 113: 99-104.
- [18] Murano, E., 1998. Use of natural polysaccharides in the microencapsulation techniques. J. Appl. Icthyol. 14: 245-249.
- [19] Kelco Division of Merck & Co. Inc. 1995. Gellan gum: multifunctional polysaccharide for texturizing. *Technical Monograph*. San Diego, California, 92123, U.S.A.
- [20] Kang, K.S., Veeder, G.T., Mirrasoul, P.J., Kaneko, T. and Cottrell, I.W. 1982. Agar-like polysaccharides produced by a *Pseudomonas* species: production and basic properties. *Appl. Environ. Microbiol.*, 43: 1086-1091.
- [21] Giavasis I., Harvey L.M. and McNeil B. 2000b. The effect of different process conditions on the biosynthesis of gellan gum. Oral presentation, Conference Abstracts, Biotechnology 2000-International Symposium and Exhibition, Sept. 2000, Berlin, Germany.
- [22] Wooldridge, M. & Jennings, N. R. (1995)
 'Intelligent Agents: Theory and Practice', *Knowledge Engineering Review*,10(2):115 – 152.
- [23] Finin T., Labrou Y. and Mayfield J., KQML as an agent communication language, in Bradshaw J., Software Agents, MIT Press, Cambridge, 1997
- [24] Mangina E. E., McArthur S. D. J., and McDonald J. R., Autonomous agents for distributed problem solving in condition monitoring. 2000. IEA/AIE2000 (The thirteen International Conference on Industrial & Engineering Applications of Artificial Intelligence & Expert Systems), New Orleans, USA, pp: 383 - 393