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Comparison of Two Different Educational Approaches to the Experimental Teaching of a Luminometric-based Analytical Method

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Abstract

In spite of the increasing presence of luminometric-based analytical methods, their application to the laboratory training of science undergraduate students is almost anecdotic. In the last years, we have implemented at the University of Malaga a new laboratory experiment devoted to illustrate the principles and applications of bioluminescence to undergraduate chemistry and biochemistry students. With the final objective of detecting microbial contaminations in water samples, students quantify ATP by means of the luciferase-catalyzed reaction. We have applied two different educational approaches to carry out this lab experiment: 1) By using a short protocol, carried out in a single laboratory session, in which students follow a "recipe", and 2) In a Problem Based Learning (PBL) context, as a full practical project developed by students during a 7 weeks period.

Our results show that those two experimental approaches may be useful to teach the principles and applications of bioluminescence, helping to develop some foundational scientific competencies that characterize the Process of Science and Quantitative Reasoning core competencies. They include evaluation and use of scientific information, critical thinking, performance of basic calculations, drawing of graphs and data presentation and interpretation. Nevertheless, the PBL learning objectives are much more ambitious, promoting the development of a series of additional skills. They include those learning objectives related to the simulation of a real-world problem, which requires the students to develop skills related to the treatment of information or the design and practical implementation of a protocol, with a high level of autonomy and personal initiative. Working in groups helped the PBL students to cultivate the Communication and Collaboration core competency, by interacting and communicating the research results to biology experts and to the general public. Many of these competencies have an intrinsic relationship with the future development of students as future teaching, technical or scientific professionals.

Keywords: PBL, Luminometry, Undergraduate students, Laboratory experiment, Educational strategies

1. Introduction

There is a lack in the formation of our chemistry and biochemistry undergraduate students, caused by the shortage of practical laboratory experiences to teach the basis and applications of luminometry, a technique that is increasingly used in many experimental and health sciences laboratories. Bioluminescence is a phenomenon observed in some animals, such as fireflies, which emit light for the recognition and attraction of their mates. This light is the result of a chemical reaction catalysed by the enzyme luciferase (EC 1.13.12.7), which requires the presence of the luciferin substrate and ATP, which can be quantified by means of this enzymatic reaction (Fig.1) [1].

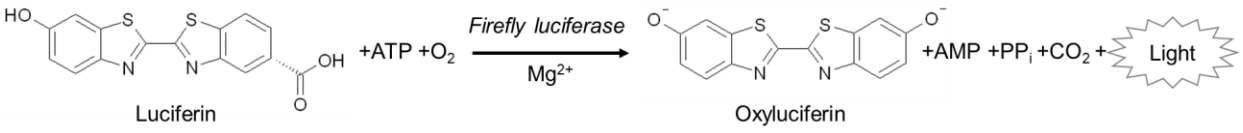

Figure 1. Luciferase-catalysed enzymatic reaction

The correlation of ATP concentration and bacteria content is the basis for the development of luciferase-based rapid methods to detect microbial contamination in drinking or stored water, skipping the long delays required by the traditional microbiological methods [2]. Throughout the two last academic years, we have implemented at the University of Malaga (Spain) a new laboratory experiment focused to illustrate the use of bioluminescence in analytical chemistry to undergraduate chemistry and biochemistry students. Based on the luminometric measurement of ATP for the

detection of bacterial contamination in water, it has been implemented in two different formats, a short protocol and a complete PBL experience.

2. Brief description of the two versions of the laboratory experiment

2.1 Short protocol

Performed by twenty 4th year chemistry students in the 2018-19 course, and by sixty 3rd year biochemistry students in the 2019-20 course, within the "Applied Biochemistry" subject. The characteristics of those groups of students conditioned the design of the protocol, so that it could fit into a short laboratory session (1-2 hours), with an estimated dedication of no more than two homework hours for the students.

After an introductory lecture in which the teacher summarized some key principles and applications of luminometry, students followed the supplied protocol to measure ATP concentrations by using luciferase. They got results to draw a calibration curve and analysed water samples from different sources. They presented their results in a final report, discussing the applicability of this method to the detection of microbial contaminations in tap water, and basing on bibliography to suggest some improvements to the protocol they had followed.

2.2 PBL

This experience was completed in the 2019-20 course by the ten 4th year biochemistry/biotechnology students that were enrolled in the subject “Advanced Instrumental Techniques”. Under the guidance of the responsible teacher in the role of the facilitator, those students carried out independently the activities detailed below, developed over a period of 7-8 weeks.

