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Substitution of commercial enzymes for native ones from Ecuador: a university innovation

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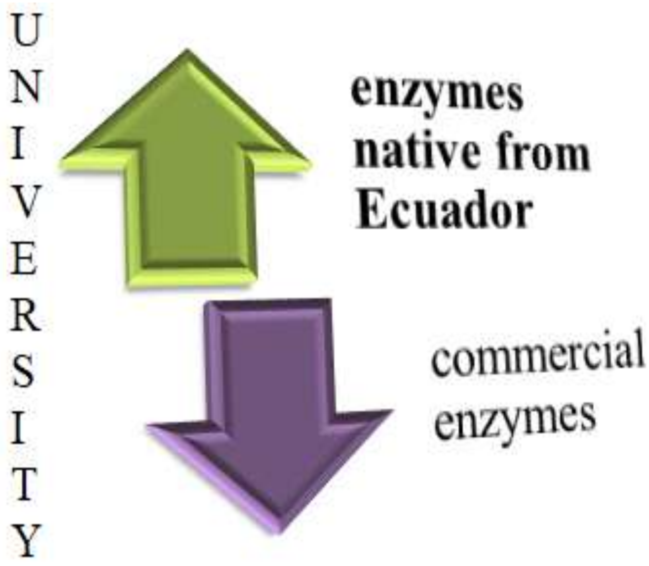
Graphical Abstract



Abstract.
The essence of one of the university's extensionist functions lies in sharing knowledge and fostering permanent dialogue with all actors in society. University is the repository of an arsenal of information that, if used efficiently, can contribute to social development, a purpose that becomes its fundamental objective and the reason upon which its relevance is based.
It has been proven that the effectiveness in the delignification of bagasse (cellulignitic mass) is much greater in enzymatic hydrolysis, by avoiding the use of large amounts of energy and chemicals that make hydrolytic processes more expensive. This condition places them in an advantageous position by allowing the saccharification of the cellulose present in the lignocellulitic waste, with the aim of obtaining in this way a variety of chemical products. This article deals with the possibility of assessing the substitution of commercial enzymes by native enzymes from Ecuador and their economic and

social impacts, from an academic perspective in which an alternative option is promoted for the creation of intangibles that make possible the strengthening of the University's role to advance towards the model of sustainable development, based on the usefulness of knowledge.
Keywords: enzymes, lignocellulitic waste, hydrolytic processes, intangible

Introduction

Greek mythology tells that Prometheus gave wisdom to men. But the reality is that mankind has developed knowledge. At present, it is essential to steer wisely the direction of knowledge, since the vertiginous advance of science and technology has placed humanity at the peak of good and evil [1].

It is necessary to better know the technical processes, their impact and their interrelations with the economic evolution so that both individuals and governments can understand and influence technical progress and channel it [2]. Science, turned into a productive force based on the generation of knowledge to search for solutions to these problems, will be the basis that can ensure sustainable development in the mid term and can also solve the development issues [3].

It is important to remember Fidel Castro's immense efforts to make Cuba a country of scientists, an idea that he tried to expand for the rest of the peoples of America that have opted for the noble purposes of sustainability, because, as he stressed, teachers must feel the need to know where they their reasonings are heading, “because all of us who teach have a task ahead: to lead those who come to us and guide them along the dark path”.

In the recently closed 11th. International Congress of Higher Education, Dr. Eusebio Leal Spengler, referring to the personal commitment of today's science people, emphasized that this commitment cannot be passive and said that "It is necessary to live, create and found, and each one of us must do it to the best of our ability”.

This warning calls for everyone, from the position they occupy in their professional range, to take into account how to contribute for their work to lead to solve a problem in society, even more so when these times require from the committed professional, their contribution to the sustainability of the world.

Enzymatic hydrolysis: overview

Enzymes are degrading compounds. They belong to globular proteins and work as functional units of cellular metabolism, act by accelerating biological chemical reactions as regulators of metabolic pathways and for this purposes they need optimal pH and temperature values.

There are synthetic enzymes and also enzymes of microbial origin. In this study, those of microbioal origin that are capable of degrading in a process known as hydrolysis are studied.

