Computers in Industry 128 (2021) 103431

Contents lists available at ScienceDirect

Computers in Industry

journal homepage: www.elsevier.com/locate/compind

Fermentation 4.0, a case study on computer vision, soft sensor, connectivity, and control applied to the fermentation of a thraustochytrid

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ARTICLE INFO

Article history:
Received 4 November 2020
Received in revised form 10 February 2021
Accepted 16 February 2021
Available online 27 February 2021

Keywords:
Industry 4.0
Thraustochytrids
Fermentation
Computer vision
Soft sensor
DHA content

ABSTRACT

In this work, the incorporation of I4.0 technologies in fermentation was studied. The work aimed to explore if I4.0 technologies could be used to solve problems related to the modernization of fermentation processes, particularly, 1) the interconnection of incompatible components (sensor, compressor, and feeding pump), 2) the implementation of fermentation conditions, relevant to the process efficiency, and 3) making the fermentation an I4.0 compatible component. The technologies were tested on a lab-scale thraustochytrid fermentation, an example of a complex and economically valuable bioprocess. The results showed that the incorporated I4.0 technologies allowed acquiring the dissolved oxygen values from an incompatible equipment screen and implementing a control algorithm for obtaining; high carbon and nitrogen, and low dissolved oxygen concentration automatically after the growth phase. An automatic supervision tool allowed communicating relevant information about the fermentation state to humans and other computers. We conclude that incorporating I4.0 technologies in complex fermentation processes can improve process and equipment integration and allow the implementation of culture conditions that cannot be obtained using I2.0 and I3.0 technologies.

1. Introduction

Industry 4.0 (I4.0) is a paradigm about incorporating modern digital technologies into the manufacturing industry. Although no consensus definition of I4.0 has been reached, it is recognized that its implementation improves the process efficiency through the synergy derived from extended use of data, sensing, computation, and the intelligent interconnection of different equipment (Kagermann et al., 2013; Kamble et al., 2018; Lasi et al., 2014; Lins and Oliveira, 2020). I4.0 is the next step into the modernization of industrial processes, from the previous Industry 2.0 (I2.0) and Industry 3.0 (I3.0). I2.0 was developed when electricity became the primary source of power and is characterized by the use of electric motors manually operated. I3.0 was developed to reduce operators and thus costs and is characterized by the use of automatic machines (Vinitha et al., 2020). It has been pointed out that I4.0 requires a context-specific approach, in the sense that the implementation has to be adapted to the particularities of the field through multidisciplinary work (Culot et al., 2020). It has been considered that an I4.0 component is primarily characterized by its communication capabilities and by having a digital representation accessible by other computers (Tantik and Anderl, 2017), meaning that an I4.0 component can communicate its state and cooperate with humans and other machines or computers. I4.0 also helps to materialize process capacities such as monitoring, control, optimization, and autonomy (Moeuf et al., 2018). Implementing an I4.0 process starts with the interconnection of different equipment. This interconnection is not trivial (Lins and Oliveira, 2020) because many machines in operation have ports and communication protocols incompatible with those of new equipment. For solving the interconnection issue, the upgrade of existing equipment is an attractive solution compared to its replacement, especially for restricted budgets in small and medium-sized companies (Arjoni et al., 2017; Birtel et al., 2019; Lins and Oliveira, 2020; Peukert et al., 2020; Schlechtendahl et al., 2015).

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https://doi.org/10.1016/j.compind.2021.103431

Fermentation is the stage of a bioprocess in which large volumes of microorganisms are produced. In fermentation, living cells produce metabolites or cell biomass; the synthesis of each metabolite requires specific growth conditions (substrates, medium composition, temperature, pH, among others). Most fermentations are carried in batch mode (non-steady-state process). In aerobic fermentation, oxygen needs to be supplied at a rate equal to that demanded by cells, although this rate increases as the cell concentration increases. Cell growth conditions determine the cost of the end product and product quality. Most fermentations, especially those in which low price products are produced, are still operated at the I2.0 level. In a fermentation I2.0, skilled operators use empirical knowledge to manually operate the equipment (pumps, compressors, agitation motors, and sensors) with simple control actions during the batch process. Fermentation processes have a low technological level, even in the biopharmaceutical sector, for which there is current interest in how to incorporate modern monitoring and control techniques (Goldrick et al., 2019). It has already been suggested that I4.0 technologies could further expand the cell bioprocess capabilities (Narayanan et al., 2020).

The production of microbial lipids rich in docosahexaenoic acid (DHA) is an economically relevant cell bioprocess that requires fermentation. DHA is a long-chain omega-3 fatty acid currently used as a nutraceutical supplement and food additive, with annual sales of 3 billion USD and global annual demand growth of 16 % (de O.Finco et al., 2017). Current research in the production of DHA-rich microbial lipids aims to improve the yield to decrease production costs (Qu et al., 2011; Ren et al., 2009; Sun et al., 2016; Wang et al., 2018). Microorganisms used for DHA production are marine thraustochytrids. Biomass and lipids yields obtained in thraustochytrid fermentations are highly dependent on the dissolved oxygen and substrate concentration (Chi et al., 2009; Jakobsen et al., 2008; Qu et al., 2011). Conditions such as a high concentration of carbon and nitrogen sources and low dissolved oxygen concentration have been shown to favor DHA accumulation in the lipids of thraustochytrids, at the expense of a low biomass concentration if these conditions are implemented from the start of fermentation (Jakobsen et al., 2008). The dissolved oxygen concentration is generally controlled, increasing the agitation rate as the cell concentration increases (Heggeset et al., 2019; Jakobsen et al., 2008; Ye et al., 2020); however, high agitation rates could be detrimental to shear-sensitive cells. Another way to increase dissolved oxygen concentration is by manipulating the aeration rate. It has been shown that the feeding of the substrate at the end of the growth phase in a batch fermentation favors the lipid yield (Jakobsen et al., 2008; Wang et al., 2018; Ye et al., 2020). However, detecting the end of the growth phase typically requires manual sampling and sample processing, which delay the implementation of control actions, thus reducing the feeding strategy's effectiveness. A method to obtain relevant information from the data generated in the fermentation, to automatically determine the end of the growth phase, would exceed the capabilities offered by traditional control and automation systems (I3.0), such as those presented in Table 1.

