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Immobilized Cell Bioreactor Industrialization in the Development of an Innovative Optical Biosensor Technology

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Abstract

This commentary shows the development of a new optical biosensor, based on cell immobilization of *Pseudomonas Fluorescens HK44*, in nano and micro-tubular cellulose (TC) and a mixture of carbohydrate nanotubes (CHNTs) and carbohydrate micro-tubes (CHMTs). Methodology follows, this biocatalyst can be industrialized with the use of a single tank immobilized cell bioreactor (ICB). A techno-economic analysis was conducted within the framework of it by designing a process flow sheet with mass and energy balance. According to its case study, the investment is 227,800 euros, and the daily production cost is 1434 euros, with a maximum daily added value of 25,000 euros. The discussion revealed that novel research proposals and a novel study concept are being developed in the field of biosensors. The results are supported by papers published on ICB area development. The problem that leads to this commentary is industrialization of ICB, in the case of a simple biosensor development using immobilized cells and it is the objective.

Keywords: Bioreactor, Biosensor, Cell, Immobilization, Optical

Introduction

Immobilized cell bioreactor development has been studied in the past. Specifically, Continuous [1], the multistage fixed bed tower bioreactor (MFBT) [2] and two layer fermentations [3], fluidized bed [4-7] based on cell immobilization [8], membrane [9] and biofilm reactors [10] have been developed. Flocculated engineered *Saccharomyces cerevisiae* cells have been used in fluidized bed bioreactor systems for continuous lactose fermentation [11,12]. Immobilized cells have been used for the production of chemicals [13]. Moreover, artificial bio-electrodes have been developed by immobilizing *Geobacter sulfurreducens* cells on composite materials [14]. Continuous and batch bioreactors for alcoholic fermentation by immobilized cell on tubular cellulose (TC) have also been reported [15,16]. TC has various applications in ICB related to nano and microstructure of tubes [17]. Likewise, freeze dried immobilized cells have been used in wine making [18] and brewing [19]. TC produced after delignification of sawdust was employed for the production of carbohydrate nanotubes (CHNTs) and carbohydrate micro-tunes (CHMTs) after its hydrolysis using cellulases enzymes [20]. Finally, researchers have constructed an optical biosensor based on cell immobilization of *Pseudomonas fluorescens HK44* to thick silica films prepared using pre-polymerized tetramethoxysilane [21]. Taking into consideration the aforementioned publications on ICB development and having the potential to lead to the industrialization of one of them, the combination of bio-electrodes and optical biosensor creation employing immobilized cells can lead to the industrialization of ICB. The industrialization of ICB in the case of optical biosensor development using immobilized cells to develop a simple biosensor is a big problem. This can be supported by a low-volume ICB, and due to the high added value of the product [22] and the support of CHNTs, CHMTs, or nano and micro TCs, the ICB could operate as a fluidized bed bioreactor system. Therefore, the aim of this commentary is to prove that the optical biosensor development based on cell immobilization could be produced through a fluidized ICB in a single tank and can be industrialized in a cost-effective way. It will be examined through a techno-economic analysis in the frame of this commentary.

Optical Biosensor Based Technology

Rational

Previously published results in various interdisciplinary papers can be used to promote the development of optical biosensors. Specifically, the contribution of this commentary is supported on CHNTs, CHMTs, and TC production [23], cell [15] and enzyme immobilization in TC [24], freeze drying of immobilized cells [18,19], and thermal drying of immobilized cells [25]. Furthermore, it will be supported on specific immobilization of *Pseudomonas fluorescens HK44* on tetramethoxy silane for optical biosensor development [21] and on bio-electrode development [14]. The combination of those interdisciplinary approaches will result in the development of an original optical biosensor based on cell immobilization of *Pseudomonas fluorescens HK44* in CHNTs, CHMTs, and TC. The originality of this technology is attributed to the combination of the use of CHNTs, CHMTs, and TC, which are competitive with carbon nanotubes (CNTs) and are used for electrochemical biosensors [26], with cell and enzyme immobilization and thermal drying of immobilized cells and enzymes. The techno-economic considerations of biosensor development will demonstrate why CHNTs, CHMTs, TCs, or mixtures of these materials will be employed.

