UNIVERSITY OF LIFE SCIENCES
"KING MIHAI I" FROM Timisoara
Multidisciplinary Conference on Sustainable Development
30-31 May 2024

2024 MULTIDISCIPLINARY CONFERENCE ON SUSTAINABLE DEVELOPMENT

Preliminary results on the detection of adenosine triphosphate using the luminescent D-luciferin-luciferase reaction

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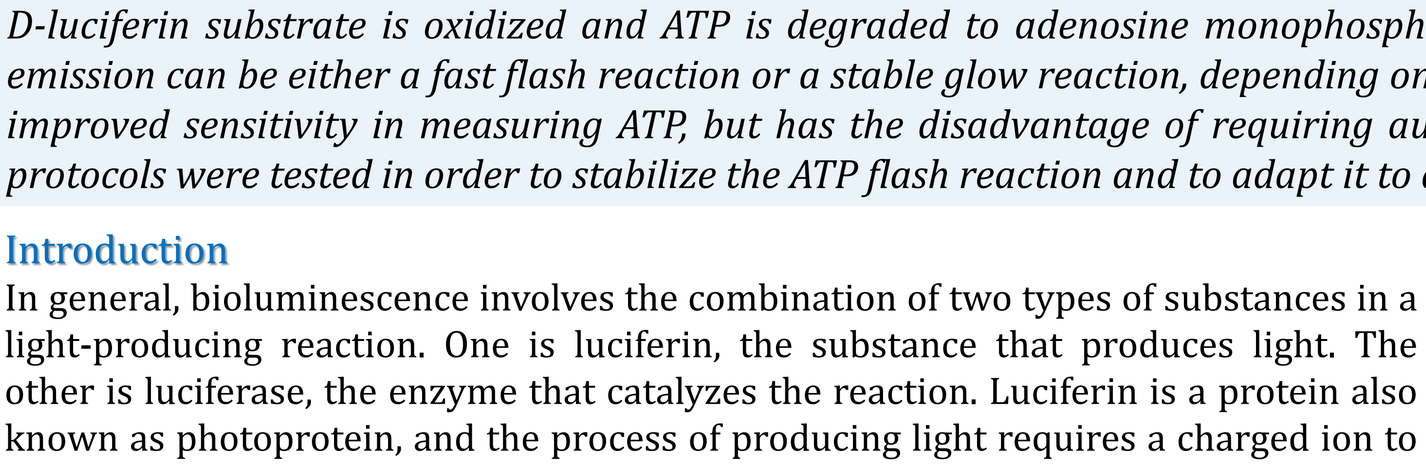
Abstract: *Living bacteria contain adenosine triphosphate (ATP), and the number of bacteria is related to the level of ATP under certain conditions, therefore the luciferase method, which catalyzes the luminescent reaction of D-luciferin in presence of ATP, is widely used to detect bacteria in different types of samples. Rapid and high-throughput ATP-based bacterial detection technologies are currently being sought. The D-luciferin substrate is oxidized and ATP is degraded to adenosine monophosphate (AMP), with energy released as visible light. The light emission can be either a fast flash reaction or a stable glow reaction, depending on the specific assay environment. The flash reaction provides improved sensitivity in measuring ATP, but has the disadvantage of requiring automatic dispensers for substrate adding. In this work, five protocols were tested in order to stabilize the ATP flash reaction and to adapt it to an equipment without automatic dispenser.*

Introduction

In general, bioluminescence involves the combination of two types of substances in a light-producing reaction. One is luciferin, the substance that produces light. The other is luciferase, the enzyme that catalyzes the reaction. Luciferin is a protein also known as photoprotein, and the process of producing light requires a charged ion to activate the reaction. Neurological, mechanical, chemical, or as yet undiscovered factors may trigger the reactions that create light. Often, the process requires the presence of other substances, such as oxygen or adenosine triphosphate (ATP). ATP is a molecule that stores and transports energy in most living organisms, including the human body. The luciferin-luciferase reaction can also create byproducts such as oxyluciferin and water.