A research gap regarding how I4.0 technologies can be incorporated into a fermentation process has been identified. To the best of the authors' knowledge, no research had discussed the characteristics of a fermentation I4.0.

In terms of the specific thraustochytrid fermentation, research gaps are that no works 1) have implemented dissolved oxygen control through the manipulation of the aeration, 2) have used data analysis for identifying the state of the fermentation, 3) have automated known conditions that favor DHA content in the accumulated lipids, considering variables related to the state of the fermentation for the close loop control.

In this work, we designed a thraustochytrid fermentation in order to evaluate how I4.0 technologies can be incorporated in a complex fermentation, in particular, to solve problems related to:

1) The interconnection of incompatible fermentation equipment,
2) The implementation of conditions favorable to the fermentation that can not be met using I2.0 and I3.0 technologies.
3) Making the fermentation an I4.0 compatible component, allowing remote access and control, identifying and communicating the fermentation state to humans and other equipment and machines.

The paper is organized as follows. In Section 2, a brief description of I4.0 technologies that in this work were applied to the fermentation process is presented. In Section 3, the fermentation design, materials, and methods are presented. In Section 4, the results from the experiments are presented. In Section 5, the implications of the analyzed I4.0 technologies over the modernization of the study case are discussed. Finally, conclusions are presented in Section 6.

2. Digital I4.0 technologies considered in the fermentation proposed design

2.1. Computer vision

Computer vision is a technology that can mimic the human visual system, allowing, for example, the translation of the information contained in images into text. Computer vision uses cameras for image acquisition and algorithms for image processing. A standard codification in image processing is RGB, which accounts for red, green, and blue light detected by light sensors in a camera. Typical computer vision processes are image acquisition, preprocessing, segmentation, feature extraction, classification, and post-processing (Islam et al., 2017; Qadri and Asif, 2009). When using video or images, a challenge is to reduce the amount of data transmitted. In computer vision, large amounts of data are transmitted between the camera and the computer vision processing unit. However, after image processing, the text descriptions obtained can be used instead of the images, reducing the amount of data to be transmitted. Reductions in the amount of data transmitted can also be obtained if the amount of images taken and processed in a period is reduced. This method is analogous to sample frequency reduction in sensors (Rahman et al., 2019). In cell bioprocesses, computer vision has been used as a standalone application to quantify and characterize cells in culture samples (Garófano et al., 2005; Ren et al., 1994; Ronen et al., 2002). However, computer vision can also be used as part of I4.0 processes (Coffey, 2018; Penumuru et al., 2020).

2.2. Neural networks

An artificial neuron is a mathematical representation of a simple model neuron. The artificial neurons can be interconnected in a logic network design, forming a neural network. A neural network, after training, can transform the inputs into the expected output. In the training stage, the parameters of the network are adjusted using sets of input-output data, capitalizing on past process experiences. Neural networks have been used in image recognition, soft sensors, and data analytics (Alford, 2006; Biechele et al., 2015; Sabanci et al., 2017; Sun et al., 2019). Neural networks have also been used to implement I4.0 processes (Elhoone et al., 2020; Hesser and Markert, 2019).

Table 1
The technological level used for the production of DHA rich lipids using thraustochytrid strains. Manual and automatic operation are referred to the manipulation of electric peristaltic and agitation pumps, and electronic sensors.

Parameter	Technological level	Reference
Manually determined substrate fed-rate	I2.0	(Jakobsen et al., 2008; Qu et al., 2011; Ren et al., 2010; Ye et al., 2020; Zhang et al., 2018)
Manually operated aeration and agitation rate	I2.0	(Abad and Turon, 2015; Bi et al., 2018; Chen and Yang, 2018; Guo et al., 2018; Jakobsen et al., 2008; Qu et al., 2013, 2011; Shene et al., 2013; Sun et al., 2016; Xiao et al., 2018; Zhang et al., 2018)
Automatic substrate fed-rate	I3.0	No works found
Automatic temperature control	I3.0	(Abad and Turon, 2015; Jakobsen et al., 2008; Qu et al., 2013, 2011; Shene et al., 2013; Xiao et al., 2018; Ye et al., 2020; Zhang et al., 2018)
Automatic pH control	I3.0	(Jakobsen et al., 2008; Shene et al., 2013; Song et al., 2007; Ye et al., 2020; Zhang et al., 2018)
Automatic dissolved oxygen control manipulating agitation	I3.0	(Heggeset et al., 2019; Jakobsen et al., 2008; Ye et al., 2020)
Automatic dissolved oxygen control manipulating aeration	I3.0	No works found

2.3. Soft sensors

A soft sensor is a technique in which a variable (output) that typically requires analytical methods for its determination is estimated using online measurements of related variables (inputs). Soft sensors solve the problem of providing estimates for variables for which no direct sensor is available (Chéruy, 1997). To relate the input and outputs variables, a soft sensor is designed offline combining previous expert knowledge of the process, recorded data, phenomenological or empirical mathematical models, or data mining methods. Soft sensors have been successfully applied in bioprocesses for monitoring the substrates, biomass, and metabolite concentration in the fermentation of various microorganisms (Biechele et al., 2015; Chéruy, 1997; Goldrick et al., 2019; Gu and Pan, 2015; Marafioti et al., 2009; Sun et al., 2019). Soft sensors have been classified as an I4.0 technology (Cruz Salazar et al., 2019; Mayr et al., 2018).

