Use of Operational Training Simulation in the Study of Ethanol Operating Conditions: A Powerful Tool for Education and Research Performance Improvement

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Abstract. Currently, virtual education has reached a significant boom in higher education institutions. The latter, considering health emergency due to coronavirus pandemic, which makes it difficult to re-open physical infrastructures. As a consequence of the above, there is concern about experimental development taught by higher education and research institutions, since the execution of the experimental component is still considered a challenge due to uncertainty of on-going infections. Currently, the University of Bremen, Germany has implemented an operational training simulator for the study of bioprocess based on mathematical equations and experimental confirmations. The software enables teaching of virtual handling and operation of bioreactors. Students and engineers can deal with real situations that arise in the plant operation. Fermentations in different modes to reflect its effects on productivity could be studied. The main purpose of this research is determining the best operating conditions for the ethanol continuous production using the BioProcess Trainer software. One of the most important findings suggest a bioreactor feed rate of 5 mL/min charged with 20 g/L of glucose to reach a productivity of 0.037 g/L.h. Results found here demonstrates the Bioprocess Trainer potential capacity not only in the study of industrial plant operating conditions but also regarding educational institutions since mentioned tool improves engineers’ expertise.

1. Introduction
Higher education and research are one of the sectors most impacted by the coronavirus pandemic. The above, considering its restructuring and adaptation to virtual modalities for continuing research and teaching activities. One of the government's strategies to face this harsh reality is the gradual reactivation of presentality type classes without the use of 100% of their capacity, but rather the implementation of semi-attendance. The foregoing represents a replica for the application of biosafety protocols in higher education institutions, specific in areas characterized by little available space, stories such as research laboratories for the development of various experimentations. That is why it is unlikely in the short term a future scenario with opening 100% of the physical infrastructure of institutions. This problem is even more pronounced in professional careers including bioprocess research within their fields such as Biotechnological, Chemical, Environmental, Biological Engineering and Industrial Microbiology, among others. The above considering equipment and infrastructure requirement for classroom teaching and research activities development. An operation training simulator is based on virtual simulation for activities development related to industrial sector. Therefore, it allows the operational training of engineers and operators [1-2] without significant physical infrastructure requirements.
Typically, an OTS interface is characterized by virtual visualization and valves, fermentation equipment and storage tanks operation, among others. Consequently, training and interaction with production plants are enhanced to visualize different operating conditions, equipment operation and control techniques that allow identifying possible problems related to metabolites production. Currently, the University of Bremen, Germany has implemented an operation training simulator (OTS) for the study of bioprocess based on mathematical equations and experimental confirmations. The mentioned computational tool is commercialized by Ingenieurbüro Dr.-Ing. Schoop GmbH.

Students and engineers can deal with real situations that arise in plant operation. Achieving fermentation in different operational modes for simulating its effects on productivity and understanding the most influential parameters. In this way, inoculum can be added virtually, controlling the pH, stirring and aeration level and observing its effects on molecules production. Some examples have been addressed from bio-digesters operation [3] to ethanol production [4-5] and recombinant proteins processing [6]. OTS is considered of great help as a complement to the virtual training currently taught in the universities and research institutes which closed their infrastructure due to the coronavirus pandemic. The latter are some of potential advantages of OTS computational tool in the field not only of teaching, but also in metabolites research and industrialization obtained from laboratory equipment. As a practical example, the study of the operating conditions in a continuous bioreactor for ethanol production was proposed in this research [7-8]. The above, considering the compatibility of the software regarding control routines already implemented, which facilitates the evaluation of operating conditions effect on productivity. Saccharomyces cerevisiae [9-10] are generally used to make this bio-product using glucose [11], due to its high resistance to low pH (4.5 to 5.0) and tolerance to high ethanol concentrations (10 to 12 %) [12-13]. However, evaluating the effectiveness of a bioprocess in the laboratory on a large scale can be expensive, slow and ineffective, generating irreversible effects on the product. The bioprocess simulation allows water, energy and time savings and improves the final product quality and performance by controlling factors such as temperature, pH, aeration and mixing [13-14]. For that reason, the main purpose of this research is determining the best operating conditions for the ethanol continuous production using the BioProcess Trainer software to assess its potential use in the academic and research sector.

