UNIVERSITI PUTRA MALAYSIA

OPTICAL BIOSENSOR BASED ON IMMOBILIZED COPPER SENSITIVE OPERON REPRESSOR PROTEIN FOR DETECTION OF COPPER IONS IN WATER

HASSAN ISMAIL

FBSB 2016 2

OPTICAL BIOSENSOR BASED ON IMMOBILIZED COPPER SENSITIVE OPERON REPRESSOR PROTEIN FOR DETECTION OF COPPER IONS IN WATER

By

HASSAN ISMAIL

Thesis Submitted to School of Graduate Studies,Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2016

COPYRIGHT

DEDICATION

I dedicated the entire work to my lovely parents and my late brother Abubakar Hassan.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

OPTICAL BIOSENSOR BASED ON IMMOBILIZED COPPER SENSITIVE OPERON REPRESSOR PROTEIN FOR DETECTION OF COPPER IONS IN WATER

By

HASSAN ISMAIL

May 2016

Chairman : Abu Bakar B. Salleh, PhD
Faculty : Biotechnology and Biomolecular Sciences

Water pollution, from heavy metals, embodies a latent risk for both terrestrial and aquatic organisms. As such, continuous, simple and sensitive detection tools for toxic metals have been a great challenge of the existing methods used in monitoring of these metals. In this case, biosensors stand worthwhile for constant monitoring of metals in polluted areas. Here a new biosensor based on a tapered Multi-Mode Fiber (MMF) coated with $CsoR_{GZ}$ protein from *Geobacillus zalihae* ($CsoR_{Gz}$) as a bioreceptor is presented. The optical property of the coated layer changes once it was subjected to copper, resulting in an increase, at the UV-region (240 nm), of the absorption of evanescent waves. This increase of absorption is proportional to the concentration of copper added. The biosensor displayed a continuous response over the range of 5 - 40 µM copper. The limit of detection and limit of quantification were 5 µM and 40 µM copper respectively, with the sensitivity of 0.045 μM^{-1} based on 20 µm sensor. The biosensor showed a fast response time of 19.8 s at room temperature and pH of 7.0. The biosensor retained its selectivity and did not respond to equivalent additions of cobalt (II) and Nickel (II). This work also revealed, for the first time, the prospect of remote, selective and sensitive monitoring of copper ions in water, from a distance of 50 m, where a continuous response in the range of 5 - 40 µM copper was obtained with no significant difference ($p > 0.06$) with the non-remote one.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

BIOSENSOR OPTIKAL BERASASKAN PROTEIN PENGAWALSELIAAN KUPRUM PEGUN UNTUK PENGESANAN ION KUPRUM DALAM AIR

Oleh

HASSAN ISMAIL

Mei 2016

Pengerusi : Abu Bakar B. Salleh, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Pencemaran air, dari logam berat, mempunyai risiko yang berbahaya untuk kedua-dua organisma daratan dan akuatik. Untuk mencipta alat pengesan logam toksik yang berterusan, mudah dan sensitif menjadi cabaran yang amat besar. Dalam kes ini, biosensor sangat sesuai digunakan untuk pemantauan berterusan logam di kawasan tercemar. Di sini biosensor baru berdasarkan fibre tirus pelbagai mod (MMF) disalut dengan CsoR protein sebagai bioreseptor dicipta. Ciri-ciri optik lapisan yang bersalut berubah apabila kuprum ditambah, menyebabkan peningkatan penyerapan gelombang di kawasan UV (240 nm). Peningkatan penyerapan gelombang adalah berkandar langsung dengan kepekatan kuprum yang ditambah. Biosensor memaparkan tindak balas yang berterusan di sepanjang julat 5 - 40 μM kuprum. Had pengesanan adalah 5 μM tembaga dengan kepekaan 0.045 μM^{-1}. Biosensor itu menunjukkan masa tindak balas yang cepat iaitu 19.8 s pada suhu bilik dan pH 7.0. Biosensor berupaya mengekalkan pemilihan dan tidak bertindak balas terhadap penambahan kobalt (II) dan nikel (II). Kerja ini juga mendedahkan, buat kali pertama, prospek pemantauan jarak jauh, yang terpilih dan sensitif ke atas ion kuprum di dalam air, dari jarak 50 m, di mana tindak balas yang berterusan dalam lingkungan 5 μM kepada 40 μM kuprum telah diperolehi dengan tiada perbezaan yang signifikan ($p > 0.06$) dibanding dengan yang dipantau pada jarak dekat.

ACKNOWLEDGEMENTS

I would like to acknowledge Geran Universiti Putra Malaysia, Geran Putra Berkumpulan (IPB) (Project number: GP-IPB/2013/9413500) for supporting this research. I hereby, thank my employer; Bauchi State University Gadau, Nigeria for the fellowship. I am also gratefull to my supervisory committee for their good and helpful supervision. I also owe appreciations to my senior coleague; Nasihah Bte Musa for her help and the generous gift of the recombinant gene. I also say thanks to my co-reseachers, Dr. Muhammad Hafiz Abu Bakar and Dr. Hanif Yaqub for allowing me to work in their labs and their observations. I also extend my gratitude to all my freindly lab mates whom i worked with in protein engineering lab (Biotech 3, FBSB, UPM), lab 140; lab 209 (Biotech 2, FBSB, UPM) and photonic lab (ALNAIR lab, Faculty of Engineering, UPM). Finally, i am highly indebted to my family for their unrelenting support, pateince and prayers throughout the study period. *Alhamdulillah Allazee bini'imatihi tatimmus salihat*.