2.4. Data storing and connectivity

Sensors can generate large amounts of data from the periodical measurements. If the processes are operated distributively, the data is primarily stored in the same industrial equipment in which it was measured. Most equipment has limited data storage capacity, meaning that most of the data acquired is lost if no central data storage system has been implemented (Villalobos et al., 2020). The centralization of the data requires improving the plant technological level, for which the installation of dedicated computers is required. The introduction of cloud computing in industrial processes appears as a solution for data access and storage (Kamble et al., 2018). An example of cloud computing is Google Drive software (Google LLC, USA). Google Drive offers the automatic synchronization of the data stored between private and remote servers. Remote access is also a powerful I4.0 tool that allows manipulating remote computers and their connected equipment (Lins and Oliveira, 2020). For the remote manipulation, the equipment has to be previously configured to allow this capability. An example of software for remote access to a local server is TeamViewer (TeamViewer AG, Germany).

3. Materials and methods

3.1. The I4.0 fermentation for the production of DHA rich lipids

A thraustochytrid fermentation for the production of DHA rich lipids was designed considering the internal integration between all equipment involved in the process, the implementation of conditions favorable for cell growth and lipid production, and a supervisor system for the external integration of the fermentation process, as an I4.0 component (Fig. 1).

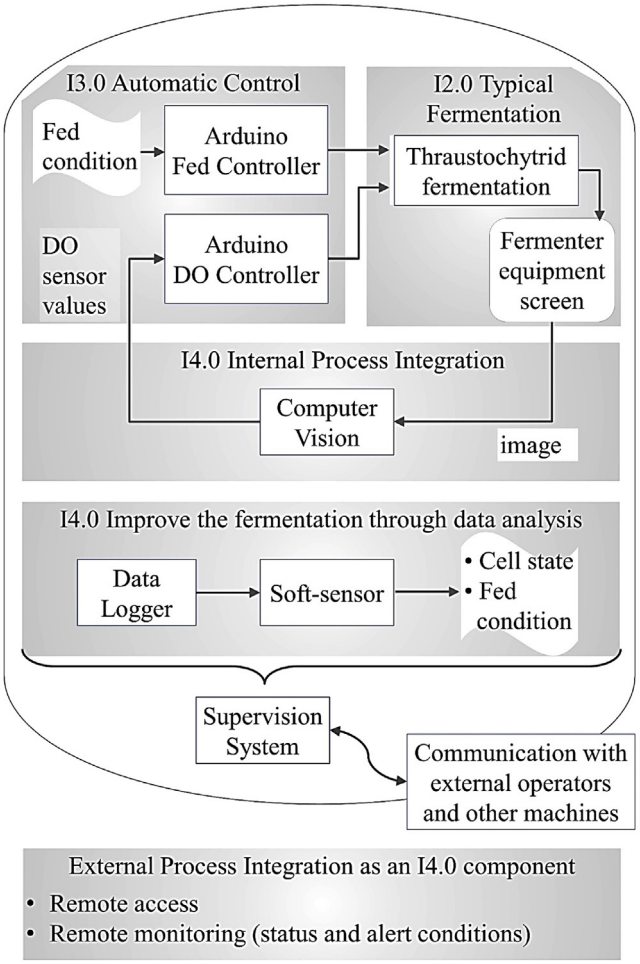

Fig. 1. I4.0 thraustohytrid fermentation process design.

The internal integration aimed to interconnect the typical I2.0 process (fermentor monitoring equipment, aeration compressors, and feeding pump) with the I3.0 automatic controllers (substrate feeding and dissolved oxygen levels) and the I4.0 computer with digital applications. Due to the communication incompatibility in terms of the physical/logical interfaces, a computer vision algorithm was developed to transmit to the computer, the values shown on the screen panel in the equipment, specifically, the dissolved oxygen values.

To improve the lipid and DHA yields of the fermentation, the favorable conditions aimed would be an initial high concentration of carbon and nitrogen, and a low level of dissolved oxygen (Jakobsen et al., 2008); however, not from the beginning of the culture, but after the end of the growth phase (cell state) in batch fermentation. The complex control strategy proposed ensures a cell growth phase, followed by a lipid accumulation phase at the beginning of which the required substrates would be added. For the automatic implementation, the automatic determination of the end of the growth phase is required. A soft sensor was designed to automatically estimate the end of the growth phase through online data analysis. The data analyzed were the values of dissolved oxygen and compressor duty cycle values stored since the start of the fermentation, obtained from a parallel running dissolved oxygen control system that manipulates the aeration compressor. The soft sensor output signaled the change from the growth phase to the lipid accumulation phase (cell state), which was used as the peristaltic feeding pump's turn-on signal. The feeding of the substrate rich medium would increase the cell activity, increasing the dissolved oxygen requirements.

The supervisor system allowed remote access to the process computer and remote monitoring of the fermentation's state, communicating the status and alert signals in case of malfunction or if requested by operators or other machines.

For implementing the design shown in Fig. 1, excepting for the I2.0 typical fermentation, the following materials were used: cellphone camera (E5, Motorola Inc, USA), computer I (240 G6, HP Inc, USA), computer II (Pavilion, HP Inc, USA), Arduino Uno microcontroller (Arduino CC, Italy), a local wireless router with internet access (N300, D-Link Corporation, Taiwan), computer software Google Drive (Google LLC, USA), TeamViewer 11 (TeamViewer AG, Germany), and Matlab (version 2019a, MathWorks, USA), Neural Network Toolbox (MathWorks, USA) and software IP Webcam (Pavel Khlebovich Google Play, USA).

The cellphone camera was used to acquire and transmit images from the monitoring equipment screen to the computer I. The cellphone periodically took pictures of the screen using the IP Webcam software. WiFi was used to provide internet access to the cellphone and computer I. The functions performed by the computer I were: executing the computer vision algorithm, computing the dissolved oxygen control algorithm, estimation of the end of the growth phase using a soft sensor, computation of the actions on the feeding pump (when needed), and the implementation of the supervisor system; all the actions were performed by codes developed in Matlab. All the data obtained and processed by computer I, including the dissolved oxygen level and the aeration duty cycle, was automatically translated and stored as a text file in a shared Google Drive folder. Team Viewer was used for full remote access to computer I (through the internet), to allow code edition, monitoring, and the manipulation of the compressors and peristaltic pump. The Arduino microcontroller was used to interconnect computer I and the peristaltic pump and compressors, allowing the implementation of the on/off control actions. Computer II was used to implement the monitoring system (developed as code in Matlab) that triggered alert signals to the process operator in the cases of malfunction during the fermentation.