Material and method

Table 1. Luminescence activity – ATP concentration variation

Variation of ATP	PBS pH=7,4 (µL)	$MgSO_4$ 0,01 mM (µL)	Luciferin 0,01 mM (µL)	Luciferase 0,01 mM (µL)	ATP 0,01 mM (µL)
1	37	1	1	1	**5**
2	27	1	1	1	**10**
3	42	1	1	1	**20**
4	17	1	1	1	**30**

Table 2. Luminescence activity – Luciferin concentration variation

Variation of luciferin	PBS pH=7,4 (µL)	$MgSO_4$ 0,01 mM (µL)	ATP 0,01 mM (µL)	Luciferase 0,01 mM (µL)	Luciferin 0,01 mM (µL)
1	37	1	1	1	**5**
2	27	1	1	1	**10**
3	42	1	1	1	**20**
4	17	1	1	1	**30**

Table 3. Luminescence activity – Standard Sigma protocol

Luciferase buffer		**D-Luciferin buffer**	
Glycine 1 M	3,75 g	Glycine 50 mM	0,2 g
EDTA 10 mM	0,02 g	Luciferin 0,15 mM	5 mg/mL
$MgSO_4$ 100 mM	1,5 g	$MgSO_4$ 5 mM	0,03 g
Luciferase	0,001 mL	EDTA 0,55 mM	0,008 g
		BSA 0,1%	0,05 g
		NaN_3 1%	0,05 g
		Tris 1 mM	0,006 g
		ATP 0,01 mM	0,99 mL

