A

M.Sc. Thesis

On

Development of optical biosensor for the detection of pesticides

For the Degree of
Master of Science

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Under the Supervision
Of

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In the end, I wish to express my deep sense of gratitude to my family, for supporting and encouraging me at every step of my work. It is power of their blessings, which has given me the courage, confidence and zeal for hard work.

Date: July, 2014

Place: Patiala

Regards,

Gurpreet Singh

(GURPREET SINGH)

CANDIDATE'S DECLARATION

I hereby declare that the work presented in this thesis entitled, **"Development of optical biosensor for the detection of pesticides"** in partial fulfillment in the requirement for the award of Degree of **Master of Science in Chemistry**, submitted in the School of Chemistry and Biochemistry, **Thapar University, Patiala**, is an authentic record of my own work carried out under the supervision and guidance of **Dr. Susheel Mittal**, Senior Professor, School of Chemistry and Biochemistry, Thapar University, Patiala and refers other researcher's work which are duly listed in the reference section. The matter embodied in this thesis has not formed the basis for the award of any other degree of this or any other university.

Date: July, 2014

Gurpreet Singh
(GURPREET SINGH)

Place: Patiala

This is to certify that the above statement made by the student concerned is correct and true to the best of my knowledge.

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CERTIFICATE

This is to certify that the thesis entitled **"Development of optical biosensor for the detection of pesticides"** being submitted in partial fulfillment of requirements for the award of degree of **Master of Science in Chemistry, submitted in the School of Chemistry and Biochemistry, Thapar University, Patiala** is a bonafide work carried out under the supervision of **Dr. Susheel Mittal**, Senior Professor, School of Chemistry and Biochemistry, Thapar University Patiala and that no part of this project has been submitted for the award of any other degree.

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Contents

Abstract

An optical biosensor was designed for determination of Pesticides and herbicides . Detection was obtained with immobilised *Chlorella vulgaris* microalgae entrapped by Ca-alginate and in free state. Effects of pH, algal density, substrate concentration, inhibition of alkaline phosphatase by organophosphate pesticides like acephate, chlorpyrifos, triazophos and malathion and herbicides like atrazine, metribuzin and glyphosate were studied. Detection limit for herbicides like atrazine, metribuzin, glyphosate was found to be in free state 10^{-10} M, 10^{-8} M, 10^{-6} M and in immobilized state 10^{-10} M, 10^{-9} M, 10^{-7} M respectively. Detection limit for organophosphorus pesticides like acephate, chlorpyrifos, triazophos was found to be in free state 10^{-8} M, 10^{-7} M, 10^{-6} M and in immobilized state 10^{-9} M, 10^{-7} M, 10^{-7} M respectively. Malathion did not inhibit the alkaline phosphatase detected by this optical biosensor.

1. List of Abbreviations

p-PNP	-	Para nitro phenyl phosphatase
PNP	-	Para nitro phenol
ALP	-	Alkaline phosphatase
OPH	-	Organophosphorus hydrolase
AChE	-	Acetylcholinesterase
BTB	-	Bromothymol blue
UV-Vis spectrophotometer	-	Ultraviolet - Visible spectrophotometer
DNOC	-	Dinitro-ortho-cresol
2,4-D	-	2,4-Dichlorophenoxyacetic acid
QCM	-	Quartz crystal microbalance
Tris - HCl	-	Tris - Hydrochloric acid

1 Introduction

Population on the earth is increasing day by day, but the sources are limited. To fulfill the needs of food, farmers continuously strive to increase the production of crops not by natural fertilizers but with the help of chemical fertilizers (urea) and pesticides (insecticides, herbicides, fungicides) which are being used for the protection of crop from harmful insects, pests and fungi. Although, these chemical substances increase the crop production but on the other hand decrease the quality of food. Due to excessive use of fertilizers and pesticides, these chemicals enter the food chain, as sometimes these are non-biodegradable and ultimately affect the metabolic process of human beings, plants and animal.

Pesticide means any chemical substance used for protecting the crop from pests like insects, fungi, bacteria, rodents and nematodes and unwanted species of plant like weeds and herbs etc. Pesticides are classified into various classes on the basis of their mode of action on plants and animals which include insecticides for control of insects; fungicides used to prevent from fungus; herbicides for destroying the weeds; disinfectants used for preventing from bacteria, mice and rats; nematodes for destroying the nematodes. Pesticides are further classified into sub-classes on the basis of chemical structure:

Insecticides like organophosphates (acephate; Fig. 1, chlorpyrifos; Fig. 2, triazophos; Fig. 3, malathion; Fig. 4), organochlorines, carbamate.