The enzymatic hydrolysis of cellulose is based on the ability of fungi and bacteria to produce a set of extracellular enzymes capable of degrading the D-glucose molecules [4] that make up the structure of cellulose.

Enzymatic hydrolysis is gaining ground (Morales et al., 2010), because the acid hydrolysis used to obtain fermentable sugars [5] has generated some problems in terms of cost and performance.

Although a large number of microorganisms (fungi, bacteria and actinomycetes) are capable of degrading cellulose, such as the cellulolytic microfungi belonging to the genera Trichoderma and Aspergillus that are producers of remarkable cellulases [6], there is the problem that only a few of the microorganisms produce significant amounts of free enzyme fractions capable of complete cellulose hydrolysis *in vitro*.

The continuous search for highly secreting organisms of cellulolytic enzymes to optimize production [7] is a priority of industrial biotechnology applied to the obtention of reducing sugars.

The understanding of these microorganisms not only plays a very important role for their industrial applications but also in the recycling process of several products such as cellulose in the biosphere [8].

Some microorganisms with potential degrading capacities of the biomass material have also been found in the symbiotic microbiota of animals such as the earthworm [9-11]. The modern tools of metagenomics, interactome, metabolomics and pyrosequencing manage to study the different degrading genomes existing in the intestines of the animal, which can be highly efficient in enzymatic hydrolysis. However, the economic cost of these technologies is quite high.

For the complete enzymatic hydrolysis, the activity of three cellulolytic enzymes is required, consisting of endo and exoglucanases supplemented with ß-glucosidases whose objective is to ensure the breakdown of the cellobiose molecules generated by the action of the other enzymes [11].

In this regard, it is difficult for a single microorganism to naturally produce the cellulolytic enzymes needed for successful degradation. The synergistic action of several microorganisms is important [12].

This has also led to the need to create genetically modified microorganisms [13], taking advantage of technological tools that were known in the 70s, with the discovery made by Stanley Cohen and Herbert Bayer using the isolation and insertion of a genome into another [14]. That is why they reprogram organisms producing cellulolytic enzymes to obtain high amounts of the enzymes under study.

Despite the fact that with the advent of modern genetics, the costs of sugar production from these recalcitrant fractions have been significantly reduced [15,16], the process is still not efficient at an industrial scale.

There is a lack of biocatalysts that can tolerate both laboratory and industrial conditions, and the enzymes of interest that are produced show low activity and therefore they are consumed in a high amount.

In order to reduce the cost of enzymes, new enzyme sources can be investigated and / or enzymes can be recycled using their relatively high stability and high affinity for cellulose.

In this regard, at a worldwide and Ibero-American level, more than half of patents related to the study of microorganisms or enzymes [17] are classified under the code C12N, representing 50% and 53%, as observed in the Figure 1.

Enzymes

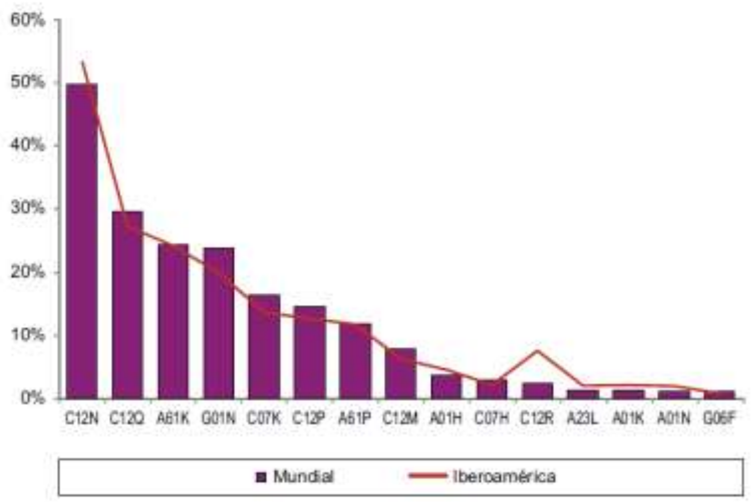


Figure. 1: Main classification codes (4 digits) for the world and Iberoamerica for Biotechnology (accumulated 2007-2013).

It is a priority to study the interaction of enzymes that degrade lignin (laccases) and cellulose (cellulases) along with xylanases to improve the process of biomass degradation and it is convenient to address the following question: Why using enzymes to degrade biomass?