Following, the details on the components of the I4.0 thraustochytrid fermentation design and the experiments are presented.

3.2. Typical I2.0 fermentation for the production of DHA rich lipids

In this work, a native *Thraustochytrium striatum* was used (Shene et al., 2013). The laboratory fermentor consisted of a 3 L stirred vessel coupled with the LiFlus GX monitoring equipment (Biotron, Kyunggi-Do, Korea). A touch screen showed the current temperature, pH, dissolved oxygen level, and agitation rate. An autoclavable dissolved oxygen sensor was used (Hamilton Company, Boston, MA, USA). Fermentations were performed at 15 °C, with a fixed stirring rate of 100 rpm; aeration was supplied by two small compressors (Mini Plus, Apex, Taiwan, and FE2106, Biotron) that combined provided a maximum aeration rate of 8 L/min. A peristaltic pump (Campbell Scientific Inc, Logan, USA) allowed the feeding of substrate rich medium when required. The equipment was installed in a laboratory of 25 m^2. Data of similar manually operated (I2.0) cell cultures can be found elsewhere (Shene et al., 2013).

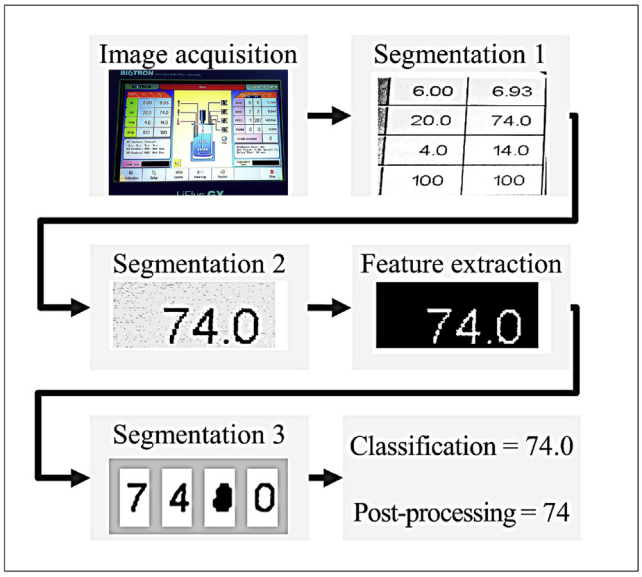

Fig. 2. Stages in the computer vision algorithm developed, starting with a picture of the LiFlus GX monitoring equipment (Biotron, Kyunggi-Do, Korea) and finishing with the dissolved oxygen concentration value.

3.3. Computer vision algorithm

A Matlab code was developed from scratch to implement the computer vision algorithm for acquiring the dissolved oxygen values shown on the equipment screen panel. Fig. 2 shows the main steps of the developed computer vision algorithm. First, the images were acquired in Matlab through the RGB codification. In the first segmentation step (segmentation 1), the gray square area where the dissolved oxygen sensor values are displayed was identified. In the segmentation 2 step, the gray square was horizontally divided in half and vertically into four equal-sized sub-images. The feature extraction step automatically analyzed the sub-images to find the shapes of the values contained. The digits were separated from the background using a threshold value, calculated using the mean value of the pixels in the image; the result was a black and white image. Then, every identified shape was separated from each other, following the shape's border. Segmentation 3 ends with sub-images consisting of a black shape (a potential digit) in a white background, used as inputs to the algorithm code that identified its values. Neural Network Toolbox of Matlab was used to train eleven neural networks that detected the numeric characters (0–9) and the dot point from the images obtained. Each neural network had ten neurons in the hidden layer. Finally, the routine classified each number by its associated variable, allowing the acquisition of the dissolved oxygen value from the screen.

3.4. Dissolved oxygen controller

A dissolved oxygen control system was designed for maintaining the dissolved oxygen in a fixed value through the fermentation. For manipulating the aeration rate, the dynamic on/off operation of the compressor was used. The closed-loop control law for dissolved oxygen with on/off operation of the compressor had the following form:

$$Duty\,cycle\,(t) = Duty\,cycle\,(t - \Delta t) + p\,(t) + i(t) \quad (1)$$

The duty cycle was defined as the number of seconds the compressor was turned on in a 20 s period. For calculating the duty cycle at time t, the value obtained at the previous sampling time $(t - \Delta t)$ was used, being Δt the sampling period. A proportional-integral control law was used. The proportional component of the controller $p(t)$ was obtained from:

$$p(t) = kp\left(DO_{ref} - DO(t)\right) \quad (2)$$

Where DO_{ref} is the desired dissolved oxygen value, DO the dissolved oxygen level at time t, and kp a tuning parameter. The integrative component $i(t)$ in Eq. (1) was calculated from:

$$i\,(t) = i\,(t - \Delta t) + ki\left(DO_{ref} - DO(t)\right) \quad (3)$$

Where ki is another tuning constant. Values used for kp and ki were 0.02 and 0.001, respectively. An Arduino electronic board was used for implementing the variable duty cycle, connecting it to a relay switch for turning on and off the compressors. The Arduino board was connected to the computer through a USB connection with the serial computer port. The Arduino was programmed for implementing the desired on-off duty cycle over a relay, following the instruction received by the serial port from the control law in the computer. If no new value was received, then the previous duty cycle value was maintained indefinitely. The Arduino board initialized two timers; the first was used for implementing the sample time Δt, while the second initialized the duty cycle.