I certify that a Thesis Examination Committee has met on 31 May 2016 to conduct the final examination of Hassan Ismail on his thesis entitled "Optical Biosensor Based on Immobilized Copper Sensitive Operon Repressor Protein for Detection of Copper Ions in Water" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Mohd Shukuri bin Mohamad Ali, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Jaafar bin Abdullah, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Azila Abd. Aziz, PhD
Associate Professor
Bioproducts Development Institute Kuala Lumpur
Universiti Teknologi Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 July 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Abu Bakar B. Saleeh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Normi Muhammad Yahaya, PhD
Senior lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PHD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other
- degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date:_____

Name and Matric No: Hassan Ismail , GS39946

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Abu Bakar B. Saleeh

Signature: _____
Name of Member of Supervisory Committee: Dr. Normi Muhammad Yahaya

TABLE OF CONTENTS

			Page
ABSTRACT			i
ABSTRAK			ii
ACKNOWLEDGEMENTS			iii
APPROVAL			iv
DECLARATION			vi
LIST OF TABLES			xi
LIST OF FIGURES			xii
LIST OF APPENDICES			xv
LIST OF ABBREVIATIONS			xvi
CHAPTER			
1	**INTRODUCTION**		1
	1.1	Problem statement	1
	1.2	General objective of the study	2
		1.2.1 Specific objectives	2
2	**LITERATURE REVIEW**		3
	2.1	Heavy Metals	3
	2.2	Copper as a heavy metal	4
	2.3	Sources of copper	5
	2.4	Health Effects of copper	5
		2.4.1 Beneficial Effects	5
		2.4.2 Chronic Effects	5
		2.4.3 Carcinogenicity	6
		2.4.4 Carcinogenicity	6
		2.4.5 Reproductive/Developmental Effects	6
	2.5	Sources of heavy metal contamination	6
		2.5.1 Natural Sources	6
		2.5.2 Anthropogenic Sources	6
	2.6	Determination of Heavy metal	7
	2.7	Biosensors	10
		2.7.1 Definition of Biosensors	12
		2.7.2 Bioreceptor	13
		2.7.3 Transducers	13
	2.8	Biosensors for Heavy metals	13
		2.8.1 Protein Based Biosensors	14
		2.8.2 Whole Cell Biosensor	16
		2.8.3 DNA based Metal Biosensor	17
	2.9	Fibre optic biosensors	17
	2.10	Immobilization	18
		2.10.1 Physical methods	18
		2.10.2 Chemical Methods	19
	2.11	CsoR Protein	19
	2.12	Optical fibre as transducer	20

2.12.1 Single mode fibre optic cable 20
2.12.2 Multimode fibre optic cable 21
2.13 Tapering of fibre 21
2.14 Conclusion and scope 22

3 METHODOLOGY 24
3.1 Preparation and production of bioreceptor 24
3.1.1 Materials, chemicals and reagents 24
3.1.2 Expression of the recombinant CsoRGZ protein 24
3.1.3 Sodium dodecyl sulphate - polyacrylamide gel electrophoreses 24
3.1.4 Purification of the CsoRGZ protein 25
3.1.5 Determination of protein concentration – Bradford protein assay 26
3.1.6 Characterization of the $CsoR_{GZ}$ protein 26
3.1.7 Isothermal titration calorimetry (ITC) 27
3.2 Fabrication of tapered-fibre transducer 28
3.3 Biosensor integration 28
3.3.1 Functionalization of the Tapered Fibre 28
3.3.2 Immobilization of CsoRGZ Protein on Tapered Fibre 28
3.3.3 Test approach for the biosensing 28
3.3.4 Absorbance Measurement 29
3.4 Biosensor performance 29
3.4.1 Linearity 29
3.4.2 Sensitivity 29
3.4.3 Sensor Dynamic Response 30
3.4.4 Limit of Detection (LOD) and Limit of Quantification (LOQ) 30
3.4.5 Interference and Selectivity 30
3.4.6 Reusability and Reproducibility 30
3.4.7 Storage Stability 31
3.4.8 Remote Sensing 31

4 RESULTS AND DISCUSSION 32
4.1 Bioreceptor preparation and production 32
4.1.1 Expression and Purification of the CsoR protein 32
4.1.2 Characterization of $CsoR_{GZ}$ protein for Binding to metals 36
4.1.3 Isothermal Titration Calorimetry 47
4.2 Transducer preparation 47
4.2.1 Tapering of multi-mode fibre (MMF) 47
4.3 Biosensor integration 48
4.3.1 Immobilization and Optimization of CsoRGZ protein 48
4.3.2 Optimization of tapering diameter of MMF for copper detection 49
4.3.3 Selectivity and interference analysis of the biosensor 54

4.4 Biosensor performance 55
4.4.1 Linearity and sensitivity of the developed biosensor 55
4.4.2 Biosensor response time and recovery time 55
4.4.3 Limit of detection and limit of quantification 57
4.4.4 Reusability and reproducibility 57
4.4.5 Storage stability 59
4.4.6 Remote sensing of copper (I) 59
4.4.7 Biosensor set-up 60

5 SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 61

REFERENCES 62
APPENDICES 70
BIODATA OF STUDENT 82

LIST OF TABLES

Table		Page
2.1	Toxic levels and permissible limits for the most hazardous and common heavy metals	8
2.2	Biosensors used for copper detection	11
2.3	Principal transduction systems used in biosensors	14
2.4	Heavy metal biosensor	15
4.1	Purification table of CsoRGZ protein	35

