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Technical and Economic Viability Analysis of Optical Fiber Sensors for Monitoring Industrial Bioreactors

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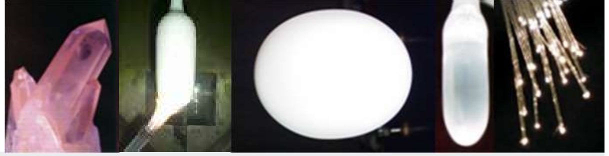


1. Introduction

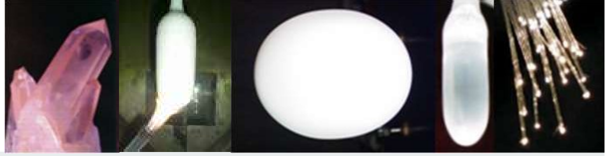
Bioprocesses:

- Relevant to different industries: pharmaceutics, energy, biomedic and food [1].

- These processes are still difficult to monitor.

- Assessment is usually performed by techniques unsuitable for automatic control, like: microscopes, centrifuges, spectrophotometers, etc. [2,3].

[1] Shuler, M.; Kargi, F. *Bioprocess Engineering. Basic Concepts*. Second Edition. Prentice Hall: 2002.
[2] Bailey, J.; Ollis, D. *Biochemical Engineering Fundamentals*. McGraw-Hill: 1986.
[3] Soares, M.C.P. *et al. Sensors* **2019**, *19*, 2493. doi:10.3390/s19112493.

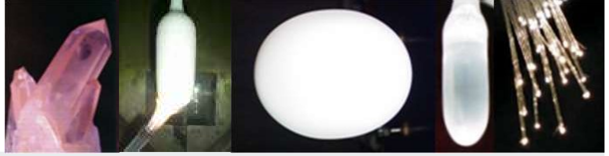


1. Introduction

Traditional procedures for analysis:

- Traditional procedures include the quantifying of the dry mass from the broth, or evaluating the glucose concentration with colorimetric assays [4].
- They are based on manual and time-consuming procedures [2].

[4] Merck Sigma Aldrich. Glucose (GO) Assay Kit. Available online: https://www.sigmaaldrich.com /catalog/product/sigma/gago20 (accessed on 6 August 2020).



1. Introduction

Traditional procedures for analysis:

- More accurate techniques, capable of evaluating multiple parameters: high performance liquid chromatography (HPLC); gas chromatography coupled to mass spectrometry (GC-MS); and the enzyme-linked immunosorbent assay (ELISA).
- They are also widely applied to chemical and biochemical analysis, since they are sensitive and reliable.
- Require expensive and bulky instrumentation, highly specialized technicians, and procedures hard to automate and to perform field analysis [3,5].

[5] Ju, H.; Kandimalla, V.K. In *Electrochemical Sensors, Biosensors and their Biomedical Applications*; Zhang, X.; Ju, H.; Wang, J. Ed.; Elsevier: 2008.

1. Introduction

Use of Sensors for Fermentation Monitoring:

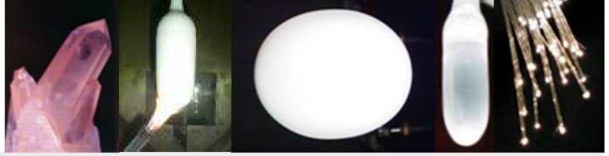
- The best alternative;
- The **use of in-line sensors** allow:
  - obtaining useful data with shorter operation times,
  - It is useful not only for the monitoring, but also for the **preliminary screening** prior to the investment on more precise equipment [3,5].

1. Introduction

Optical Fiber Sensors (OFSs):

- Biocompatible;
- Immune to electromagnetic interference;
- Show chemical and thermal stability;
- Show lower fabrication costs, being suitable for the mass-fabrication of devices [3,6,7].

[6] Li, X. *et al. Sens. Act. B: Chem.* **2018**, *269*, 103–109.
[7] Gong, C. *et al. Lab Chip* **2017**, *17*, 3431–3436.

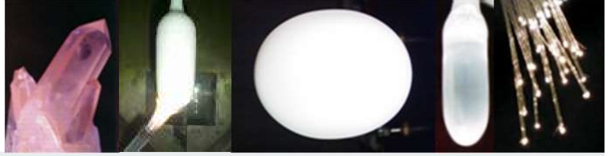


1. Introduction

Objective of this Work

- Analysis of the technical and economic viability of implementing fiber optics fed-batch ethanol fermentation systems.
    - It is compared to traditional ELISA and HPLC systems.
- Fed-batch is very prevalent in different fermentation industries.
- Ethanol production represents a **major sector of the Brazilian economy**, with ***an annual production in excess of 35 billion liters*** [8,9].
- A simple fiber sensing system is proposed and the advantages of real-time process control are verified.

[8] Souza, G.M.; Victoria, R.L.; Joly, C.A.; Verdade, L.M. Ed. *Bioenergy and Sustentability: bridging the gaps*. Scope - FAPESP - BIOEN - BIOTA - FAPESP Climate Change: São Paulo, SP, Brazil, 2015.
[9] International Sugar Association (ISO), *ISO Ethanol Yearbook 2019*, ISO 2020.

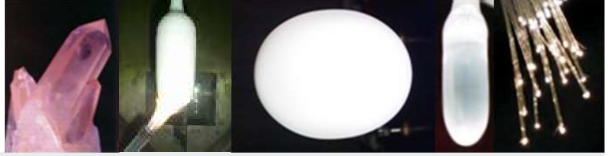


1. Introduction

Photonic Industry Facts

- Important economic aspects regarding the photonic industry itself make it attractive for new investments such as the proposed in this work.
- In the **United Kingdom (2017)**, it was estimated that the photonic industry:
  - contributed with more than **£12.9 billion**;
  - with an annual **growth rate over 5%**;
  - Employed more than **65,000 people**;
  - At least of **75%** of the production destined **to exportation** [10].

[10] Optoelectronics Research Centre (Part of the Russell Group). Key photonics industry facts (June 2017). Available online: https://www.orc.soton.ac.uk/who-we-are (accessed on 14 August 2020).