Materials

Multimode Microplate Reader (Biotek Sinergy H1)
Black microtiter plates

Results and discussions

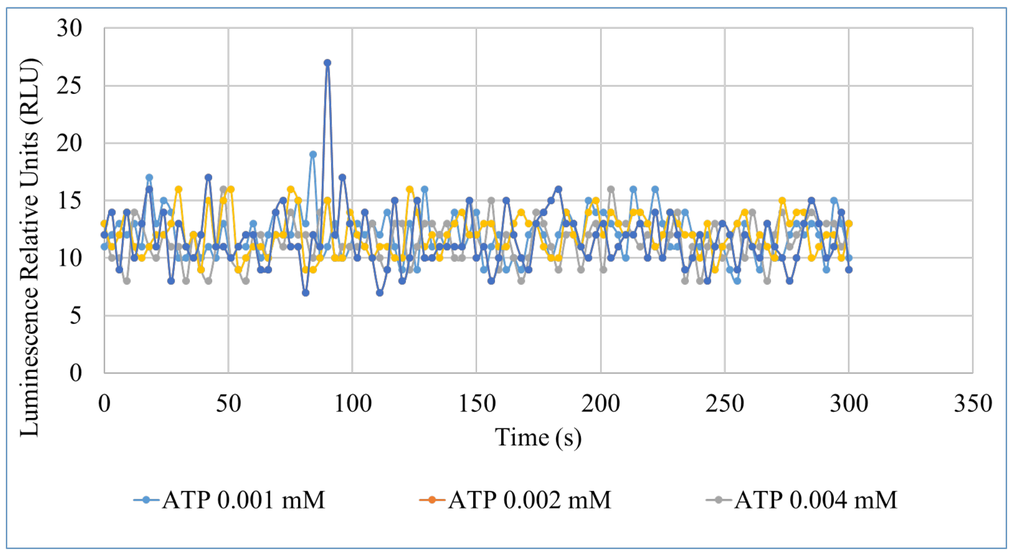


Fig. 1. Luminescence activity - ATP concentration variation

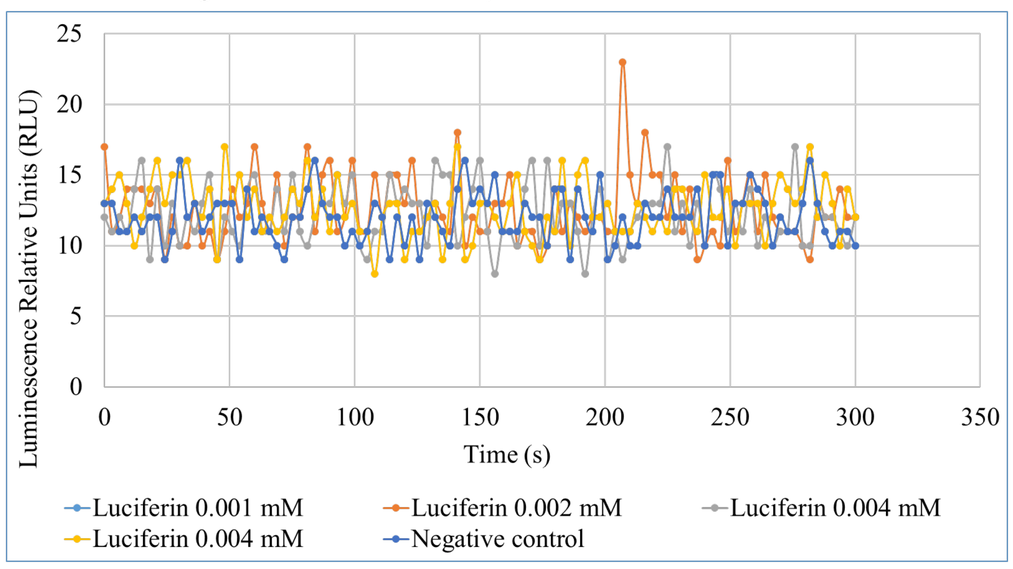


Fig.. 2 . Luminescence activity - Luciferin concentration variation

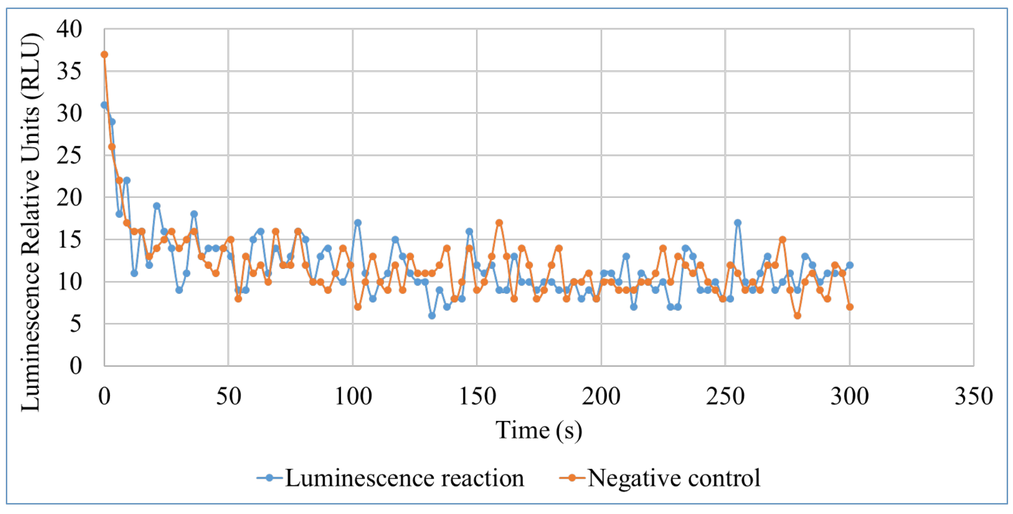


Fig. 3. Luminescence activity - standard Sigma protocol

Acknowledgement: This work was financially supported by the Project RoRS 337- ROmania Serbia NETwork for assessing and disseminating the impact of copper mining activities on water quality in the cross-border area (RoS-NET2), implemented under the Interreg-IPA Cross-border Cooperation Romania-Serbia Programme that is financed by the European Union under the Instrument for Pre-accession Assistance (IPA II) and co-financed by the partner states in the program.

Conclusions

Even the concentration of ATP or luciferin were varied, the luminescence reaction did not appeared . For the tests carried out, the results revealed that the reaction is still very fast and still challenging. Slowing of the reaction is considered where the readout devices do not have injector