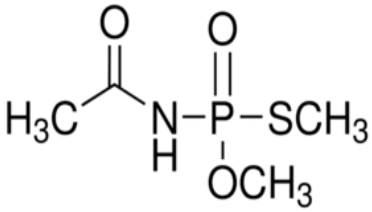

Figure.1 Acephate

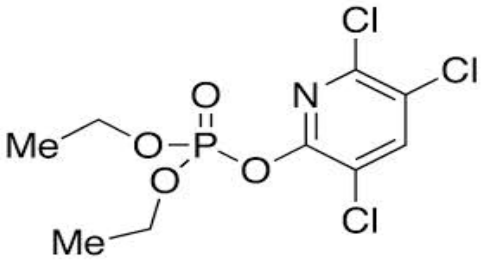

Figure.2 Chlorpyrifos

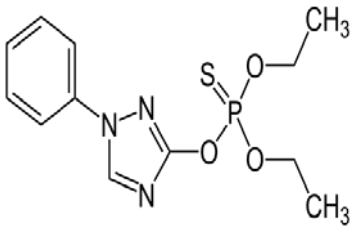

Figure 3. Triazophos

Figure 4. Malathion

Herbicides: Triazines (atrazine; Fig. 5, metribuzin; Fig. 6), organophosphorus (glyphosate; Fig.7), substituted ureas.

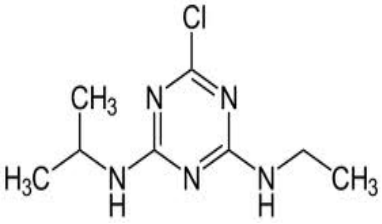

Fig.5 Atrazine

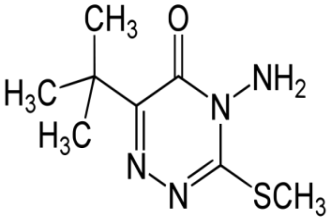

Fig. 6 Metribuzin

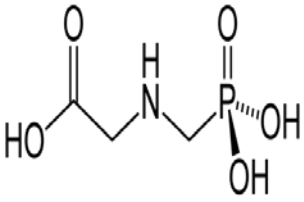

Fig.7 Glyphosate

Fungicides: Hexachlorobenzene, organomercurials, pentachlorophenol, pthalimides, dithiocarbamates.

Nematicides: Isothiocyanates, carbamates, halogenated hydrocarbons.

Organophosphates: Pesticides inhibit and destroy the pest in the several ways. Acephate is used for control of a wide range of biting and sucking insects, especially aphids, including resistant species, in fruit, vegetables (e.g. potatoes and sugar beets) and in horticulture (e.g. on roses). Chlorpyrifos and triazophos acts on the nervous system of insects by inhibiting acetylcholinesterase. Malathion is a parasympathomimetic which binds irreversibly to cholinesterase.

Herbicides can kill the plant either by contact process (in contact with the weed destroys the plants) or inhibiting the metabolic processes particularly enzyme system. Atrazine is used to stop pre and post-emergence broadleaf and grassy weeds in crops. In the United States as of 2014, atrazine was the second most widely used herbicide after glyphosate[1]. Glyphosate is

used to kill weeds. Metribuzin acts by inhibiting photosynthesis by disrupting photosystem II [2]. Herbicides like atrazine, metribuzin and glyphosate are widely used because of their cost effective, supreme quality and safe from undue reaction, packed in good quality material and easily used. Organophosphates pesticides like acephate, triazophos, chlorpyrifos and malathion are widely used due to different insecticidal effect, a high speed kill performance, resulting in less resistance and the use of low concentration.

For the detection and determination of toxicity and inhibition of enzyme activity by the pesticides even at low concentration in our environment on living beings many analytical methods are used including colorimetry, capillary electrophoresis, mass spectrometry, gas chromatography(GC), high-performance liquid chromatography(HPLC), thin layer chromatography coupled with different detectors, cyclic voltametry and some flow injection analysis. These all analytical methods are expensive, time-consuming and usability only in highly specialized laboratories with very expensive equipment and trained personnel. However, biosensors overcome these limitations.