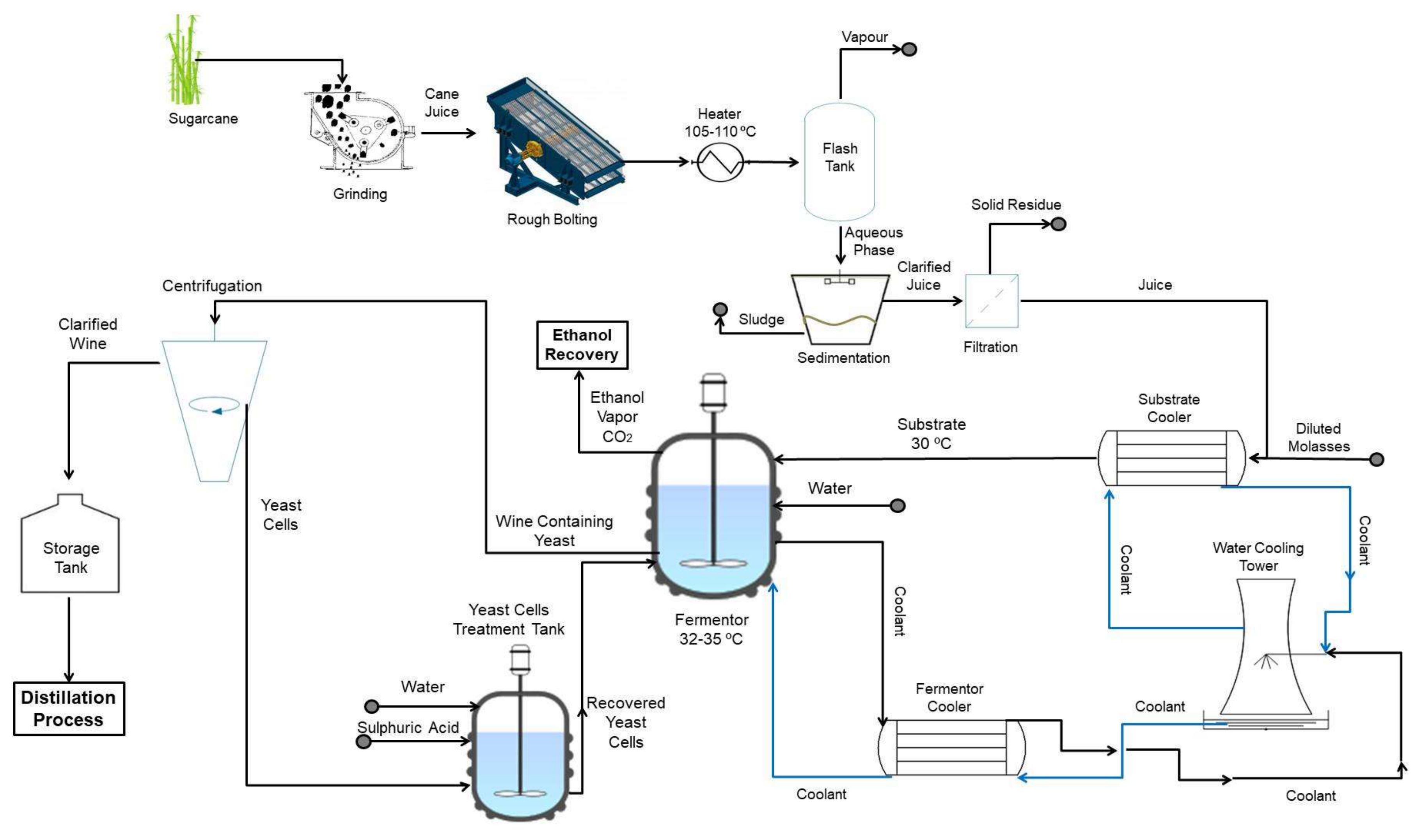


1. Introduction

Photonic Industry Facts

- According to Gong et al. [7], fiber optic sensors are also advantageous in terms of the **low costs involved in the waveguide production**:
  - Common optical fibers may be produced with lengths as high as ~50 km;
  - Due to the economies of scale, the average fabrication cost is in the order of **US$ 0.01 per meter of fiber**.
  - On the other hand, mass production does not compromise the quality of the materials: authors cite the model “SMF-28e” (Corning), which presents diameter variations of only ~0,56% of its nominal length [7].
- These data clearly show the ***potential of developing low-cost photonic sensors***.

2. General Ethanol Fermentation Industry



Adapted from [11] Basso, L.C.; Basso, T.O.;Rocha, S.N. Ethanol Prouction in Brazil: The Industrial Process and Its Impact on Yeast Fermentation. In: *Biofuel Production. Recent Developments and Prospects*; Bernardes, M.A.S. Ed.; IntechOpen: Rijeka, Croatia, 2011. doi: 10.5772/17047

2. General Ethanol Fermentation Industry

Costs – Preliminary Analysis

- Once the main objective is to analyze the gains involved in adopting an instrumentation system, it is possible to admit that both the instrumented and non-instrumented processes present same costs of:
  - Acquisition of reactants and raw materials;
  - Acquisition of utilities (electric energy, steam, and cooling water);
  - Other production costs.

2. General Ethanol Fermentation Industry

Costs Previously Evaluated

- Vieira et al. [12]: analyzed a fermentation process plant similar to the one of the last figure, capable of **processing 123.6 tons of sugarcane molasses/year**.
    - Cost estimated for **plant construction**: **USD 88.7 millions**, whereas **USD 39.4 millions** are referent to the **equipment acquisition** (including a fermentor fabricated in stainless steel 316L).
    - **Operation costs**: estimated in **USD 13.3 millions/per year for reactants and raw materials'** acquisition, and **USD 7.8 millions/year** for the acquisition of **utilities**.

[12] Vieira, J.P.F. *et al. Ind. Crops Prod.* **2016**, *89*, 478–485, 2016. doi: 10.1016/j.indcrop.2016.05.046.