LIST OF FIGURES

Figure		Page
2.1	Schematic diagram of an optical biosensor	12
4.1	SDS-PAGE of crude CsoR protein, M is protein marker; S1 and S2 are supernatant	32
4.2	SDS-PAGE of purified CsoRGZ protein, M is protein marker, S1, S2 and S3 are elutes from different fractions of affinity chromatography	33
4.3	Purification chromatogram of CsoRGZ protein using step elution	34
4.4	SDS-PAGE of CsoRGZ protein. M is Protein Ladder, S1 and S2 are uncleaved purified CsoRGZ protein and CP is cleaved purified CsoRGZ protein (11 kDa)	35
4.5	Binding analysis of $CsoR_{GZ}$ to Copper (I) (0-100 μM Cu^{+}) carried out at the wavelength range of 200 nm – 300 nm	37
4.6	Binding analysis of copper (I) to $CsoR_{GZ}$ protein at a single wavelength of 240 nm	37
4.7	Binding analysis of copper (I), (0 – 1 mM Cu^{+}), to $CsoR_{GZ}$ protein at 200 nm – 300 nm as monitored by UV-vis Spectrophotometer	38
4.8A	Binding analysis of copper (I) to 20 μm $CsoR_{GZ}$ protein at 240 nm showing the saturation point at 0.6 mM Cu^{+}	38
4.8B	Linearity graph of binding analysis of copper (I) (0 – 1 mM Cu+) to $CsoR_{GZ}$ protein at 240 nm	39
4.9	Binding analysis of copper (II) (0-100 μM Cu^{2+}) to $CsoR_{GZ}$ protein as monitored by UV- vis Spectrophotometer in range of 200 nm to 350 nm	40
4.10	Binding analysis of copper (II) (0-100 μM Cu^{2+}) to $CsoR_{GZ}$ protein at a single trend of 240 nm	40
4.11	Binding analysis of copper (II) (0 – 1 mM), to $CsoR_{GZ}$ protein as monitored by UV-vis Spectrophotometer in the range of 200 nm to 300 nm	41
4.12A	Binding analysis of copper (II) (0 – 1 mM), to $CsoR_{GZ}$ protein at 240 nm showing the saturation point at 0.7 mM Cu^{2+}	41

4.12B	Linearity graph of binding analysis of copper (II) (0 – 1 mM), to $CsoR_{GZ}$ protein at 240 nm	42
4.13	Binding analysis of Zinc (0 - 1mM) against $CsoR_{GZ}$ protein at wavelength 240 nm to 400 nm	43
4.14	Binding analysis of zinc (II); (0 - 1mM) against 20 µM $CsoR_{GZ}$ protein at 240 nm	43
4.15A	Binding analysis of nickel (II); (0-100 µM), to 20 µM $CsoR_{GZ}$ protein at 200 nm – 300 nm	44
4.15B	Binding analysis of nickel (II); (0-1 mM) aganist 20 µM $CsoR_{GZ}$ protein at 240 nm	45
4.16	The result of binding analysis of 0-100 µM Co^{2+} to $CsoR_{GZ}$ protein at different wavelengths as monitored by UV-vis Spectrophotometer	46
4.17	Binding analysis of 20 µM $CsoR_{GZ}$ protein against cobalt (II); (0-100 µM) at 240 nm	46
4.18	Binding analysis of 5 µM $CsoR_{GZ}$ protein titrated against 0- 100 µM copper using single injection nano ITC	47
4.19	Scanned image of MMF tapered down to a diameter of 20 µm	48
4.20	Optimization of $CsoR_{GZ}$ protein concentrations	49
4.21A	Performance analysis of 40 µm sensor showing the saturation point of the sensor, due to copper concentration in the range of 5 – 40 µM at 240 nm (n=3)	50
4.21B	Performance analysis of 40 µm sensor, showing the change in the sensor cumulative absorbance due to copper concentration at 240 nm	50
4.22A	Performance analysis of 30 µm sensor, showing the change in the sensor cumulative absorbance due to copper concentration in the range of 5 – 40 Mm	51
4.22B	Performance analysis of 30 µm sensor after it was exposed to different copper concentrations	52
4.23A	Performance analysis of 20 µm sensor showing the saturation point due to addition of copper in the range of 5 – 40 µM	53
4.23B	Performance analysis of 20 µm sensor showing the change in the cumulative absorbance of the sensor at different copper concentrations	53

4.24A	Absorbance spectrum of the biosensor exposed to 5 - 40 μM nickel (II) concentrations within the wavelength range of 200 nm to 300 nm	54
4.24B	Absorbance spectrum of biosensor when exposed to 5 - 40 μM cobalt (II) concentrations within the wavelength range of 250 nm to 330 nm	54
4.24C	Interference analysis of Co^{2+} and Ni^{2+} at different concentrations against Cu^{+} using a 20 μm sensor	55
4.25A	Strip chart of the response time (s) of the biosensor towards 10 μM copper at a single trend of 240 nm	56
4.25B	Response and recovery time (s) taken from a single absorbance peak in the strip chart	56
4.25C	Strip chart of the dynamic response of the biosensor towards 10 μM - 40 μM copper at single trend of 240 nm using 20 μm and 30 μm sensors	57
4.26A	The reusability of the biosensor obtained from the absorbance spectra (240 nm) of three cycles of sensor's response under exposure to 10 μM copper for 2 minutes and followed by 2 mM EDTA for 10 minutes	58
4.26B	The reproducibility of the biosensor obtained from the UV-vis absorption spectrum (240 nm) of ten replicate sensor's response under exposure to 10 μM copper (RSD 2 %)	58
4.27	Storage Stability of the biosensor after six weeks	59
4.28	Copper remote sensing analyses using 20 μm sensor. The change in absorbance due to copper detection was presented	60