To protect the human health from possible hazards, there is a need to develop sensitive, fast and easy methods for determination of pesticides in water, vegetables and fruits. One of these methods is biosensor. A biosensor is a device that integrates a free and immobilized biological element (e.g. enzyme, DNA probe, antibody) that recognizes the analyte (e.g. enzyme substrate, complementary DNA, antigen) and a transduction element used to convert the (bio) chemical signal resulting from the interaction of the analyte with the bioreceptor into an electronic one. According to the signal transduction technique, biosensors are classified into electrochemical, optical, piezoelectric and mechanical biosensors. Optical biosensors (UV spectrophotometer, fluorescence spectrophotometer) have been used for pesticides detection due to their high sensitivity. UV spectrophotometers have been used for pesticides detection due to their easily handling system, sensitivity, less time consuming, cheaper and without trained personnel. UV spectrophotometers for pesticides detection are based on measurements of absorbance of products (PNP) at 400 nm of the inhibition of enzymatic activity (alkaline phosphatase) by different pesticides.

3 Literature Survey

Pesticides analysis is essential due to their extensive use and bad impact on health and environment. The detection of pesticides can be done by several analytical techniques. A wide number of analytical methods like HPLC, GC - MS or various combinations of these are employed for the detection of pesticides [3–7]. These methods suffer disadvantage of high cost, need for trained personnel and laboratory bound tests. From last decade various types of biosensors were developed for the detection of pesticides as these provided a fast, portable, with high sensitivity and cost-effective. Enzymes commonly used for the detection based on inhibition are cholinesterase, alkaline phosphatase, acid phosphatase, tyrosinase, aldehyde dehydrogenase, acetolactate synthase. Enzyme biosensors for pesticide detection are based on measurements of enzyme inhibition, to develop a microbial biosensors microorganisms have to be immobilized on to a transducer using immobilized techniques (e.g. cross-linking, entrapment) [8] .

J. M. Abad et al. (1998) developed piezoelectric biosensor for detection of organophosphatase pesticides: paraxon and carbaryl, based on inhibition activity of acetylcholinesteras(bioreceptor). The detection limit is reported as 5.0×10^{-8} and 1.0×10^{-7} M for paroxon and carbaryl, respectively [9].

Ashok Mulchandani et al. (2001) with electrochemical and optical transducers studied the inhibition of organophosphorous hydrolase (OPH) enzyme by different organophosphate pesticides. Potentiometric OPH-based enzyme electrode using organophosphatase hydrolase enzyme immobilized by cross-linking OPH with bovine serum albumin and glutaraldehyde. This biosensor was able to detect as low as 2 µM of paraoxon, ethyl parathion, methylparathion and diazinon but other non-organophosphate pesticides such as simazine, triazine, atrazine, sevin and sutan, did not interfere in approximately 2 min. while optical OPH-based biosensor was able to detect as low as 2 µM of paraoxon and parathion and 5 µM of coumaphos, without interference from carbamates and triazines in less than 2 min. [10].

Christophe Vedrine et al. (2003) designed an optical biosensor (fluorescence-based biosensor) for determination of herbicides like DNOC and atrazine, simazine, isoproturon,

diuron using immobilised Chlorella vulgaris microalgae entrapped on a quartz microfibre filter and placed in a five-membrane homemade flow cell with maximum biosensor response at pH-7 at 20 °C. Detection limit for DNOC, atrazine, simazine, isoproturon and diuron was found to be 5, 0.25, 0.5, 0.025, and 0.025 µg/L respectively [11].

Celine Chouteau et al. (2005) developed conductometric biosensors based on immobilized whole cell *Chlorella vulgaris* microalgae (enzymes can be immobilised on electrodes using bovine serum albuminand glutaraldehyde as a crosslinker using as bioreceptor and conductometric electrode as transducer for detection of alkaline phosphatase activities with heavy metal of cadmium ions , substrate pNPP and detection limit was 1 ppb of Cd^{2+} [12].
Brandy J. White et al. (2005) developed optical sensor for detection of organophosphates based on inhibition of organophosphorus hydrolase by organophosphates pesticides paraoxon, coumaphos, diazinon, or malathion at absorbance intensity 412 nm and detection limit found 7 parts per million, 250 ppt, 800 ppt, 1 part per billion [13]. Mamta Awasthi et al. (2005) studied the effect of heavy metals: zinc (Zn), nickel (Ni) and cadmium (Cd) on enzyme activity of nitrate reductase and alkaline phosphatase by UV-Visible spectrophotometer biosensor in free and immobilized state of *Chlorella vulgarius* as biomaterial and studied the effect on growth and photosynthetesis activities of *Chlorella vulgarius*. In this experiment Zn and Cd was more sensitivity showed to alkaline phosphatase and Ni towards nitrate reductase enzyme [14].