2. Methodology

For the development of this research, the virtual study of operating conditions effect on ethanol productivity was evaluated. For this case, glucose concentration as a limiting substrate and flow rate are chosen as main fermentation parameters influencing ethanol formation. Once simulation matrix design allowing the interaction of mentioned parameters has been defined, simulations are implemented in the Bioprocess Trainer software. To carry out the simulations in continuous mode, the data in Table I was taken based on a factorial design with a central point. That is why, 5 simulations were performed. Data is shown in Table 1 and Figure 1.

| Parameter       | Value | Units |
|-----------------|-------|-------|
| $S_{feed}(-1)$  | 10    | g/L   |
| $S_{feed}(0)$   | 15    | g/L   |
| $S_{feed}(+1)$  | 20    | g/L   |
| $F_0(-1) = F_{out}$ | 1    | mL/min |
| $F_0(0) = F_{out}$ | 2    | mL/min |
| $F_0(+1) = F_{out}$ | 5    | mL/min |

For ethanol production in continuous type mode, a bioreactor is programmed to be operated with a constant glucose supply and simultaneously, an extraction of the exhausted medium is set up (see Figures 1-2).
Prior to continuous mode, process must be simulated in batch mode (see Table 2) to obtain as many cells as possible that will serve as biocatalysts during the fermentation process. That is why Table 2 refers the operating conditions used for this first stage.

Table 2. Initial operating conditions for ethanol production (batch mode). Inoculum concentration (X0); Glucose concentration (S0) and Air feed (VVM).

| Parameter   | Value | Units  |
|-------------|-------|--------|
| X0 (batch)  | 0.9   | g/L    |
| S0 (batch)  | 10    | g/L    |
| VVM         | 0.1   | mL/mL/min |
| Air Feed    | 1.0   | L/min  |

Once the microorganism is inoculated (Inoculation valve), samples are taken every two hours (Sample port). *Saccharomyces cerevisiae* yeast cells use glucose as an energy source for reaching all the biochemical reactions that finally converge in ethanol production.

As a consequence of the fermentation process pH tends to destabilize, in such a way it must be controlled in a range between 4.0-4.5. To target this objective of maintaining the pH at the mentioned
levels, the Bioprocess Trainer software has implemented a control routine that allows the controlled KOH (Base) dosing (see Figure 2).

![Bioprocess set up for ethanol production using a continuous mode. Source Bioprocess Trainer.](image)

**Figure 2.** Bioprocess set up for ethanol production using a continuous mode. Source Bioprocess Trainer.

As a result of metabolic bio-conversion process from glucose to ethanol, energy is released into the environment. The latter is known as an exothermic process and the temperature tends to be increased in the equipment.

That is why a temperature control at 35ºC is programmed as the optimal production level. To achieve this end, cold water is fed through bioreactor using a cooling jacket. Here, water it is pumped at 2 L/min in all the cases evaluated. The input operating parameters, such as stirring speed, cooling water, inoculum load and substrate, among others, are presented in Figure 3.
3. Results and Discussions
The main goal of this research is studying the best operating conditions for the ethanol continuous production using the BioProcess Trainer software. Special emphasis is highlighted on demonstrating its potential use for the academic and research sector. The latter with the aim of contributing in education and research improvements for engineers and students. Therefore, OTS are considered as a promising tools to overcome difficulties reached at engineering teaching and researching institutions as a consequence of the limited usage of equipment and infrastructure due to coronavirus pandemic.

The results regarding all simulated fermentation are shown in Figure 4. For first bioprocess (Figure 4-a) operated at the lowest operating conditions, biomass reached an average concentration of 5.64 g/L from first to eleventh fermentation day. Then, it started to decline until it showed a value of 2.70 g/L on day 20. Ethanol was estimated with a highest concentration of 2.23 g/L at 16 hours after starting the batch bioprocess and it decreased to an average concentration of 0.016 g/L. Subsequently, ethanol concentration was increased to 1.23 g/L on day 19 of continuous operation. Finally, substrate showed a level of 0.15 g/L up to day 17. Therefore, it increased for a final value of 1.19 g/L on day 20 with a dilution rate of 0.006 h⁻¹.
Figure 4. Results of kinetic study for ethanol production. (a) S=10 g/l, F=1 mL/min; b) S=20 g/l y F=5 mL/min; c) S=10 g/l, F=5 mL/min; d) S=20 g/l, F=1 mL/min; e) S=15 g/l, F=2 mL/min.
By contrast, simulating the process at maximum flow rate (5mL/min) and glucose feed (20 g/L), biomass concentration achieved a value of 4.32 g/L in the middle of the first day (see Figure 4-b). Ethanol concentration obtained its highest value of 2.93 g/L during the first day. Bioreactor reached dilution rate of 0.03 h\(^{-1}\). Figure 4 (c) shows the results of simulation operated at S=10 g/l and F=5 mL/min. Similar results were shown: biomass showed a maximum concentration of 4.13 g/L on day 1 and ethanol reached a value of 2.27 g/L at 16 hours. Dilution rate was estimated at 0.03 h\(^{-1}\). However, a different scenario is shown at a dilution rate of 0.006 h\(^{-1}\) (Figure 4-d). It can be seen that the biomass reached a maximum value of 8.70 g/L on the sixth day and maximum product obtained was simulated at 2.53 g/L at 15 days. Therefore, simulating the process at central point operating conditions (S=15 g/l, F=2 mL/min), a maximum ethanol level is calculated (2.12 g/L) on the sixth day and also a maximum biomass concentration of 6.34 g/L was obtained. At this operating conditions the dilution rate is estimated at 0.012 h\(^{-1}\).