LIST OF APPENDICES

Appendix		Page
A	Optical fibre cables	70
A1	Single mode fibre optic cable	70
A2	Multimode fibre optic cable	70
B	Equipment in $CsoR_{GZ}$ protein production and purification	71
B1	BioRad mini protean electrophoresis kit	71
B2	UV transluminater	71
B3	Branson sonicator	72
B4	Aktaprime plus purifier	72
B5	XK 16 column	73
C	Standard curve from Bradford assay with bovine serum albumin (BSA) as standard protein	73
D	TA microcalorimeter used during Nano isothermal titration calorimetry	74
E	Optical fibre processor GPX-3000 (Vytran) used to taper the MMF into desired parameters.	74
F	Typical view of Vytran GPX-3400 graphical user interface for inputting the desired parameters of fibre tapering	75
G	Handy cleaver FC-7 (Sumitomo Electric), used for cleaving the ends of fibres prior to slicing	75
H	Fiber fusion slicer (Sumitomo Electric, Japan) used for slicing the tapered fibre to the pig tails.	76
I	AvaLight-DH-S-BAL (Avantes, The Netherlands), light source used during the biosensing	76
J	AvaSpec-2048 (Avantes), spectrometer used during the sensing experiment.	77
K	Biosensor set-up; (a) light source, (b) sensing chamber, (c) UV- spectrometer and (d) computer display screen	77

K1	Mill jig used as sensing chamber, (JETRO ENGR, Malaysia); (a) Arial view of the sensing chamber and (b) Side view of the sensing chamber.	78
L	Chemicals and reagents used	79
L1	Equipment used	81

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AChE	Acetylcholinestrate
AES	Atomic Emission Spectroscopy
APTES	Aminopropyl triethoxysilane
BSA	Bovine Serum Albumin
CATV	Cable Television
DDVP	Dimethyl-2.2-dichlorovinyl phosphate
EDTA	Ethylene diamine tetraacetic acid
EPA	Environmental Protection Agency
HCL	Hydrochloric acid
ICP-MS	Inductively coupled plasma mass spectrometry
IPTG	Isopropyl thiogalactoside
ITC	Isothermal Titration Calorimetry
IUPAC	International Union of Pure and Applied Chemistry
kDa	Kilo Dalton
MMF	Multi-Mode Fibre
MOPS	Morpholine propane sulfonic acid
MWCO	Molecular Weight Cut Off
NA	Numerical Aperture
pI	Isoelectric point
RDA	Recommended Daily Allowance
ROS	Reactive Oxygen species
RT	Room Temperature

SMF	Single-Mode Fibre
WD	Wilson's disease

CHAPTER 1

INTRODUCTION

Environmental pollution from toxic metals is a global phenomenon coupled with industrial developments. Heavy metals mostly constitute the most toxic pollutants in the environment, and also in other areas such as in food industry and medicine. Some heavy metals like Cadmium (Cd), and Lead (Pb) pose a great threat due to the toxicity and extensive industrial application. There are several methods used for detection of heavy metals such as electrochemical or spectroscopic; such as atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) and inductively coupled plasma mass spectrometry (ICP-MS) etc. (Lia et al., 2006, Askari et al., 1983). These techniques, however, are either expensive or lack sensitivity. Furthermore, the techniques cannot account for the real time concentrations of heavy metals that are accessible to the aquatic organisms. Therefore, it is highly desirable to develop simpler approaches of monitoring pollution caused by heavy metals. Sensitive biosensors employing metal binding proteins, such as methallothioneins (SmtA) or regulator proteins (MerR), combined to a remarkably sensitive capacitative transducer were recently labelled to access heavy metals (Bontidean et al., 2004).

1.1 Problem statement

Heavy metals bioaccumulate higher in the upper trophic levels making organism in the upper trophic more prone to the toxicity (Ilangovan et al., 2006, Krawczyk et al., 2000).

The conventional approaches employed in the monitoring of heavy metals using spectrophotometry, chromatography, mass spectrometry techniques involve sophisticated and expensive equipment, vastly skilled personnel and they are generally laborious (Chouteau et al., 2005). The total amount of heavy metals detected by such means might not always relate to toxicity of these samples as the original bioaccessibility of the metal ions cannot be accounted for. Thus, there is need for the cheaper and simpler methods that can be used to monitor heavy metals.

Biosensor, being a promising tool in this regard, is an investigative device, which translates a biological response into a measurable signal. Several patterns of biosensors have been defined previously for detection of heavy metals. Extensive varieties of bioreceptor and transducer set-up have been applied for the construction of biosensors (Amine et al., 2006).

Enzymes are more commonly employed as bioreceptor in construction of biosensors. Although enzyme-based biosensors enjoy relatively high degree of substrate specificity, their use in biosensors design may be hindered due the laborious, longer

and expensive purification steps, coupled, sometimes, with the need to have coenzyme/cofactor to produce detectable response. Microorganisms provide an ideal alternative (Marzuki et al., 2012). The ability of the cells to uptake and therefore detect great amount of chemicals from the various enzymes and co-factors that co-exist in the cells; conversely, affects the selectivity.