Biomass degradation through enzymes

Biomass is the most abundant raw material on the planet. It is considered to play the same role as petroleum in the 20th century.

Although several studies on its management have been carried out, a significant practical application has not yet been found [13]. However, it is considered a by-product of enormous availability that is generated from different sources such as agroindustrial [18].

Plant biomass represents one of the most important sustainable sources of organic fuels, chemicals and materials. The use of cellulosic biomass continues to be a topic of global interest in view of the rapid depletion of oil [19] . and the high potential chemical energy of this raw material.

The bioconversion of lignocelluloses is being studied as a means to alleviate the shortage of food and energy, and to reduce pollution. However, the conversion of biomass based solely on the use of cellulose has few opportunities to become economically attractive [20].

The search for new enzymatic crudes of microbiological origin, which allow to take advantage of the cellulolytic capacity of fungi and bacteria existing in forest degradation material, urban solid waste and agricultural residues, in the context of a biorefinery, constitutes an interesting alternative to the challenges that the current model of development imposes.

Consequently, the characterization of the enzymatic sources, their optimization in the hydrolysis process and their recirculation is a priority objective, since the lignocellulosic biomass also has enzymes [21].

The technological proposal on which the study is based, from the perspective of university science, establishes a strategy in which new enzymatic crudes are generated from traditional and modern biotechnological techniques and information is offered on the mechanisms of action of the enzymes studied.

The method to be used includes a study of technological surveillance, the development of experiments with input and output variables to understand the parameters that affect the enzymatic production and the types of enzymes produced.

However, like with all novel studies, there are contradictions to be solved by means of the representation of the phenomenon in mathematical models and the best conditions of obtaining the product with degrading capacity are determined for further experimental verification.

What impacts can the technological proposal generate?

According to the study carried out, the expected outcomes are not only thought to respond to the scientific-technical realm as explained, but also to the economic, social and environmental scope.

The reasons underlying this approach are first due to the fact that the raw material used does not compete with food biomass, an important aspect if we take into account the need to find renewable sources of materials to obtain products with high added value, which can be considered as innovative, of greater profitability for their use and that do not compromise the nutrition of the population.

This alternative contributes to reducing the volume of waste (approximately at a 40%), an aspect that reduces the problems involving its treatment, management and risk of diseases. It is worth remembering that the different types of urban, agricultural and forest waste, have many well known negative efects on the environment, such as production of greenhouse gases that contribute to climate change; soil deterioration due to deforestation; leachates that pollute groundwater, etc. To counter these negative impacts, in addition to the many measures put in place by the different countries, this study may well offer an additional tool, especially considering a reduction in the pressure caused by the agricultural frontier expansion, aspect that correspond to the actions aimed to comply with the Sustainable Development Goal 12 of the United Nations to ensure sustainable consumption and production patterns. This aspect has also been discussed at the UN Conference on Sustainable Housing and Urban Development, “Habitat” III that took place in Quito in 2016. Ecuador's National Plan of Good Living promotes sustained, inclusive and sustainable economic growth, for which this proposal would help by means of decreasing the production costs, therefore increasing profitability and stimulating investment.

Finally, it is important to highlight that these topics are a support for the development of high impact studies and higher education that guarantee the relevance of university scientific research focusing on responding to the new emergencies, demands and challenges of the society.

Conclusions

1. It is a challenge for university science to play its role as a knowledge generation center, characterized by its quality and relevance, backed by solid and reliable processes, and striving to achieve a harmonious integration with all social actors that really drives local and national development.
2. The existence, proven through scientific research, of new enzymatic crudes with high catalytic activity, makes feasible their incorporation into the industrial design of a technology of in situ production of enzymes to decompose biomass waste in a context of product diversification.
3. Obtaining an enzymatic technology of its own is a powerful tool. The fact that it is the result of international collaboration and cooperation, mainly among developing countries, involving universities, is a triumph for the entire society of the countries involved, which highlights the importance of establishing alliances with the strategic objective of advancing the development processes, with greater insertion in social and productive dynamics.
4. The introduction of this result will benefit both the development of knowledge that opens the possibilities of obtaining new products and technological facilities, and the creation of employment and probable social benefit products.

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