2. General Ethanol Fermentation Industry

Costs Previously evaluated

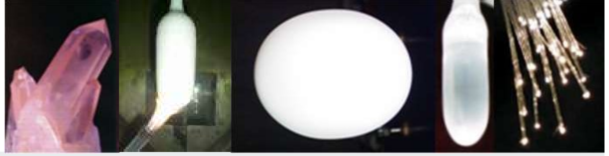
- Humbird et al. [13]: analyzed the ethanol production from corn, for a plant capable of processing 2205 tons of corn/day, **producing 61 million gallons of ethanol/year**.
    - Cost estimated for **plant construction**: **USD 422.5 millions**, whereas **USD 154.5 millions** are referent to the **equipment acquisition**.
    - **Operation costs**: estimated in **USD 65.64 millions/per year for reactants and raw materials'** acquisition, and **USD 4.72 millions/year** for the acquisition of **utilities**.

[13] Humbird, D.; Davis, R.; Tao, L.; Kinchin, C.; Hsu, D.; Aden, A.; Schoen, P.; Lukas, J.; Olthof, B.; Worley, M.; Sexton, D.; Dudgeon, D. *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol. Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover.* U.S. Department of Energy: National Renewable Energy Laboratory (NREL), Golden, CO, USA. Technical Report NREL/TP-5100-47764, Contract No. DE-AC36-08GO28308, 2011.

2. General Ethanol Fermentation Industry

Costs Previously evaluated

- Once this basic costs are **essentially constant**, they:
  - Impact the overall company cash flow;
  - **Do not directly affect the analysis of the viability** in adopting a new instrumentation strategy.
- It is important to evaluate if the premature detection of undesirable disturbances may result on real gains, affecting the cash flow.



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Benefits of Real-Time Monitoring over HPLC

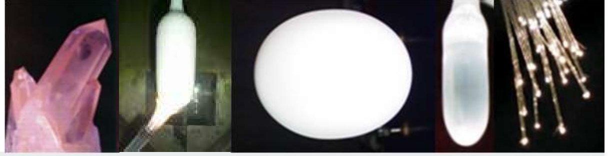
- To demonstrate the benefits of monitoring ethanol bioreactors in real time: conduction of a simulation study of a **fed-batch reactor subjected to a disturbance**.

- The model is derived from the general fermentative reaction:

$$X + S \rightarrow P + (X + \Delta X)$$

- X is the microorganisms concentration (*Saccharomyces cerevisiae*), S is the substrate concentration, P is the products concentration (ethanol) and ΔX an increase in cell concentration due to reproduction. [2,3,14].

[14] Doran, P. *Bioprocess Engineering Principles*, 2nd ed; Elsevier, 2013.



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Benefits of Real-Time Monitoring over HPLC

- Applying the Monod model [2,14], we arrive at the set of Equations (1) through (4) [15]:

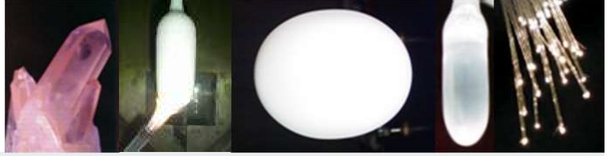
$$\frac{dV}{dt} = F \Rightarrow V = V_0 + F \; (1)$$

$$\frac{dX}{dt} = \mu X - \frac{FX}{V} \; (2)$$

$$\frac{dP}{dt} = q_p X - \frac{FP}{V} \; (3)$$

$$\frac{dS}{dt} = -\mu_s X + \frac{F(S_F - S)}{V} \; (4)$$

[15] Soares, M.C.P. *et al. Blucher Chem. Eng. Proc.* **2018**, 1, 2010-2014. doi: 10.5151/cobeq2018-PT.0532.



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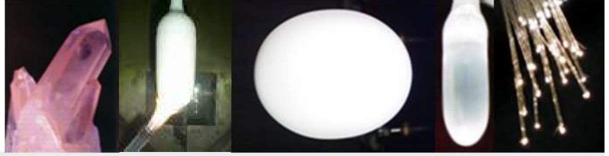
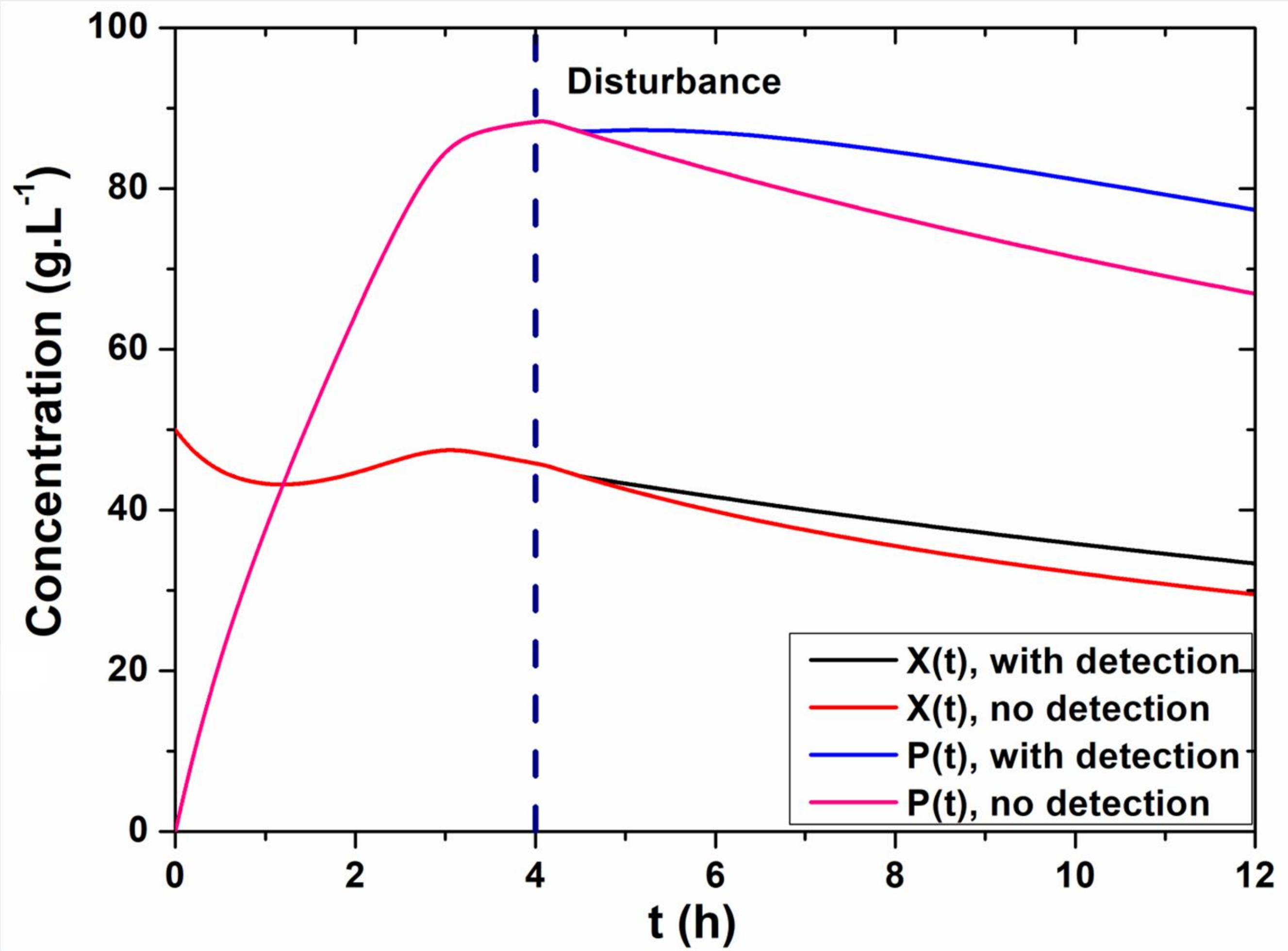
- μ_s = specific rate of substrate consumption;
- μ = specific cell growth rate;
- q_p = specific rate of product formation.
- On fed-batch operation mode:
  - initial concentrations X_0, P_0 and S_0;
  - initial volume of fermentation broth V_0;
  - A constant feed flow F supplies the reactor with fresh substrate with concentration S_F.