Metalloproteins are another category of the bioreceptor used for the integration of the heavy metal sensors and their specificity for metal binding make them more attractive tool (Corbisier et al., 1999). For developing heavy metal biosensors, different metalloproteins/peptides have been employed (Cherian et al., 2003, Chow et al., 2005). The good selectivity shown by the metalloproteins even though in more multifaceted natural environments such as in blood or marine water especially when integrated to a seemly transducer has made it a very good biorecepter that may provide an alternative to the existing measuring techniques of metal ions concentrations (Barondeau et al., 2002).

1.2 General objective of the study

To develop an optical biosensor based on $CsoR_{GZ}$ protein as bioreceptor that will be used for selective detection of copper ions in water.

1.2.1 Specific objectives

1. To characterize the bioreceptor $CsoR_{GZ}$ protein for its binding potential to copper ions.
2. To immobilize the $CsoR_{GZ}$ protein on optical fiber and integrate the biosensor.
3. To assess the performance of the biosensor for the detection of copper ions in water.

REFERENCES

Alpat, S. K., Alpat, S., Kutlu, B., Ozbayrak, O., Buyukisik, H. B. (2007) Development of biosorption-based algal biosensor for Cu(II) using *Tetraselmis chuii*. *Sens. Actuators B* 128 (1), 273–278.

Amaro, F., Turkewitz, A. P., Martín-González, A., Gutiérrez, J. C. (2011). Whole-cell biosensors for detection of heavy metal ions in environmental samples based on metallothionein promoters from Tetrahymena thermophila. *Microbial Biotechnology*, *4*(4), 513–522.

Amine, A., Mohammadi, H., Bourais, I., Palleschi, G. (2006). Enzyme inhibition-based biosensors for food safety and environmental monitoring. *Biosensors and Bioelectronics*, *21*, 1405–1423.

Appenroth, K. (2010). Soil Heavy Metals. *Soil Biology 19*, 19–30.

Barondeau, D. P., Kassmann, C. J., Tainer, J. a., Getzoff, E. D. (2002). Structural chemistry of a green fluorescent protein Zn biosensor. *Journal of the American Chemical Society*, *124*(14), 3522–3524.

Belkin, S. (2003). Microbial whole-cell sensing systems of environmental pollutants. *Curr. Opin. Microbiol*. 6 (3), 206–212.

Bontidean, I., Ahlqvist, J., Mulchandani, A., Chen, W., Bae, W., Mehra, R. K.,Csöregi, E. (2003). Novel synthetic phytochelatin-based capacitive biosensor for heavy metal ion detection. *Biosensors and Bioelectronics*, *18*(5-6), 547–553.

Bontidean, I., Berggren, C., Johansson, G., Csöregi, E., Mattiasson, B., Lloyd, J. R., Brown, N. L. (1998). Detection of heavy metal ions at femtomolar levels using protein-based biosensors. *Analytical Chemistry*, *70*(19), 4162–4169.

Bontidean, I., Lloyd, J. R., Hobman, J. L., Wilson, J. R., Csöregi, E., Mattiasson, B., Brown, N. L. (2000). Bacterial metal-resistance proteins and their use in biosensors for the detection of bioavailable heavy metals. *Journal of Inorganic Biochemistry*, *79*(1-4), 225–229.

Bontidean, I., Mortari, A., Leth, S., Brown, N. L., Karlson, U., Larsen, M. M., Csöregi, E. (2004). Biosensors for detection of mercury in contaminated soils. *Environmental Pollution*, *131*(2), 255–262.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.

Brena, B., González-Pombo, P., Batista-Viera, F. (2013). Immobilization of enzymes: a literature survey. *Immobilization of Enzymes and Cells*, *1051*, 15–31.

Burgess, R. R. (2009). *Chapter 4 Preparing a Purification Summary Table*. *Methods in Enzymology* (1st ed., Vol. 463). Elsevier Inc.

Campanello, G. C., Ma, Z., Grossoehme, N. E., Guerra, A. J., Ward, B. P., Dimarchi, R. D., Giedroc, D. P. (2013). Allosteric inhibition of a zinc-sensing transcriptional repressor: Insights into the arsenic repressor (ArsR) family. *Journal of Molecular Biology*, *425*(7), 1143–1157.

Çetinus, Ş. A., Şahin, E., & Saraydin, D. (2009). Preparation of Cu(II) adsorbed chitosan beads for catalase immobilization. *Food Chemistry*, *114*(3), 962–969.

Cherian, S., Gupta, R. K., Mullin, B. C., Thundat, T. (2003). Detection of heavy metal ions using protein-functionalized microcantilever sensors. *Biosensors and Bioelectronics*, *19*(5), 411–416.

Chouteau, C., Dzyadevych, S., Durrieu, C., Chovelon, J. M. (2005). A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. *Biosensors and Bioelectronics*, *21*(2), 273–281.

Corbett, D., Schuler, S., Glenn, S., Andrew, P. W., Cavet, J. S., Roberts, I. S. (2011). The combined actions of the copper-responsive repressor CsoR and copper-metallochaperone CopZ modulate CopA-mediated copper efflux in the intracellular pathogen listeria monocytogenes. *Molecular Microbiology*, *81*(2), 457–472

Corbisier, P., Van Der Lelie, D., Borremans, B., Provoost, A., De Lorenzo, V., Brown, N. L., Mattiasson, B. (1999). Whole cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. *Analytica Chimica Acta*, *387*(3), 235–244.