3.5. Soft sensor for estimating the end of the growth phase and feeding condition

A soft sensor was designed for estimating the end of the growth phase in the fermentation. The design was based on expert knowledge of the process; in a thraustochytrid fermentation with dissolved oxygen control, a peak in the aeration rate occurs at the end of the growth phase. In our implementation, the peak in aeration rate corresponds to a maximum value in the duty cycle of the compressor. In this regard, the soft sensor has to find the peak in the compressor duty cycle for estimating the end of the growth phase. For implementing the soft-sensor, stored measurements of the compressor duty cycle, used for implementing the dissolved oxygen control, were used. To avoid a false detection, the algorithm calculated the average duty cycle required for maintaining the dissolved oxygen level at the set value in periods of 30 min; the increase or decrease of the average value was evaluated in periods of the same length. The end of the growth phase was estimated when a continuous decrease in the average duty cycle in six consecutive periods of 30 min was detected, indicating a past peak value. The detection implied a 3 h delay between the time of the end of the growth phase and the start of the lipid accumulation phase.

3.6. Fed controller for the thraustochytrid fermentation

The strategy used for the thraustochytrid fermentation consisted of keeping the dissolved oxygen constant, followed by a second stage in which substrate medium was automatically fed to the culture, causing a cell state with increased aeration requirements. For implementing the automated substrate feeding, a relay that activated the feeding pump at a rate of 20 mL/min was used. The fed consisted of 120 mL of the sterile medium containing 5.2 g of yeast extract, 0.24 g monosodium glutamate, and 12 g glucose in artificial seawater.

3.7. Remote monitoring for detecting process malfunction

A monitoring tool was designed to communicate the fermentation state and give an alert signal to the process operator and other machines. The fermentation state included if the fermentation was in the growth or in the lipid accumulation phase and the data obtained during the fermentation. The conditions for alert triggering were: if a new value was not stored in the last 10 min (FC1), and if the dissolved oxygen value reached a critical deviation, defined by $\left|DO_{ref} - DO(t)\right| > 10$ (FC2). The alert algorithm was executed every 30 s, with an alert consisting of a beep sound. The monitoring tool was implemented in Matlab in computers I and II. Computer II had internet access through an independent connection and was physically located out of the laboratory. Table 2 shows the process malfunctions events, a possible cause, the verification method, and the most likely solution shown to the operator in computer II. Methods for automatically detecting failures relied on the continuous analysis of the process data stored in a shared Google Drive folder. Google Drive software made continuous synchronization of this folder between computer I and computer II through the internet.

3.8. Experiments

A series of four fermentations were performed in order to test the incorporation of I4.0 technologies.

The first fermentation was used to acquire data for training the computer vision algorithm, to evaluate the error rate for detecting the DO values shown on the monitoring screen, and for determining an appropriate sample time for image acquisition. For training, 500 different images were acquired, manually identified, processed, and divided into training (70 %), testing (15 %), and validation (15 %) sets. The neural network output was manually assigned to -1 (non-detection) or 1 (detection of the correct number). A threshold output value of 0.3 was established, over which a value detection was considered valid. For evaluating the error rate of the trained computer vision algorithm, images were acquired during 13 h of a batch fermentation experiment at the maximum algorithm processing speed. The images obtained were also manually identified for detecting errors with the computer identification. The maximum error (erroneous digit identification) allowed for the computer vision algorithm was 5%. The evaluation of the computer vision algorithm also aimed to calculate the maximum sample rate for which the process of image acquisition, processing, and storing could be done in computer I.

Two 120 h fermentations were performed in order to test the dissolved oxygen control system, using the data obtained from the computer vision algorithm. The mean square error was used for calculating the controller error following the reference value.

A final 120 h fermentation was performed to test the soft sensor component and the fed control strategy. In this experiment, the dissolved oxygen level was controlled at 20 %.

In the last three fermentations, the supervisor system was tested for identifying error conditions with an online alarm system. In the final fermentation, the detection of dissolved oxygen deviations was deactivated after the fed addition due to the expected change in dissolved oxygen concentration due to increased aeration requirements of the cells (the aimed culture condition).

Table 2
Failure conditions, potential cause, verification method, and solutions for the designed control system, shown in computer II.

Failure condition (FC)	Possible cause	Verification method *	Solution *
FC1: Last recorded data happened more than 10 min ago	The computer or the cellphone was restarted due to an automatic update installation.	Verify if the devices were restarted	Restart the computer vision algorithm and the control system
	Computer I can not retrieve the pictures from the cellphone camera due to communication failure.	Test if computer I is able to connect to the IP address of the cellphone camera.	Restart the phone camera and the WiFi router.
		Determine if the cellphone is running without issues	Verify if the cellphone has a new IP address and connect to it
	Computer I can retrieve the pictures, but the computer vision algorithm can not identify the image values due to screen light reflections or change in the camera position.	Verify that the gray square of values is detected in the computer vision algorithm	Adjust the camera position to avoid light reflectances in the equipment screen, adjust the camera to pointing to the equipment screen
FC2: The absolute difference between the last dissolved oxygen value and the controller reference value is higher than 10 %.	The compressor does not respond to the computer I signals	Test if computer I is able to connect to the Arduino device. Test if it is possible to manipulate the compressor from direct instructions	Personal visit to the laboratory for evaluating communication malfunction between computer and Arduino

* In our implementation, it was preferable always to attempt remote access to the devices through Team Viewer. If the problem could not be solved remotely, then a personal inspection at the laboratory was required.

4. Results

The computer vision algorithm was executed at the maximum processing speed of Computer I, for 13 h. In this period, a total of 1200 images were acquired and identified by the computer vision algorithm, resulting in an average processing time of 39 s. Erroneous identification (16 cases over the 1200 images) occurred, equivalent to 1.33 % of the total cases, a value below the maximum defined error rate for the digit recognition of 5%.

The control of the dissolved oxygen level was tested at 10 % and 20 % using a sampling time equal to 120 s. The controlled system followed the reference in the two cases (Fig. 3) with mean square errors of 10.27 and 3.10 for the set values of 10 and 20 %, respectively.