Simulation parameters for a fed-batch reactor at 33 °C (temperature for maximum cell growth [3]): X_0= 50 gL^{-1}, P_0 = 0, S_0 = 30 gL^{-1}, V_0 = 1 L, F = 0.66 Lh^{-1} and S_F = 192 gL^{-1}.



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

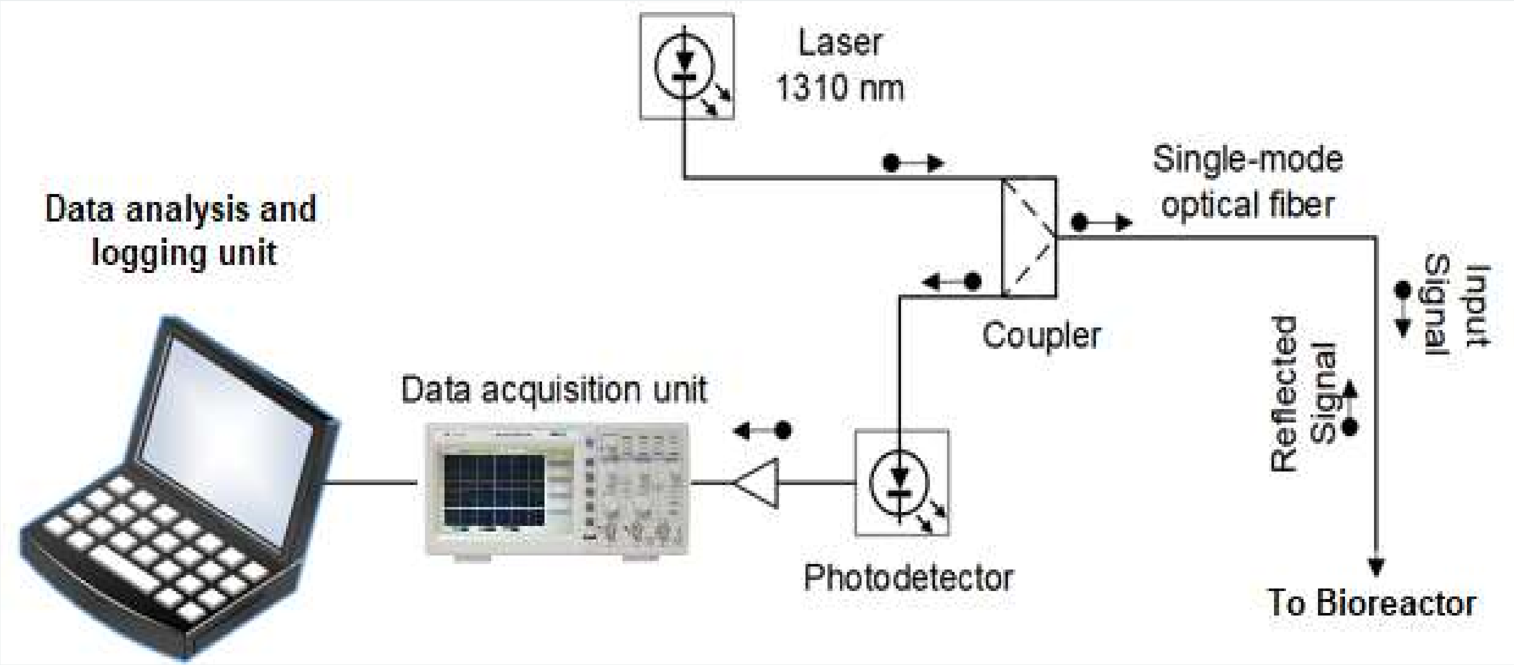


- Without real-time monitoring, (such as using HPLC for process control): X follows the red curve and P the pink curve;
- If in-line real-time instrumentation can detect the disturbance and $\boldsymbol{S_F}$ **is restored to the original levels just after 20 minutes**: X and P follow the black and blue branching curves, respectively.
- After a **12 hours cycle**: 11.6% reduction in the concentration of cells and 13.5% reduction in ethanol production for the system without real-time monitoring in relation to one with in-line real-time assessment.