Cutler, P. (1996). Affinity chromatography. *Methods in Molecular Biology (Clifton, N.J.)*, *59*, 157–168.

Dameron, C. T., Winge, D. R., George, G. N., Sansone, M., Hu, S., Hamer, D. (1991) A copper thiolate polynuclear cluster in the Ace1 transcription factor. *Proc. Nat Acad. Sci. USA* 88 (14), 6127–6131.

Duffus, J. H. (2002). "Heavy metals" a meaningless term? *Pure and Applied Chemistry*, *74*(5), 793–807.

García Sánchez, F., Navas Díaz, a., Ramos Peinado, M. C., Belledone, C. (2003). Free and sol-gel immobilized alkaline phosphatase-based biosensor for the determination of pesticides and inorganic compounds. *Analytica Chimica Acta*, *484*(1), 45–51.

Grabski, A. C., Burgess, R. R. (2001). Preparation of protein samples for SDS-polyacrylamide gel electrophoresis: procedures and tips. *inNovations*, *13*, 10–12.

Hartung, A., Wirth, F., Bartelt, H. (2011). Light Propagation in Tapered Optical Fibers : Spatial Light Confinement and Generation of Plasmonic Waves. *Progress In Electromagnetics Research Symposium Proceedings, Marrakesh,Morocco, Mar*. 20–23, 2011 255, 255–258.

Higgins, K. A., Giedroc, D. (2014). Insights into Protein Allostery in the CsoR/RcnR Family of Transcriptional Repressors. *Chemistry Letters*, *43*(1), 20–25.

Ibrahim, S. A., Rahman, N. A., Abu Bakar, M. H., Girei, S. H., Yaacob, M. H., Ahmad, H., Mahdi, M. A. (2015). Room temperature ammonia sensing using tapered multimode fiber coated with polyaniline nanofibers. *Optics Express*, *23*(3), 2837.

Ilangovan, R., Daniel, D., Krastanov, a., Zachariah, C., Elizabeth, R. (2006). Enzyme based biosensor for heavy metal ions determination. *Biotechnology and Biotechnological Equipment*, *20*(1), 184–189.

IPCS (2003) Elemental mercury and inorganic mercury compounds: Human health aspects. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document 50).

Isarankura-Na-Ayudhya, C., Tantimongcolwat, T., Galla, H.-J., Prachayasittikul, V. (2010). Fluorescent protein-based optical biosensor for copper ion quantitation. *Biological Trace Element Research*, *134*(3), 352–63.

Ivask, A., Green, T., Polyak, B., Mor, A., Kahru, A., Virta, M., Marks, R. (2007). Fibre-optic bacterial biosensors and their application for the analysis of bioavailable Hg and As in soils and sediments from Aznalcollar mining area in Spain. *Biosensors and Bioelectronics*, *22*(7), 1396–1402.

Jung, Y., Brambilla, G., Richardson, D. J. (2008). Broadband single-mode operation of standard optical fibers by using a sub-wavelength optical wire filter. *Optics Express*, *16*(19), 14661–14667.

Keiderling, T. A. (2002). Protein and peptide secondary structure and conformational determination with vibrational circular dichroism. *Current Opinion in Chemical Biology*, *6*(5), 682–688.

Kim, C. S., Choi, B. H., Seo, J. H., Lim, G., Cha, H. J. (2013). Mussel adhesive protein-based whole cell array biosensor for detection of organophosphorus compounds. *Biosensors and Bioelectronics*, *41*(1), 199–204.

Koyun, A., Ahlatcıoğlu, E., İpek, Y. K. (2012). Biosensors and Their Principles. *A Roadmap of Biomedical Engineers and Milestones*, 117–142.

Kumar, J., Jha, S. K., D'Souza, S. F. (2006). Optical microbial biosensor for detection of methyl parathion pesticide using *Flavobacterium sp*. whole cells adsorbed on glass fiber filters as disposable biocomponent. *Biosensors and Bioelectronics*, *21*(11).

Lee, S., Sode, K., Nakanishi, K., Marty, J. L., Tamiya, E., Karube, I. (1992) A novel microbial sensor using luminous bacteria. *Biosens. Bioelectron.* 7 (4), 273–277.

Lehmann, M., Riedel, K., Adler, K., Kunze, G. (2000) Amperometric measurement of copper ions with a deputy substrate using a novel *Saccharomyces cerevisiae* sensor. *Biosens. Bioelectron.* 15 (3-4), 211–219.

Leth, S., Maltoni, S., Simkus, R., Mattiasson, B., Corbisier, P., Klimant, I.,Wolfbeis, O. S., Csoregi, E. (2002) Engineered bacteria based biosensors for monitoring bioavailable heavy metals. *Electroanalysis* 14 (1), 35–42.

Liao, V. H. C., Chien, M.-T., Tseng, Y. Y., Ou, K.-L. (2006). Assessment of heavy metal bioavailability in contaminated sediments and soils using green fluorescent protein-based bacterial biosensors. *Environmental Pollution (Barking, Essex : 1987)*, *142*(1), 17–23.

Libertino, S., Conoci, S., Scandurra, A., Spinella, C. (2013). Biosensor integration on Si-based devices: Feasibility studies and examples. *Sensors and Actuators, B: Chemical*, *179*, 240–251.

Lin, M., Hu, X., Ma, Z., Chen, L. (2012). Functionalized polypyrrole nanotube arrays as electrochemical biosensor for the determination of copper ions. *Analytica Chimica Acta*, *746*, 63–9.