The soft sensor for detecting the end of the growth phase and the subsequent automatic feeding was tested in a fermentation carried out with dissolved oxygen controlled at 20 % (Fig. 4). The results showed that the soft sensor successfully detected the peak in the average duty cycle (Fig. 5b), as defined by the occurrence of decreases in six periods of 30 min (Fig. 4c). The end of the growth phase was estimated at 48.6 h. At time 48.6 h, the soft sensor signaled the event to the control system, which activated the feeding pump adding 120 mL of substrate rich medium in a period of 6 min. Immediately after the substrate fed, the compressor duty cycle increased, trying to catch up with the decreasing level of dissolved oxygen. Soon after, the duty cycle required for maintaining the dissolved oxygen at 20 % reached its maximum value. Under these conditions, the compressors were unable to supply air at the rate required to keep dissolved oxygen at the specified level. At this point, the following condition was meet: a high carbon and nitrogen concentration in the medium, due to the recent addition of fresh substrate, and dissolved oxygen close to 0%. The dissolved oxygen drop lasted for about 40 h (Fig. 4a) due to the high oxygen demand by the active cells. Close to the fermentation's end, the dissolved oxygen level increased, and a reduction in the duty cycle required for the dissolved oxygen control was registered.

In all previous experiments, the supervision system with alert conditions was applied. As shown in Fig. 5, in the fermentation carried out with dissolved oxygen controlled at 10 %, 524 alerts for FC1 were activated, meaning that the fermentation did not store and transmit data for 8.73 h (7.28 % of the total length period) (Fig. 5a). The alert for FC1 was activated in two periods around the time 34 h and 98 h. Moreover, one alert for FC2 was activated (Fig. 5d). In the fermentation carried out with dissolved oxygen controlled at 20 %, the alert for FC1 was activated 1434 times, meaning that the system was not connected for 12.03 h (10.03 % of the total length period), around times of 14, 29, and 91 h (Fig. 5b). The alarm for FC2 was activated 11 times (Fig. 5e). Finally, in the experiment with substrate feeding the alert for FC1 was activated 11 times, equivalent to 0.08 % in the 120 h fermentation (Fig. 5c). The alert for FC2 was activated two times, equivalent to 1 min in the 120 h experiment (0.01 %) (Fig. 5f).

5. Discussion

Although developed from scratch, the computer vision algorithm implemented in this work ended up with steps similar to the one used by Qadri and Asif (2009) and Islam et al. (2017). The algorithms performed: image acquisition, detecting where the relevant information was located in the picture, segmentation of the area selected, and the individual processing of the segmented images to recognize numbers and symbols. The present work showed that a computer vision algorithm could be a simple solution to interconnect equipment that lacks interfaces or that have communication ports incompatible with the rest of the plant equipment. Integration using computer vision can be especially useful when the replacement of the incompatible equipment is not feasible and in cases in which no physical intervention over the device is desired due to possible damage or process detention.

The dissolved oxygen control requires precise measurements because errors are interpreted as real deviations from the reference levels, activating quick changes in the manipulated variable. Also, the dissolved oxygen controller requires an appropriate sampling time. The sampling time needs to be higher than the minimum image processing time (39 s), not too high to allow controlling the system, and not too small, to avoid an excessive amount of data transmitted. For this reason, a 120 s sampling time was tested. The on-off compressor operation, although simple, was able to maintain stable the dissolved oxygen level, close to the reference value in the batch fermentations. Even when computer vision errors were detected in all experiments, the controller was sufficiently robust to avoid deviations after wrong measurements. The successful implementation of the dissolved oxygen control proved that the computer vision's error rate (1.33 %) and the sampling time of 120 s were low enough for controlling the dynamics of the fermentation process used in this work.

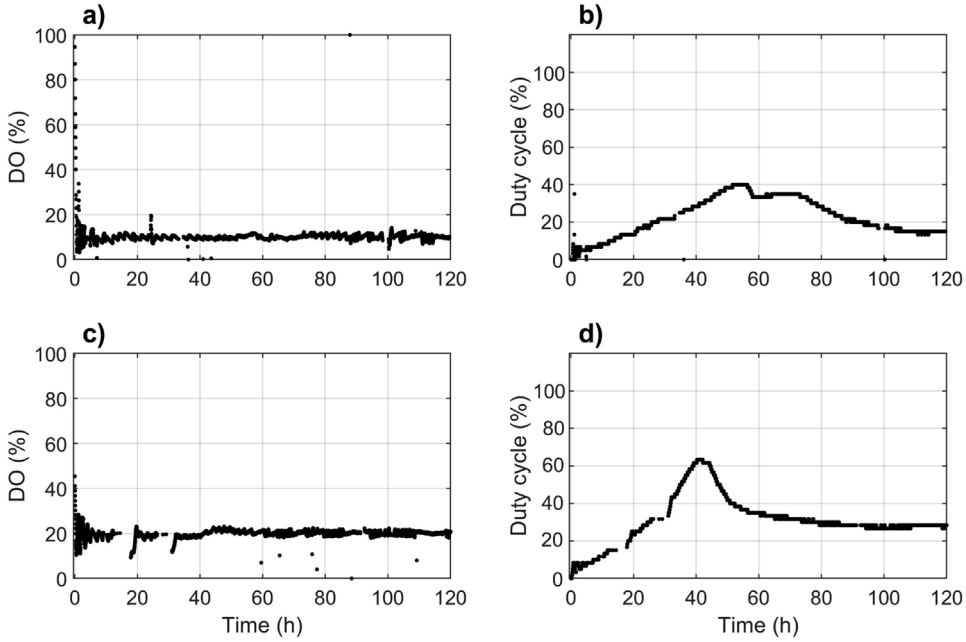

Fig. 3. Dissolved oxygen (DO) (a and c) and duty cycle of the compressor (b and d) during thraustochytrid fermentations performed with dissolved oxygen controlled at 10 % (a and b) and 20 % (c and d).