3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

- For instrumentation costs estimates, the following fiber optic reflectometer setup is considered:



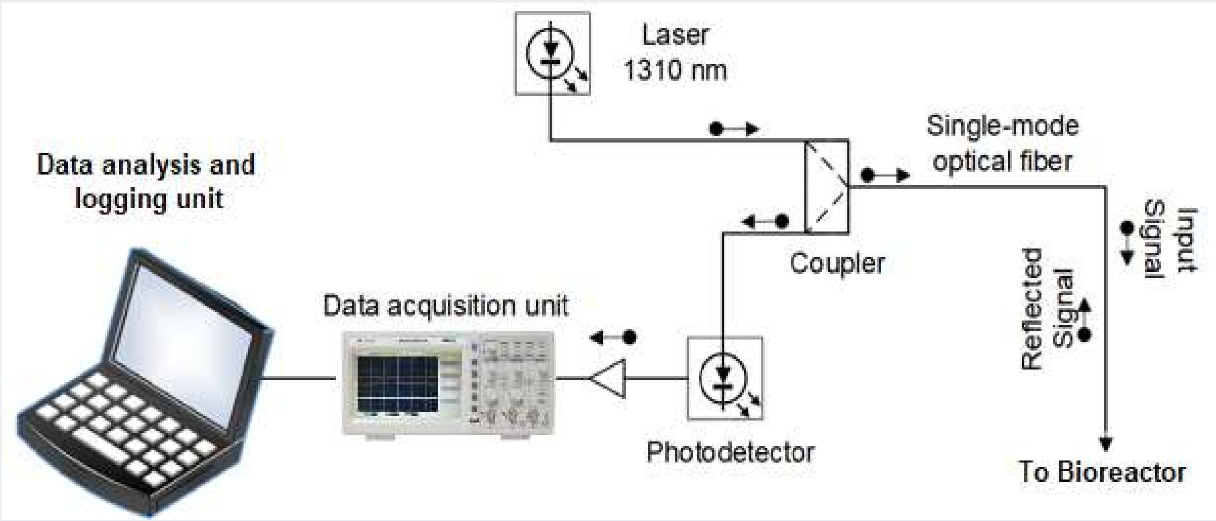
We have previously proposed it for the evaluation of silica nanofluids [16], fast screening of microbial growth parameters [3] and fed-batch bioreactors [17].

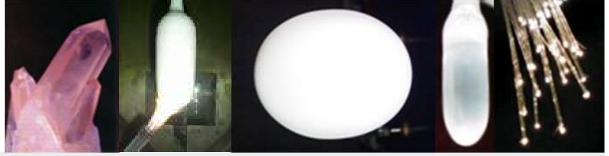
[16] Soares, M. C. P. *et al. Sensors* **2020**, 20, 707. doi: 10.3390/s20030707
[17] Soares. M.C.P.*et al. Proc. 26th International Conference on Optical Fiber Sensors* **2018**. doi: 10.1363/OFS.2018.ThE39.

3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

- Light from a 1310 nm laser is guided to the bioreactor by a single mode optical fiber submerged in the fermentation broth.
- The luminous signal is partially reflected in the fiber/broth interface and is directed to a photodetector using a fiber optic coupler or circulator.





3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

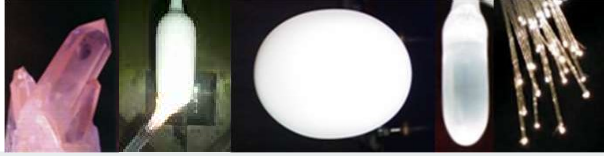
Instrumentation Setup and Costs

- The intensity of the reflected signal is given by Equation (5), which is Fresnel's equation [18]:

$$I_R = k \cdot I_0 \left[\frac{(n_f - n_b)}{(n_f + n_b)} \right]^2 \quad (5)$$

- I_R is the reflected signal intensity, I_0 is the emitted light intensity, k is a coupling coefficient that accounts for optical losses, and n_f and n_b are the refractive indexes of the optical fiber and fermentation broth, respectively.

[18] Saleh, B.E.A.; Teich, M.C. *Fundamentals of Photonics*, 1st ed; J. Wiley & Sons. 1991. doi: 10.1002/0471213748



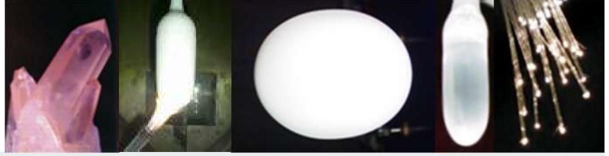
3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

- As demonstrated before in [17], $\boldsymbol{n_b}$ **is directly related to $\boldsymbol{S}$ and $\boldsymbol{P}$** concentrations in the broth, and therefore can be used as the monitoring parameter.

- On the other hand, this sensor is quite robust: as shown on [3,17], if the broth is sufficiently diluted (until ~0.12 gL^{-1}), **the sensor may be used for the direct quantifying of the biomass concentration X**.

- In a very concentrated medium, then, a small sample may be collected to another tank where the probe is inserted and diluted to a known volume to evaluate X.



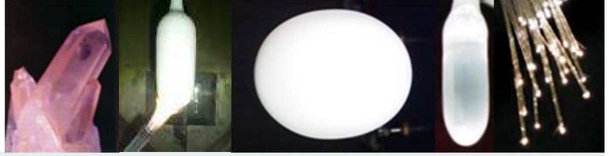
3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

- On the particulate system, light that reaches cells is scattered, generating random fluctuations that are coupled back to the fiber core [3].