Liu, T., Ramesh, A., Ma, Z., Ward, S. K., Zhang, L., George, G. N., Giedroc, D. P. (2007). CsoR is a novel Mycobacterium tuberculosis copper-sensing transcriptional regulator. *Nature Chemical Biology*, *3*(1), 60–68.

Long, F., Zhu, A., Shi, H. (2013). Recent advances in optical biosensors for environmental monitoring and early warning. *Sensors*, *13*(10),

Lovrien, R., and Matulis, D. (2005). Assays for total protein. *Current Protocols in Microbiology*, *Appendix 3*, Appendix 3A.

Ma, Z., Cowart, D. M., Scott, R. A., Giedroc, D. P. (2009a). Molecular Insights into the Metal Selectivity of the Copper (I) -Sensing Repressor CsoR from *Bacillus subtilis* Molecular Insights into the Metal Selectivity of the Copper (I) -Sensing Repressor CsoR from *Bacillus subtilis* . *Biochemistry* (I), 3325–3334.

Ma, Z., Cowart, D. M., Scott, R. A., Giedroc, D. P. (2009b). Molecular insights into the metal selectivity of the Copper(I)-sensing repressor CsoR from *Bacillus subtilis*. *Biochemistry*, *48*(15), 3325–3334.

Mahurpawar, M. (2015). Effects of heavy metals on human health. *Social Issues and Environmental Problems: September*, ISSN- 2350-0530(O) ISSN- 2394-3629(P) *0530*.

Marazuela, M. D., Moreno-Bondi, M. C. (2002). Fiber-optic biosensors - An overview. *Analytical and Bioanalytical Chemistry*, *372*(5-6), 664–682.

Marzuki, N. I., Bakar, F. A., Salleh, A. B., Heng, L. Y., Yusof, N. A., Siddiquee, S. (2012). Development of electrochemical biosensor for formaldehyde determination based on immobilized enzyme. *International Journal of Electrochemical Science*, *7*(7), 6070–6081.

Michel, C., Ouerd, A., Battaglia-Brunet, F., Guigues, N., Grasa, J. P., Bruschi, M., Ignatiadis, I. (2006). Cr(VI) quantification using an amperometric enzyme-based sensor: Interference and physical and chemical factors controlling the biosensor response in ground waters. *Biosensors and Bioelectronics*, *22*(2), 285–290.

Miller, J., Miller, J. C. (2010). *Statistics and Chemometrics for Analytical Chemistry*. *Technometrics* (Vol. 46).

Monk, D. J., Walt, D. R. (2004). Optical fiber-based biosensors. *Analytical and Bioanalytical Chemistry*, *379*(7-8), 931–945.

Pfeiffer, R. (2007). Wilson's Disease. *Seminars in Neurology*, *27*(2), 123–132.

Pohanka, M., Republic, C. (2008). Electrochemical biosensors – principles and applications. *Methods*, *6*(2), 57–64.

Pooi See, W., Nathan, S., Yook Heng, L. (2011). A disposable copper (II) ion biosensor based on self-assembly of L-cysteine on gold nanoparticle-modified screen-printed carbon electrode. *Journal of Sensors*, *2011*.

Quartacci, M. F., Cosi, E., Navari-Izzo, F. (2001). Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper deficiency or excess. *Journal of Experimental Botany*, *52*(354), 77–84.

Quinn, C. (2010). Analyzing ITC Data for the Enthalpy of Binding Metal Ions to Ligands. *TA Instruments, Application Note*, 1–6.

Rademacher, C., Masepohl, B. (2012). Copper-responsive gene regulation in bacteria. *Microbiology (United Kingdom)*, *158*(10), 2451–2464.

Ramanathan, S., Ensor, M., & Daunert, S. (1997). Bacterial biosensors for monitoring toxic metals. *Trends in Biotechnology*, *15*(12), 500–506.

Rascio, N., Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, *180*(2), 169–181.

Rensing, C., Maier, R. M. (2003). Issues underlying use of biosensors to measure metal bioavailability. *Ecotoxicology and Environmental Safety*, *56*(1), 140–147.

Rosano, G. L., Ceccarelli, E. a. (2014). Recombinant protein expression in Escherichia coli: Advances and challenges. *Frontiers in Microbiology*, *5*(APR), 1–17.

Sai, V. V. R., Kundu, T., Deshmukh, C., Titus, S., Kumar, P., Mukherji, S. (2010). Label-free fiber optic biosensor based on evanescent wave absorbance at 280 nm. *Sensors and Actuators, B: Chemical*, *143*(2), 724–730.

Samarghandi, M. R., Nouri, J., Mesdaghinia, A. R., Mahvi, A., Nasseri, S., Vaezj, F. (2007). Efficiency removal of phenol, lead and cadmium by means of UV/TiO2/H2O2 processes. *International Journal of Environmental Science and Technology*, *4*(1), 19–25.

Sarkar, A., Daniels-Race, T. (2013). Electrophoretic Deposition of Carbon Nanotubes on 3-Amino-Propyl-Triethoxysilane (APTES) Surface Functionalized Silicon Substrates. *Nanomaterials*, *3*(2), 272–288.

Schön, A., Freire, E. (2012). TA Instruments – Application Note Discovery and Characterization of Inhibitors of Protein / Protein Interactions by ITC . TA Instruments – Application Note, 1–7.

Scouten, W. H., Luong, J. H. T., Stephen Brown, R. (1995). Enzyme or protein immobilization techniques for applications in biosensor design. *Trends in Biotechnology*, *13*(5), 178–185.