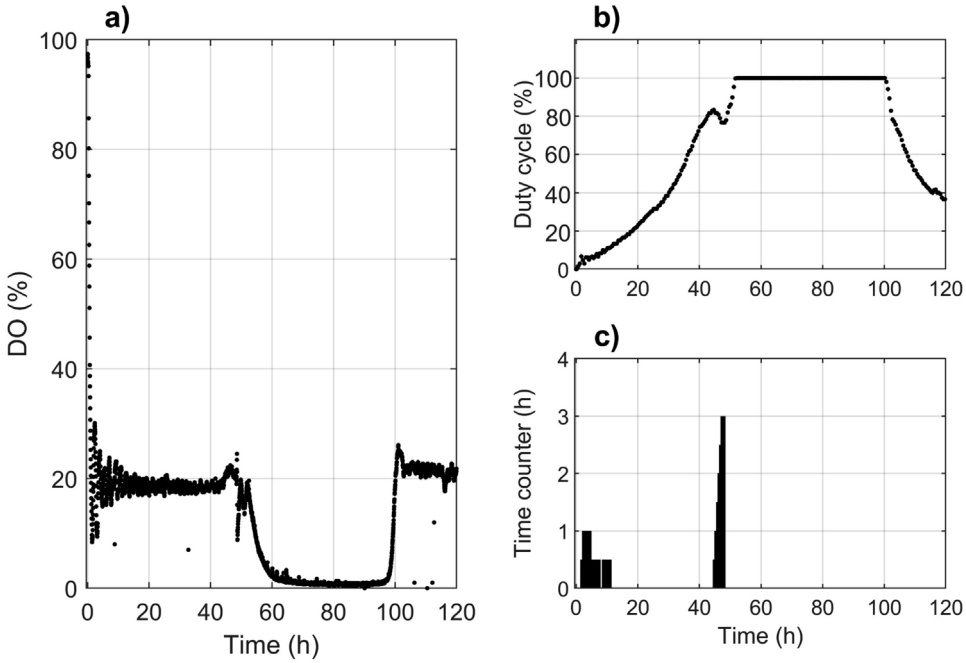

Fig. 4. (a) Dissolved oxygen (DO), (b) compressor duty cycle, (c) the time counter for detecting the end of the growth phase through decreasing average duty cycles in the thraustochytrid fermentation, to automatically fed with nutrients.

The soft sensor for estimating the end of the growth phase used the duty cycle data generated by the dissolved oxygen controller, combined with expert knowledge of the process. This knowledge-based soft sensor is a representative example of I4.0 technology. The soft sensor showed that it could not be implemented using only I2.0 and I3.0 technologies due to its reliance on data analysis. The soft sensor design represents an innovation for the thraustochytrid fermentations. The soft sensor developed can be used as a part of control systems more complex than those previously reported (summarized in Table 1). The soft sensor developed used duty cycle data generated by the dissolved oxygen controller and expert knowledge of the process.

After the end of the growth phase, substrate addition caused an increase in the compressor duty cycle. Even with the maximum duty cycle value, the dissolved oxygen dropped to values close to 0. At this point, the culture, through the automatic control system, reached a high concentration of the nutrients and a low dissolved oxygen concentration, which were the aimed favorable conditions for DHA accumulation (Jakobsen et al., 2008). The increase in the dissolved oxygen concentration and the reduction in the required duty cycle close to the end of the culture suggest a reduction in cell