There is a correlation between maximum growth rate \(\mu_{\text{max}}\) and the dilution rate \(D\) applied to reactors operated at continuous mode. According to the theory of continuous reactor, if \(\mu_{\text{max}} > D\) the biomass will accumulate in the reactor. By contrast, if \(D > \mu_{\text{max}}\), a phenomenon called reactor “Wash-out” will occur [15]. This situation is applied to a reactor reaching a low final biomass and it is caused by an abrupt increase in the flow rate provided. For avoiding the risk mentioned, it is recommended that \(D\) should be less than \(\mu_{\text{max}}\) without reaching closed values. In this research, suspended cells simulated with values less than \(\mu_{\text{max}}\). So that, dilution rate was limited by the growth rate. In all cases, it was found that the \(\mu_{\text{max}}\) value was greater than \(D\), considering a calculated value of \(\mu_{\text{max}}\) closed to 0.21 h\(^{-1}\). The latter for avoiding the wash-out phenomenon. Biomass (Qx) and ethanol (Qp) productivities rates are shown in Figure 5.

![Figure 5. Biomass and ethanol productivity calculated from simulations S1 to S5](image)

The highest product and biomass volumetric rate was also obtained at simulation S2 (Figure 4-b), being 0.0375 and 0.0508 g/L/h, respectively, at a dilution rate of 0.03 h\(^{-1}\). Contrary, lowest values are calculated in simulation S1 with a level of 0.0071 and 0.0174 g/L/h, respectively, at a dilution rate of 0.006 h\(^{-1}\). Furthermore, it is evident that dilution rate is directly related to volumetric productivity, since simulations suggest promising productivity values at the highest \(D\). The latter finding has been also verified by previous reports [16].

Likewise, a study carried out by [17] determined that dilution rate and the kinetic parameters influence the productivity of ethanol, obtaining a concentration of 13.75 g/L at \(D\) 0.005 h\(^{-1}\). Similarly, an ethanol productivity with a value of 3.8 g/L/h was obtained by operating the bioreactor at a dilution rate of 0.13 h\(^{-1}\) [18].

Figure 6 shows the ethanol and biomass yields. Simulation S1 obtained a higher biomass yield with a value of 0.40 g/g by feeding a substrate concentration of 10 g/L and a flow rate of 1 ml/min. Lowest
values were estimated at simulation S2 with a yield of 0.28 g/g when using a flow rate of 5 ml/min and a glucose feed of 20 g/L. Therefore, the highest product yield was achieved at simulation S3 with a value of 0.23 g/g operated at a flow rate of 5 ml/min. A direct relationship between Yps and feed flow is evident.

Figure 6. Biomass and ethanol yield calculated from simulations S1 to S5.

Figure 7 shows that simulation S2 reached a higher consumption rate qs with a level of 0.11 g/L.h and a Qx of 0.0375 g/L.h. On the other hand, the lowest value of qs was shown in simulation S1 being 0.015 g/L.h with a Qx of 0.0071 g/L.h. These values allow to observe a direct relationship between the specific rate of substrate consumption and the volumetric productivity of biomass. Likewise, in continuous mode operations a high qs value is obtained, since the substrate entering the reactor is exposed to a high concentration of biomass. A reactor (operated at continuous mode) is characterized by its constant volume and the continuous substrate feeding and removing. Likewise, residence time is determined by flow rate. That why residence time needs to optimized focused on targeting the best yields and the lowest operating costs. Based on the latter, and OTS simulator could offer potential capacities to be implemented on teaching and researching purposes related bioprocess engineering. The foam generated in reactor is usually controlled by adding antifoam. However, the latter reduce the oxygen transfer rate, since it affects cell growth and productivity [12]. Likewise, in an experiment carried out by [17] it could be concluded that ethanol production is also controlled by cell growth.

Figure 7. Glucose uptake rate calculated from simulations S1 to S5.
This research consisted on demonstrate the potential use of an OTS simulator for bioprocess engineering. As a typical example of application ethanol production from a continuous mode is set up. The maximum values of product were reached in simulation S2. Furthermore, the highest product yield from substrate is calculated with a value of 0.23 g/g.

4. Conclusions
In this research, BioProcess Trainer software was used to perform various simulations in continuous mode. The latter has significant theoretical advantages compared to other modes of operation; Finally, it was evident that simulation S3 was the most efficient when comparing parameters such as total process time and production capacity, showing a value of 2.27 g/L of ethanol in 4 days when using a feeding of glucose with a level of 10 g/L. Based on results found here from the Bioprocess Trainer OTS software, it is demonstrated its potential advantages to face challenges reached at teaching and researching institutions as a consequence of the limited usage of infrastructure due to coronavirus pandemic.

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