- The assessment of X is then based on obtaining the autocorrelation function of the light intensity I_R, $G_2(\tau)$, where t is the instant of the measurement, T is the time of the last measurement, N is the total of measurements collected, and τ is an arbitrary delay:

$$G_2(\tau) = \lim_{T \to \infty} \frac{1}{T} \int_0^T I(t) \cdot I(t+\tau) dt \cong \lim_{N \to \infty} \frac{1}{N} \sum_{j=1}^{N} I(j) \cdot I(j+\tau) \quad (6)$$



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

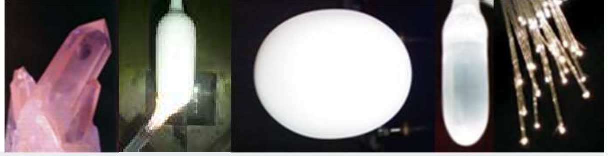
- When the quasi-elastic light scattering (QELS) phenomenon takes place, Equation (6) may be fitted by Equation (7) (Siegert Equation) [3]:

$$G_2(\tau) = \alpha + \beta \exp(-2\Gamma\tau) \quad (7)$$

- In Equation (7), α and β are the fitting adjustable parameters. The adjustment is used to calculate Γ, the **average decay rate, which is directly proportional to X**.

- Therefore, depending on the control goals and on the available infrastructure, the sensor can easily monitor **S/P and/or X**.

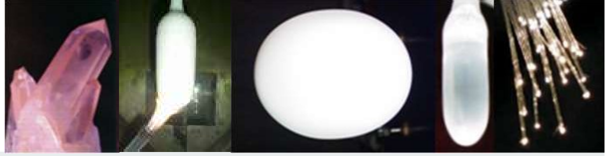
- The assessment can ***be directly performed in-line or in a parallel vessel***.



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

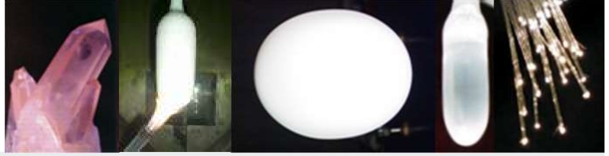
- Table 1: list of all necessary components to implement the sensor setup, along with the cost per unit and supplier.
- The total cost in Brazilian *Reais* (BRL) and United States Dollars (USD) is presented in the last row: **an exchange rate of 5.48 BRL to 1 USD (June 2020) was used**.



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

Instrumentation Setup and Costs

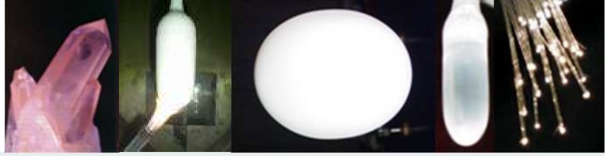
Component	Part No	Currency	Cost/unit	Supplier
1310 nm Benchtop SLD Source	S5FC1018S	USD	3375.12	Thorlabs
1310 nm Coupler	TW1300R2A1	USD	398.22	Thorlabs
1310 nm Circulator	CIR1310-APC	USD	496.70	Thorlabs
Switchable Gain Amplified Detector	PDA50B2	USD	568.12	Thorlabs
50 MHz Digital Oscilloscope	MVB-DSO-50	BRL	2500.00	Minipa
Combined Cost	-	**BRL** **USD**	**29,013.11** **5,294.36**	-



3. Real-time Monitoring of Fed-Batch Bioreactors with Optical Fibers

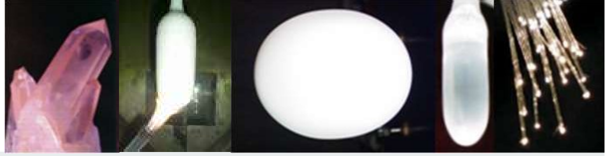
Instrumentation Setup and Costs

- Aside from the individual components, qualified personnel are required to supervise the **implementation** of the optical fiber reflectometer.
- To that end, we are also considering in our calculations the **cost of hiring a qualified engineer for a six months period**.
- According to Brazilian law 4.950-A/66, the minimum wage for a qualified chemical engineer is 9,405.00 BRL (1,716.24 USD) per month for an 8 h working shift, corresponding to 56,430 BRL (10,297.45 USD) over 6 months.
- **1st-year cost of implementing the fiber optic sensing setup** (components + qualified work): **85,443.11 BRL (15,591.80 USD)**.



4. Economic Viability of Fiber Optics Sensing

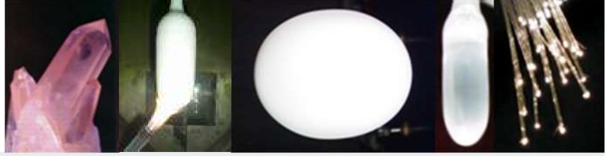
- Comparison of the costs of implementing the in-line optical fiber reflectometer with traditional HPLC analysis.
- We have considered **two scenarios**:
  - in the first scenario, an ethanol plant acquires their own HPLC equipment, but acquisition and maintenance costs for a chromatography column may be prohibitive;
  - and so in the second scenario, the plant hires a third party specialized in providing HPLC analysis services.



4. Economic Viability of Fiber Optics Sensing

First Scenario: acquiring HPLC equipment

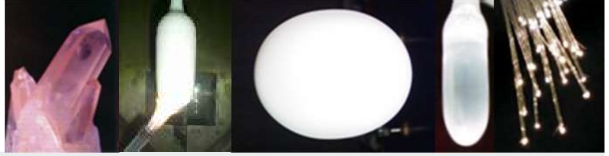
- We have contacted several suppliers and arrived at:
  - an average cost of **27,500.00 USD (150,700.00 BRL)** for a **refurbished** benchtop equipment,
  - plus 200.00 USD/hour (1,096.00 BRL/hour) **installation costs**.
  - Acquisition and installation costs already far surpass those from acquiring all required components for the fiber optics setup (the **assembly of the reflectometer represents and economy of ~81% in relation to the acquisition of a refurbished HPLC**);
  - and the ethanol plant would still have to invest more resources to maintain a suitable stock of reagents for the HPLC column.



4. Economic Viability of Fiber Optics Sensing

Second Scenario: plant hires a third party laboratory

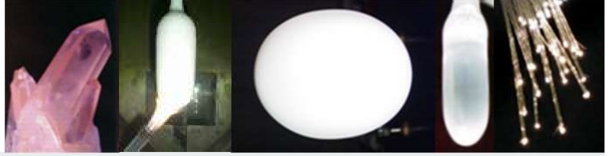
- On Brazilian market the average cost of contracting HPLC analysis services is of **100 BRL/hour (18.25 USD/hour)**.
- It is possible to estimate the yearly cost by considering the following assumptions: plant uptime = 200 days/year; batch runtime = 10 h; number of analysis = 2/day; analysis runtime = 24 h [11].
- This returns a total cost of **960.000 BRL/year (175,182.48 USD/year)**.