Carbohydrate nano tubes (CHNTs), and carbohydrate micro tubes (CHMTs) based biosensor

The original optical biosensor is based on tubular cellulose (TC), and products of its hydrolysis such as CHNTs and CHMTs can be employed for cell and enzyme immobilization [15,24]. The technology will include cell immobilization on CHNTs, CHMTs, and TC after encapsulation of cells in tubes of CHMTs and TC, thermal drying at 37°C or freeze drying. After preparing this immobilized biocatalyst, the powder will be put into a petri dish and rehydrated with water containing a small concentration of glucose or plain water. *Pseudomonas fluorescens HK44* is activated by some compounds that induce bioluminescence, which can be measured by a fluorometer. An alternative version of the original optical biosensor can be prepared by enzyme immobilization in CHNTs, CHMTs, and TC. The enzymes will be all that are contained in the cell of *Pseudomonas fluorescens HK44* and will be prepared after breaking the cell wall.

This enzymatic biosensor will also operate, as aforementioned, with the immobilized cell optical biosensor. The purpose of these cell- or enzyme-immobilized biosensors is to analyze salicylates and naphthalene [21]. The significance of this analysis is attributed to salicylates, which are contained in aspirin-containing drugs. Furthermore, naphthalene is an aromatic hydrocarbon; therefore, many polycyclic aromatic hydrocarbons can be examined for quantitative determination in foods and general in industry. The commercial product of an optical biosensor will be a kit containing the dried powder of immobilized *Pseudomonas fluorescens HK44* in CHNTs, CHMTs, or TC or a kit containing immobilized *Pseudomonas fluorescens HK44* enzymes in CHNTs, CHMTs, or TC. Thus, the techno-economic evaluation of biosensor production will be done through a case study developed within the framework of this commentary.

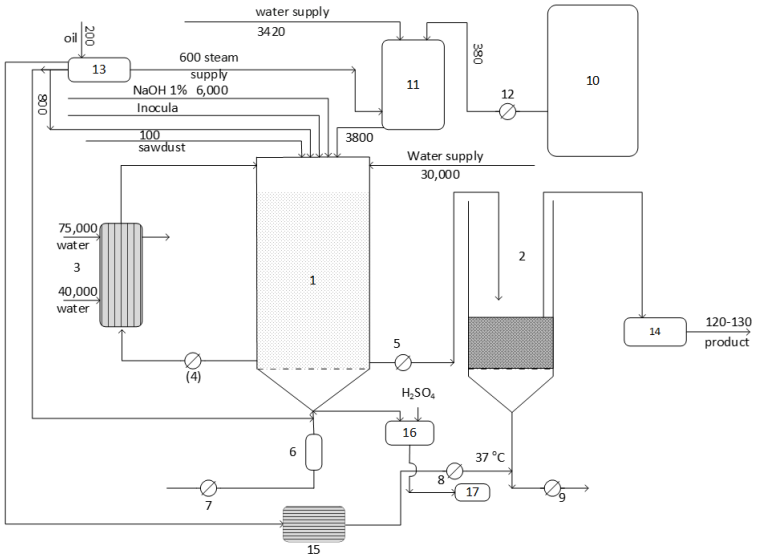
Immobilized cell bioreactor (ICB) and optical biosensor technology development