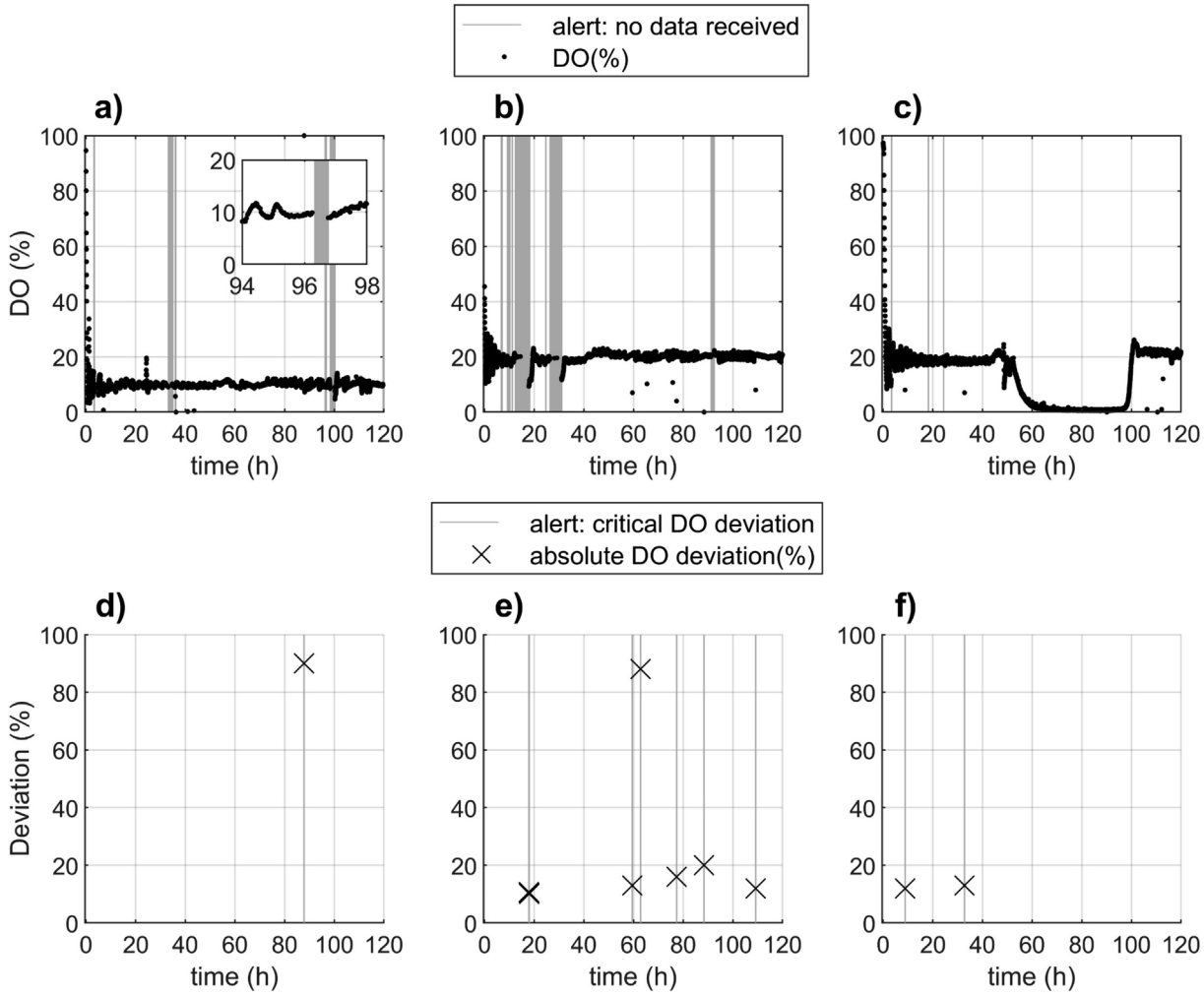

Fig. 5. Supervision system. Alert triggered when no data was received in the last 10 min (a, b, and c) and data received represented by dissolved oxygen (DO). Alert triggered when the absolute difference between the dissolved oxygen values and the setpoint was higher than 10 % (d,e,f), except for the time after the feed addition (f). Experiment with dissolved oxygen controlled at 10 % (a and d), 20 % (b and e), and 20 % with automatic start of nutrient feeding at 48.6 h (c and f).

metabolic activity. This last condition would indicate an appropriate time for harvesting the biomass, which can also be incorporated in another future automated design. The maximum rate of aeration (defined by the compressor capacity) determines the amount of feeding that allows reaching the dissolved oxygen level close to zero.

The standalone monitoring equipment of the typical I2.0 setup did not allow implementing new control schemes. In contrast, the newly added computer I was essential for implementing the I4.0 design. Remote access using Team Viewer allowed remotely watching the data processing and the pictures taken by the cellphone in real-time. The implementation of a synchronized folder using Google Drive allowed the remote supervision system to be implemented. The use of a modern computer for data storage and data processing proved useful when storing large data time series compared to monitoring equipment limitations. The incorporation of computers is a way to enhance the technological level of the fermentation process.

In terms of the supervision system, the main cause for no data received remotely during the experiments (FC1) was the restart for upgrades installation in the computer and cellphone. The identification of the malfunction sources allowed the operator to take the necessary corrective actions. The use of the Arduino board as the source of the duty cycle signal for the compressor operation allowed to maintain the duty cycles even when the computer was restarted. This feature allowed maintaining the fermentation with dissolved oxygen values close to the reference until the control system was functioning again.

The final design for the modernization of the fermentation for lipid production considered I4.0 technologies for data acquisition (computer vision algorithm), storing and connectivity (modern computer, storing with synchronized folders, WiFi, internet remote access), analysis (soft sensor, alert supervision tool), and control. The fermentation improved its capabilities concerning core process I4.0 attributes, such as process monitoring, control, optimization, and autonomy defined by Moeuf et al. (2018), showing that the proposed design is in line with the current research trends in the industry 4.0 development. The present work can also be considered a continuation of the work about equipment modernization presented by Schlechtendahl et al. (2014), showing the possibility of upgrading existing equipment instead of replacement for implementing I4.0 processes. Our approach aimed to be didactic, showing the potential of integrating old or discontinued equipment into the I4.0 processes. Finally, improvements in plant operation with remote supervision tools appear appropriate to modern times when social distancing is required.

The presented work can shed light on how a fermentation I4.0 would be like; characterized by the interconnection of the equipment, remote access, intensive computation, the use of real-time and recorded data, expert knowledge obtained from human

experience and data analysis, and also, machines that effectively communicate its state to humans and other machines or computers for increase cooperation.

6. Conclusions

In this work, the incorporation of I4.0 technologies in fermentation was studied, considering the integration of internal equipment, improvements to the fermentation culture, and the external integration of the process as an I4.0 component.

Contribution 1: The results show that computer vision can be used for interconnecting incompatible fermentation equipment. This solution is especially useful when the equipment replacement is not an option and in cases in which no physical intervention over the device is desired due to possible damage or potential process detention.

Contribution 2: Our work describes a dissolved oxygen controller that manipulates the duty cycle of the compressors responsible for aeration, which has not been previously reported for the culture of the strain under study.

Contribution 3: A soft sensor was designed using expert knowledge about the fermentation for the automatic detection of the end of the growth phase in a thraustochytrid fermentation. The soft sensor used the compressors' duty cycle computed by a dissolved oxygen control. The contribution is novel and allows using the state of the culture in a control loop for implementing favorable culture conditions for the system under study.

Contribution 4: An automatic supervisor system was designed and tested, allowing sending and receiving messages between the fermentation process, the operator, and other computers. The supervisor systems allowed implementing remote access, monitoring, and the fermentation system's external integration, showing how a fermentation I4.0 would be like.

This work contributes to reducing the knowledge gap in terms of the incorporation of industry 4.0 technologies in fermentation. We conclude that the incorporation of I4.0 technologies has direct application in solving relevant problems that have delayed the modernization of fermentation processes, such as process and equipment integration and the implementation of complex culture conditions relevant to the process efficiency that cannot be obtained using I2.0 and I3.0 technologies.

CRediT authorship contribution statement

C. Alarcon: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Visualization, Investigation, Writing - review & editing. **C. Shene:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This work was supported by the CONICYT PFCHA/Doctorado Nacional/2018-21180806 and Conicyt project: Basal Centre for Biotechnology and Bioengineering (CeBiB) FB-0001.

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