4. Economic Viability of Fiber Optics Sensing

Hypotheses used to estimate the cost of hiring third party analysis for a whole year (1st year operation)

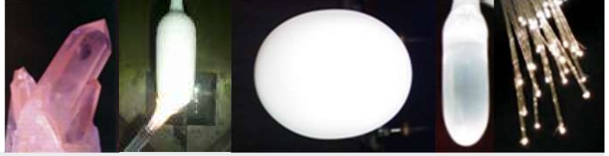
Parameter	Unit	Value	Source
Analysis runtime	Hours	24	Assumed
Plant uptime	Days/year	200	[11]
Batch runtime	Hours	10	[11]
# of contracted analysis	analysis/day	2	Assumed
Exchange Rate	USD/BRL	5,48	(Brazilian Central Bank, BACEN, 26 June 2020)



4. Economic Viability of Fiber Optics Sensing

Comparison between scenarios

Costs	HPLC	Fiber Optic Reflectometer	Currency
Acquisition	150,700.00 27,500.00	29,013.11 5,294.36	BRL USD
1st-Year operation **(third party hired)**	960,000.00 175,182.48	85,443.11 15,591.81	BRL USD



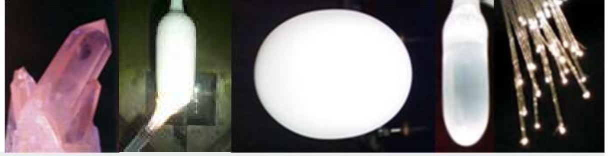
4. Economic Viability of Fiber Optics Sensing

- Finally, we can use this data to calculate, with a Weighted Average Capital Cost (WACC) model [19], **the 5-year cash flow** of an ethanol plant after **implementation of the fiber optic instrumentation** in detriment of contracting HPLC services.

- **WACC**: this methodology consists on **evaluating the contribution of the debt cost (interest on the amount) and the return demanded by shareholders (cost of capital) for the investment to be made** [19].

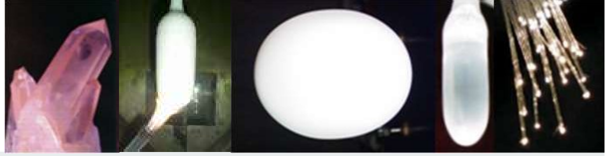
- Then, the project must show return superior than the debt and capital costs to be viable.

[19] Damodaran, A. *Damodaran On Valuation: Security Analysis for Investment and Corporate Finance*, 2nd ed; John Wiley & Sons. 2011.



4. Economic Viability of Fiber Optics Sensing

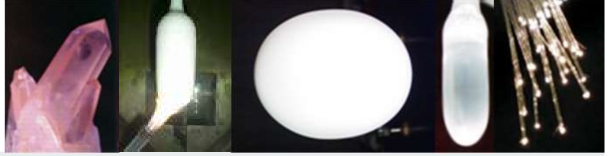
- Considering:
    - depreciation rate of **20% per year**;
    - Net present value (NPV) **discount rate of 2.25% per year** (current 2020 Brazilian rate as per Brazil's Central Bank - BACEN), we can calculate an Internal Rate of Return (IRR).
    - IRR is an estimate of the profitability of the investment in the fiber optic platform.



4. Economic Viability of Fiber Optics Sensing

Main Financial Indicators Obtained

- Internal Rate of Return (IRR): **742.11%**.
- Discounted payback (amount of time for the investment to pay for itself): **0.14 years, or approximately 50 days**.