Biocatalysts, biosensors, and bio-electrodes using immobilized cells and enzymes demand low-volume bioreactors mainly due to their high added value [22]. Single-tank batch bioreactors using free cells are extensively used in industrial-scale bioprocessing. Moreover, cell-on-cell immobilization leads to cell flocculation can operate as a fluidized bed bioreactor system which needs a single tank bioreactor, as it is applied using free cells. However, the immobilization of *Pseudomonas fluorescens HK44* in CHNTs will be performed in solution, by immobilizing CHNTs on the cell wall through hydrogen bonding. By encapsulating cells in tubes, the same microorganism will be immobilized on CHMTs and TCs. Using CHNTs is obviously necessary for a single-tank bioreactor, while employing CHMTs or TC fluidized bed systems is feasible to operate due to the light nature of cellulosic materials. Consequently, ICB can be industrialized for the production of CHNTs, CHMTs, or TC-supported *Pseudomonas fluorescens HK44*. In the case of *Pseudomonas fluorescens HK44* enzymes immobilization in CHNTs will be done by their encapsulation in tubes, and in CHMTs or TC as well; Therefore, the enzyme immobilization in these supports' bioreactors can be industrialized.

Techno-economic evaluation of optical biosensor through ICB

Description of nano and micro-TC based optical biosensor production: This optical biosensor is produced as a powder by delignified cellulosic biomass forming nano- and micro-tubes of TC. Immobilized *Pseudomonas fluorescens HK44* cells may be immobilized within the delignified cellulosic biomass. The wet immobilized biocatalyst is then thermally dried using a hot air stream, and at least 4 g of dry nano and micro-TC are packaged in a small paper bag. To create the optical biosensor, a thin layer of 4 g of dry nano- and micro-TC-supported *Pseudomonas fluorescens HK44* cells are deposited in a petri dish. Subsequently, an aqueous sample solution will be analyzed, and the bioluminescence will be measured using a fluorometer. Likewise, starch gel can be added to create a cohesive mass; in this case, thermal dehydration occurs as previously described. As shown in **Figure 1**, the process flowchart is used to conduct a techno-economic analysis to determine whether technology is cost-effective.

Industrial design for the production of optical biosensor: In the single-tank bioreactor with a capacity of 7,000 liters



1. Single tank bioreactor (7,000 L) and delignification
2. Filter and dryer (70,000 L)
3. Plate heat exchanger (30,000 kcal/h)
4. Pump (5HP)
5. Pump (5HP)
6. Sterile filter (60 m^3/h)
7. Air pump (1000 m^3/h)
8. Air pump (10,000 m^3/h)
9. Pump (3HP)
10. Molasses tank (10 m^3)
11. Molasses preparation tank (10 m^3)
12. Pump
13. Boiler (200 kg oil)
14. Packaging (25000/day)
15. Heat exchanger
16. Lignin precipitation reactor (10,000)
17. Lignin filter (7,000 L)

Figure 1. Process flow sheet with mass and energy balance (kg) for the production of an optical biosensor material.

(1), the delignification process can be conducted and will follow the immobilization of *Pseudomonas fluorescens HK44* cells in nano and micro-TC. The bioreactor is loaded with 100 kilograms of sawdust, followed by the addition of 6,000 liters of 1% NaOH. The delignification is conducted for three hours at 100°C with steam from boiler 13 [27]. The liquid is then removed, and the delignified nano and micro-TC is rinsed with 30,000 liters of tap water to cool the bioreactor and remove the NaOH. Subsequently, 3-3.5 °Be sterilized molasses from tank 11 and 0.5–1 kg pressed cells of *Pseudomonas fluorescens HK44* produced in the laboratory bioreactor are inoculated. Air passes through the sterile filter 6 and is supplied to the bioreactor for cell growth and cell immobilization. To keep the temperature constant at 30°C, the bioreactor is cooled by the plate heat exchanger 3. After about 15 hours, the bioreactor content is pumped to filter 2 by the pump 5. The wet nano and micro tubular cellulose is thermally dried with a hot air stream of 37°C heated in the heat exchanger 15. To facilitate drying, it is suggested that the biocatalyst be divided into four floors [28,29]. By this process, 120–130 kg of dry immobilized biocatalyst is produced.

Investment (Euros):

Table 1. Investment cost. Equipment cost for plant installation.