Shakya, K., Chettri, M. K., Sawidis, T. (2008). Impact of heavy metals (copper, zinc, and lead) on the chlorophyll content of some mosses. *Archives of Environmental Contamination and Toxicology*, *54*(3), 412–421.

Shetty, R. S., Deo, S. K., Liu, Y., Daunert, S. (2004) Fluorescence-based sensing system for copper using genetically engineered living yeast cells. *Biotechnol. Bioeng*. 88 (5), 664–670.

Sian, H. K., Said, M., Hassan, O., Kamaruddin, K., Ismail, A. F., Rahman, R. a., Illias, R. M. (2005). Purification and characterization of cyclodextrin glucanotransferase from alkalophilic Bacillus sp. G1. *Process Biochemistry*, *40*(3-4), 1101–1111.

Silva, C. J. S. M., Sousa, F., Gübitz, G., Cavaco-Paulo, A. (2004). Chemical Modifications on Proteins Using Glutaraldehyde. *Food Technology and Biotechnology*, *42*(1), 51–56.

Singh, R. K., Tiwari, M. K., Singh, R., Lee, J. K. (2013). From protein engineering to immobilization: Promising strategies for the upgrade of industrial enzymes. *International Journal of Molecular Sciences*, *14*(1), 1232–1277.

Smaldone, G. T., & Helmann, J. D. (2007). CsoR regulates the copper efflux operon copZA in Bacillus subtilis. *Microbiology*, *153*(12), 4123–4128.

Stockinger, E. J. (1994). Bradford Assay for Protein quantification. *Cell*, *32*(1), 3–6.

Su, L. A., Jia, W.Z., Hou, C. J., Lei, Y. (2011) Microbial biosensors: a review. *Biosens. Bioelectron.* 26 (5), 1788–1799.

Tantimongcolwat, T., Isarankura-Na-Ayudhya, C., Srisarin, A., Galla, H. J., Prachayasittikul, V. (2014). Polyacrylamide Hydrogel Encapsulated E. Coli Expressing Metal-Sensing Green Fluorescent Protein As a Potential Tool. *Excli Journal*, *13*, 401–415.

Tekaya, N., Saiapina, O., Ben Ouada, H., Lagarde, F., Jaffrezic-Renault, N. (2013) Ultra-sensitive conductometric detection of heavy metals based on inhibition of alkaline phosphatase activity from *Arthrospira platensis*. *Bioelectrochemistry* 90, 24–29.

Tencaliec, A., Laschi, S., Magearu, V., Mascini, M. (2006). A comparison study between a disposable electrochemical DNA biosensor and a Vibrio fischeri-based luminescent sensor for the detection of toxicants in water samples. *Talanta*, *69*(2), 365–369.

Theophanides, T., Anastassopoulou, J. (2002). Copper and carcinogenesis. *Critical Reviews in Oncology/Hematology*, *42*(1), 57–64.

ThermoScientific. (n.d.). SnakeSkin ™ Dialysis Tubing, *0747*(88244).

Tischer, W., Wedekind, F. (1999). Immobilized Enzymes : Methods and Applications. *Current*, *200*, 95–126.

Turdean, G. (2011). Design and development of biosensors for the detection of heavy metal toxicity. *International Journal of Electrochemistry*, *2011*, 1–15.

Usali, N., Hasmadi, M., Corresponding, I. (2010). Use of Remote Sensing and GIS in Monitoring Water Quality. *Journal of Suitanable Development 3*(3), 228–238.

Velasco-Garcia, M. N., Mottram, T. (2003). Biosensor Technology addressing Agricultural Problems. *Biosystems Engineering*, *84*(1), 1–12.

Verma, N., Singh, M. (2005). Biosensors for heavy metals. *BioMetals*, *18*(2), 121–129.

Vop ádensk á, I., Palkov á, Z. (2015). New biosensor for detection of copper ions in water based on immobilized genetically modi fi ed yeast cells. *Biosensors and Bioelectronics 72*, 160–167.

Wang, R., Wang, W., Ren, H., Chae, J. (2014). Detection of copper ions in drinking water using the competitive adsorption of proteins. *Biosensors and Bioelectronic*, *57*, 179–185.

WHO (2011) Cadmium in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/ WSH/03.04/80/Rev/1)

WHO (2003). Chromium in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/ WSH/03.04/4).

WHO (2003). Copper in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/ WSH/03.04/88).

WHO (2011) Lead in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/WSH/03.04/9/ Rev/1)

WHO (2005) Mercury in drinking-water. Background document for development of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/ WSH/05.08/10)

WHO (2005) Nickel in drinking-water. Background document for development of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/ WSH/05.08/55)

Wilcox, D. E. (2008). Isothermal titration calorimetry of metal ions binding to proteins: An overview of recent studies. *Inorganica Chimica Acta*, *361*(4), 857–867.

Wilson, G. S., Gifford, R. (2005). Biosensors for real-time in vivo measurements. *Biosensors and Bioelectronics*, *20*(12), 2388–2403.

Yagi, K. (2007). Applications of whole-cell bacterial sensors in biotechnology and environmental science. *Appl Microbial Biotechnol*, 17, 1251–1258.

Yuce, M., Nazir, H., Donmez, G. (2010) A voltammetric *Rhodotorula mucilaginosa* modified microbial biosensor for Cu(II) determination. *Bioelectrochemistry* 79 (1), 66–70.

Zeng, Y., Yi, R., Cullen, B. R. (2003). MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(17), 9779–9784.