Students, worked in groups of 5, playing a role of “corporations” that must offer a solution for a given technical issue that has to be resolved throughout the course. A meaningful driving question would guide their work: To implement a rapid method to detect microbial contaminations in tap water, at an affordable cost allowing this type of routine testing. In this question, the main requirements related to the desired analytical method were presented: quickness (results must be available in a few hours), affordability (a high number of water samples should be routinely assayed) and sensibility (sample volumes should be restricted to a few millilitres). Following these guidelines, students performed a complete bibliographic search, leading to the selection of "chemical" methods that do not require several days for their completion, unlike traditional microbiological assays. Once the potential solutions to the problem were identified (mainly methods based on the enzymatic measurement of ATP), students carried out the necessary steps for their subsequent practical implementation. They included the identification of the required reagents and instrumentation, the design and the execution of the experimental protocols leading to the measurement of ATP by means of the luciferase reaction. Post-lab activities included the drafting of both, a final report of scientific journal-quality, and an executive summary that could be understood by non-experts. Finally, each group presented in class the results obtained in a session that replicated a work corporate meeting.

3. Student’s results

3.1 Assessment tests comparison

A first estimation of the students’ progress was made by means of an assessment test composed by the following eight short open-questions:

1. What do fluorescence and luminescence have in common?
2. How are they different?
3. What do a fluorimeter and a luminometer have in common?
4. How are they different?
5. Could you indicate the substrates, effectors, products of the luciferase-catalysed reaction?
6. How are luciferases classified according to their kinetics?
7. What applications of luminometry do you remember?
8. How would you measure the presence of bacterial contamination in a sample?

This test was administered at the beginning of the introductory lecture (pretest) and repeated after students delivered their full reports (posttest). As observed in Figure 2, after performing this laboratory experience, either the PBL or the short protocol, an improvement in the students’ knowledge about bioluminescence and the principles and applications of luminometry was achieved for the three groups. Most students could identify chemiluminescence as a light-emitting phenomenon, and

distinguish it from fluorescence, describing the characteristics of the corresponding instrumentation. Although initially most of the students were not able to recall the components, the kinetic characteristics and the applications of the luciferase-catalysed reaction, their knowledge about these issues increased after they carried out this laboratory experience. However, in spite of the achievement of new knowledge by all the three groups of students, those performing the PBL reached the highest level of performance on all the questions.