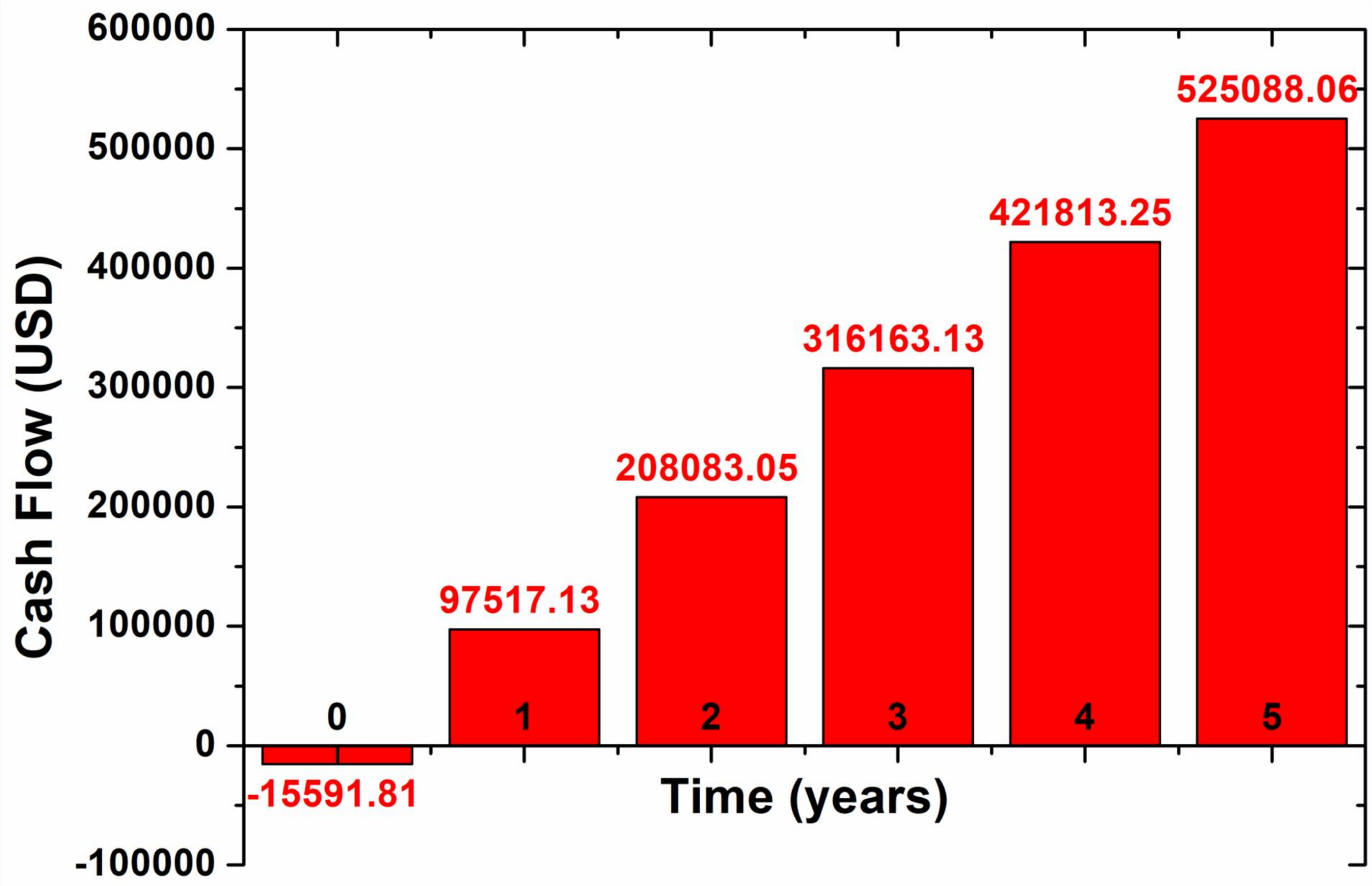


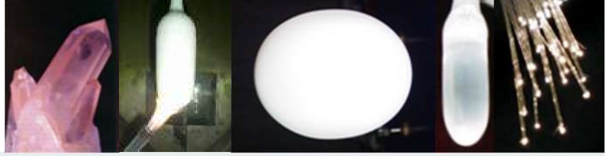
4. Economic Viability of Fiber Optics Sensing

Main Financial Indicators Obtained

Indicator	Value
NPV (BRL) (USD)	2,877,482.55 525,088.06
NPV Discount Rate (% yearly)	2.25%
IRR (% yearly)	742.11%
Spread (%)	739.81%
Discounted payback (years)	0.14

4. Economic Viability of Fiber Optics Sensing

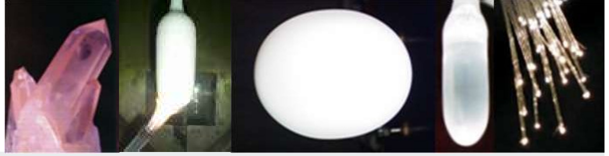




4. Economic Viability of Fiber Optics Sensing

Other monitoring alternatives

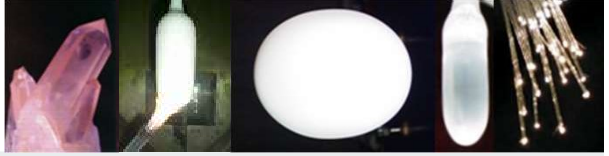
- For the sake of completeness, we also provide a quick assessment of alternative solutions to HPLC and the proposed fiber optic reflectometer.
- That is important especially for the case where the industry only wishes to access glucose concentration in the fermentation broth.
- There are two main choices in this case, an optical density measurement with a spectrophotometer or the use of colorimetric kits such as [4].



4. Economic Viability of Fiber Optics Sensing

Other monitoring alternatives

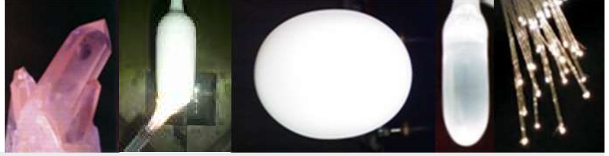
- Spectrophotometers: available for reasonably low prices starting at only a few thousands of dollars.
  - Disadvantages of requiring regular sampling of the broth, and the measurements are not as direct, **since different concentrations of other components will also affect the optical density**.
- Use of colorimetric kits:
  - Also requires taking samples of the broth in regular intervals.
  - The sample is mixed with the kit and analyzed on the spectrophotometer for a given wavelength, using a calibration curve.



4. Economic Viability of Fiber Optics Sensing

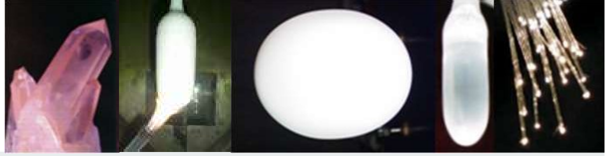
Other monitoring alternatives

- Use of colorimetric kits:
  - If each test should be conducted once an hour (which is simple to do with the optical fiber setup):
    - Each kit in [4] can be used for analyzing 20 samples.
    - Considering 12 analysis/day, 200 days per year, it results in a total usage of 120 kits per year, or about **9,360.00 USD/year (51,292.80 BRL/year)** in costs.
    - In this scenario, the costs of the kits alone, not including inflation and other required consumables or analytical equipment, **is almost twice the costs of acquiring all the components for the fiber optic reflectometer**.
  - It is clear the fiber optic sensing approach is still very attractive when compared to optical density and colorimetric analysis.



5. Conclusions

- There is considerable medium- and long-term financial gains from implementing fiber optic systems for monitoring fed-batch bioreactors as a substitute for traditional HPLC analysis.
- The average Brazilian ethanol plant is expected to see a **return for their investment in about 50 days, together with a 5-year NPV of 525,088.06 USD, corresponding to an IRR of 742%**.
- Similar results are expected for any industry worldwide that utilizes HPLC for monitoring their bioreactors.
- The proposed fiber optic setup is comparatively a very low-cost analytical solution:
  - It does not demand highly trained personnel to operate and maintain it;
  - Fiber optic systems are highly flexible and can be adapted for most industrial needs using commercially available components, being able to detect from sucrose to even biomass concentration [3,12-14].



Thank you for your attention!

Questions?

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