Machinery	Price (€)
Single tank bioreactor	5,000
Filter and dryer	15,000
Pump	1,000
Pump	1,000
Sterile filter	15,000
Air pump	5,000
Air pump	5,000
9. Pump (800)	800
Molasses tank	10,000
Molasses handling tank	10,000
Pump	5,000
Boiler	5,000

Packaging	50,000
Heat exchanger	15,000
Lignin precipitation reactor	10,000
Lignin filter and dryer	15,000
Total	227,800

Production cost Euros/day):

Table 2. Daily production cost. Parameters affecting the production cost.

Parameter	**Cost (€/day)**
Raw material	114
Labor cost	850
Thermal energy	200
Consumables	160
Electricity	**30**
Investment payment	**30**
Water requirements	**50**
Total	**1434**

Discussion

The article based on a single tank ICB, leads to industrialization of immobilized cells, for the production of an optical biosensor material packaged in small paper bag each containing 4g. Therefore, the daily added value created is raised to 25,000 €. Taking into account the daily production cost of 1,434 € according to **Table 2**, it is obvious that the process is cost effective by a substantial margin. Moreover, because the required investment to implement the plant is estimated to be 227,800 euros (**Table 1**), the investment payment in this case will be obtained within one month. Food ingredients and pharmaceutical active compounds which are contained in drugs, could be examined for possible analysis by this biosensor. Such compounds could be aspirin and derivatives of salicylic acid that are contained in aspirin containing drugs and polycyclic aromatic hydrocarbons such anthracene, phenantrhene, fluoranthene, pyrene, fluorene, chrysene, acenapthene, benzanthracene, benzofluoranthene, benzopyrene, dibenzoantracene, methyl holanthrene, 1-methylphenanthrene, 9-ethylphenanthrene, 5-methylchrysene, 6-ethylchrysene, 1,4 anthraquinone, tetrachloro-dibenzo-dioxine (TCDD), etc. In the case that CHNTs are utilized in the production of an optical biosensor, salicylates and naphthalene derivatives will be entrapped within nanotubes. This nanomaterial can be immobilized on the cell wall of *Pseudomonas fluorescens HK44*, allowing bioluminescence to be induced. In this case, the biosensor will be a CHNTs-supported cell that has been thermally desiccated. **Figure 1**'s process flowchart requires the addition of a new bioreactor for hydrolyzing TC to CHNTs and CHMTs using cellulase enzymes (Panitsa et al., 2023), as well as a second bioreactor for cell immobilization. As with cellulosic biomass, nano and micro-TC, CHNTs, and CHMTs are produced using an abundant, sustainable, and low-cost raw material. Advantages of this biosensor are (i) simple product biosensor, (ii) technology for its production, (iii) analytical method, (iv) the very abundant raw material to produce TC, CHNTs, and CHMTs which leads to a cost-effective process.

New Aspects Raised by the Commentary

The commentary demonstrates (i) that the industrialization of single-tank ICB using immobilized cells in TC, CHNTs, and CHMTs would enable the industrialization of optical biosensors. (ii) Prepare a research proposal to investigate the effect on bioluminescence of various compounds encapsulated separately in nano and micro-TC and in a mixture of CHNTs and CHMTs. Similarly, this leads to a general concept of using TC, CHNTs, and CHMTs in the development of biosensors. Consequently, a single ICB tank can be used to industrialize immobilized cells due to the small size and minimal cost of the manufacturing facility.

Conclusion

The commentary showed that a new optical biosensor based on cell immobilization of *Pseudomonas fluorescens HK44*, in nano- and micro-TC, CHNTs, and CHMTs is possible. It is attributed (i) to cell immobilization, which has been proved experimentally by research that has been published; (ii) to the fact that ICB is a single tank bioreactor system; (iii) to the low volume of ICB and the size of the factory. (iv) Low production costs in comparison with added value are created, and low investment is necessary as well. The commentary is promising for new research proposals and concepts.

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