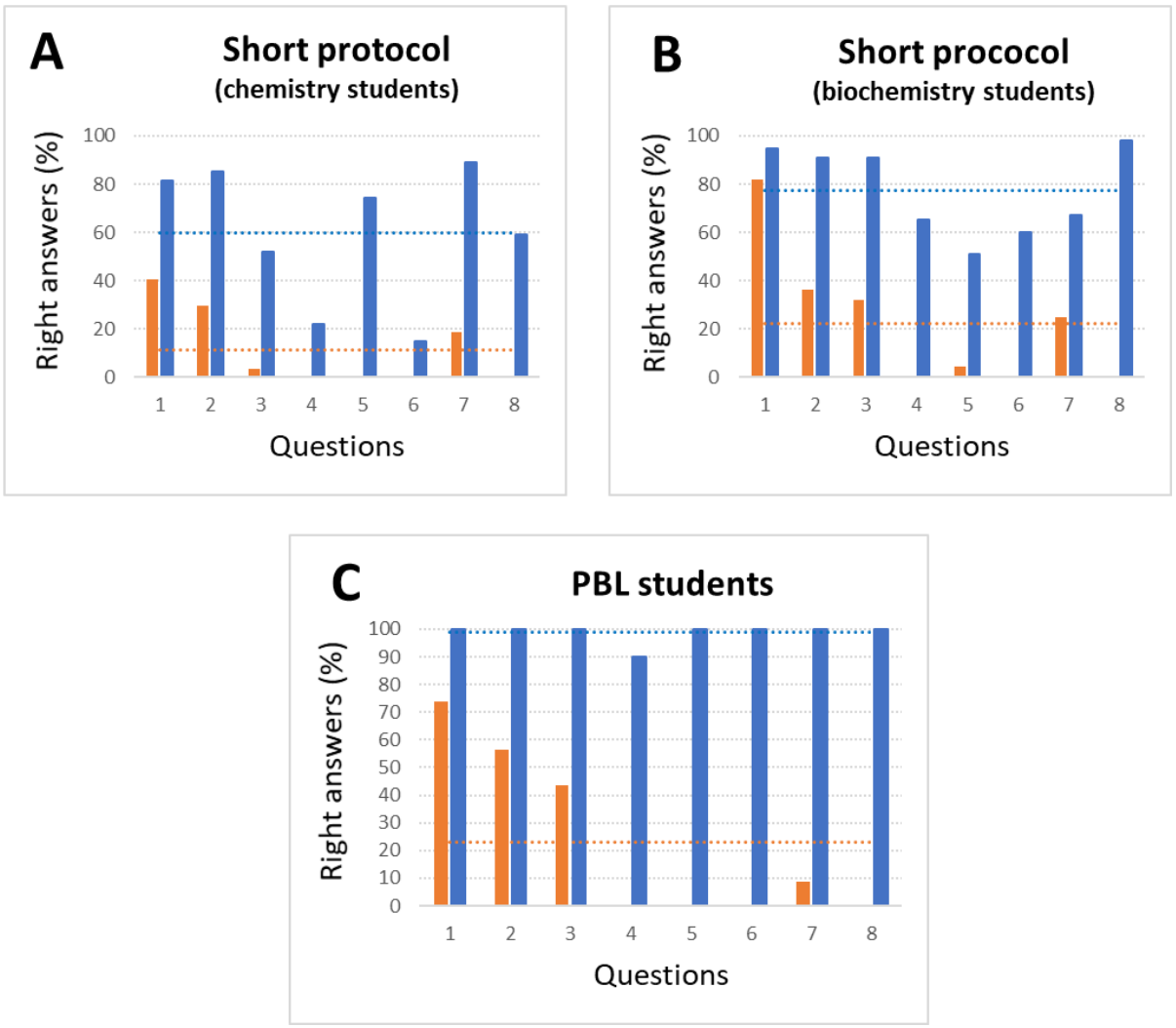

Figure 2. Student's outcome evaluation. Percentages of students who answered correctly the pretest (orange bars) and the posttest (blue bars) questions are shown. **A**. 4th year chemistry students performing the short protocol (20 students, 2018-19 course, subject "Applied Biochemistry"). **B**. 3rd year biochemistry students performing the short protocol (60 students, 2019-20 course, subject "Applied Biochemistry"). **C**. 4th year biochemistry/biotechnology students performing the PBL (10 students, 2019-20 course, subject "Advanced Instrumental Techniques"). Pretest full average is shown as an orange dotted line and posttest full average as a dotted blue line.

3.2 Students' achievement of the learning objectives

The two versions of the laboratory experiment share a number of learning objectives that are aligned with some of the core competencies included in the *Bioskills Guide*, a set of measurable learning outcomes developed and validated by over 600 college biology educators and based on the *Vision and Change* core curricular recommendations for undergraduate biology education [3]. In this regard, some foundational scientific competencies that characterize the *Process of Science*, such as evaluation and use of scientific information, critical thinking or data interpretation, are related to the following learning goals, successfully achieved by most of the students after performing this experiment:

- To search, apply and reference scientific literature
- To use a luminometer and apply luminometry to a real-world problem
- To know the principles and applications of bioluminescence

The students setting of a calibration curve, the establishment of the application range of a method and the determination of the ATP concentration of a sample, were related with the core competency *Quantitative Reasoning*, including the performance of basic calculations, drawing of graphs and data presentation. In this regard, although most students performed the protocol correctly and could draw valid calibration curves, some of those performing the short protocol made mistakes when calculating the concentration of the problem samples, which did not occur with those who followed the PBL approach.

As expected, the learning goals for the PBL approach included those from the short protocol, and others derived from the more active role played by students in their learning process, making them undertake authentic real-world tasks, similar to those that they would find in their professional future. Thus, many of these students faced for the first-time common issues for a professional, such as the acquisition of reagents and materials, the assessment of the necessary instruments and equipment, the adaptation and scaling of experimental protocols, or the analysis of costs and operational feasibility, among others. In addition, those students performing the PBL presented their results to a diverse audience, wrote a scientific journal-quality report and summarized their conclusions in an "executive report" that could be understood by non-experts. Those learning goals are related to the *Communication and Collaboration* core competency, which incorporates competencies for interacting and communicating the research results to biology experts and the general public.

4. Final conclusion

The detection of microbial contamination by measuring ATP concentration with luciferase is a useful tool to teach the use of bioluminescence to science undergraduate students. Either as a short protocol carried out in a single laboratory session, or as a long PBL experience, the students' achievement of the learning goals was very satisfactory. Students who followed the PBL approach could, in addition, adopt a series of additional transversal skills that could be useful in their future professional careers. Between these two extreme examples of the protocol, in terms of students' dedication, a whole range of intermediate options could be used by educators to suit their course programming and fulfill the learning objectives to be achieved by their students by means of this laboratory experiment.

5. Acknowledgements

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