Jitendra Kumar et al. (2006) developed a system for the detection of methyl parathion pesticide based on optical microbial biosensor using whole cells of *Flavobacterium* sp. were immobilized by trapping in glass fiber filter as biocatalyst the enzyme hydrolyzed the methyl parathion into detectable product *p*-nitrophenol at 410 nm with buffer pH 8.5 . A detection limit of methy parathion was found as 0.3 µM [15].

Hangh Nguyen-Ngoc et al (2007) developed fluorescent biosensor based on entrapment of whole cells of *Chorella vulgarius* in an inorganic translucent matrix for the detection of herbicide: diuron that inhibits the algal photosystemII observed by this experiment [16].
Bambang Kuswandi et al. (2008) developed a method for the detection of chlorpyrifos was developed optical fibre biosensor using a single sol–gel film with immobilized

acetylcholinesterase (AChE) and bromothymol blue (BTB). The detection limit was observed for chlorpyrifos as 0.04 mg/L [17].

Elena Pena-Vazquez et al. (2009) encapsulated three microalgal species (*Dictyosphaerium chlorelloides*, *Scenedesmus intermedius* and *Scenedesmus* sp.) in silicate sol–gel matrices to detect the herbicides (triazines: simazine, atrazine, propazine, terbuthylazine, urea based herbicides: linuron) and developed microalgae fiber optic biosensors. The detection limit of simazine, atrazine, propazine, terbuthylazine and linuron was 3.6, 13.5, 7.6, 3.3 and 4.1, µg/L respectively. No significant increases of fluorescence response was obtained for similar concentrations of 2, 4-D or Cu (II) [18].

Kun Jia et al. (2013) using gold QCM (quartz crystal microbalance) for the detection of carbofuran and atrazine . By this methodology the detection limit of carbofuran and atrazine was reported as 4.5 µM and 4.6 µM respectively [19].

4 Experimental section

4.1 Materials and method

Unicellular green algae *Chlorella vulgaris* was prepared in BG-11 medium. The organophosphate pesticides like acephate (97%), chlorpyrifos (94.3%), triazophos (60%), malathion (96.14%) and herbicides like atrazine (95%), metribuzin (70%), glyphosate (95%)) were purchased from Markfed (Mohali) and were prepared at different concentrations from 10^{-10}M to 10^{-2} M. For interference study concentration varied from 2×10^{-10}M to 2×10^{-2} M. $MgCl_2$ was purchased from Loba chemie (India). Substrate para-nitrophenyl phosphate (p-NPP) and Tris-HCl were purchased from Sigma Aldrich. Double beam UV- VIS spectrophotometer UV 570455 was used to measure absorbance. Inhibition of alkaline phosphatase (*Chlorella vulgaris)* activity was assayed by different herbicides like atrazine, metribuzin, glyphosate and organo-phosphorus pesticides like acephate, chlorpyrifos, triazophos, malathion. p-PNP (0.02 mg/10 mL) was used as substrate and $MgCl_2$ (2 g/100 mL) was used as enzyme activator. Tris – HCl (16 g/L) was used as a buffer at pH 8.7. The enzyme activity is based on total para- nitrophenol (PNP) formed in a known volume of culture suspension.

4.2 Cell immobilization

A widely used technique for immobilization is entrapment in Ca-alginate gels. *Chlorella vulgarius* cells (0.2 mL/million cells) obtained by centrifugation and repeated washings, were suspended in 0.05g of sodium alginate for 15 min. The mixture was pumped drop-wise into ice-cold $CaCl_2$ (2%) solution and the beads thus formed, were washed several times with deionised double distilled water and placed in refrigerator under culture conditions.

5 Results and Discussion

5.1 Measurement of absorbance using whole cells

Algal biosensor response of para-nitrophenol (p-NP) was obtained from UV-Vis spectrophotometer with absorption peak at 400 nm (Figure 8) generated by alkaline-phosphatase activity on substrate para-nitro phenyl phosphate (p-NPP). *Chlorella vulgaris* algae biomaterial 0.2 mL with cell count 10 million cells/ mL was incubated with enzyme activator 0.3 mL of $MgCl_2$ (2 mM) solution, p-NPP (0.2g per 10ml) substrate 0.4 mL and 9.1 mL Tris-HCl buffer of pH~8.7, reaction mixture was shaken for 3 hours. After filtration, the solution was separated and measured its absorbance at 400 nm. This experiment was repeated a number of times to optimize different parameters like incubation time, pH, biomaterial concentration and concentration of substrate.

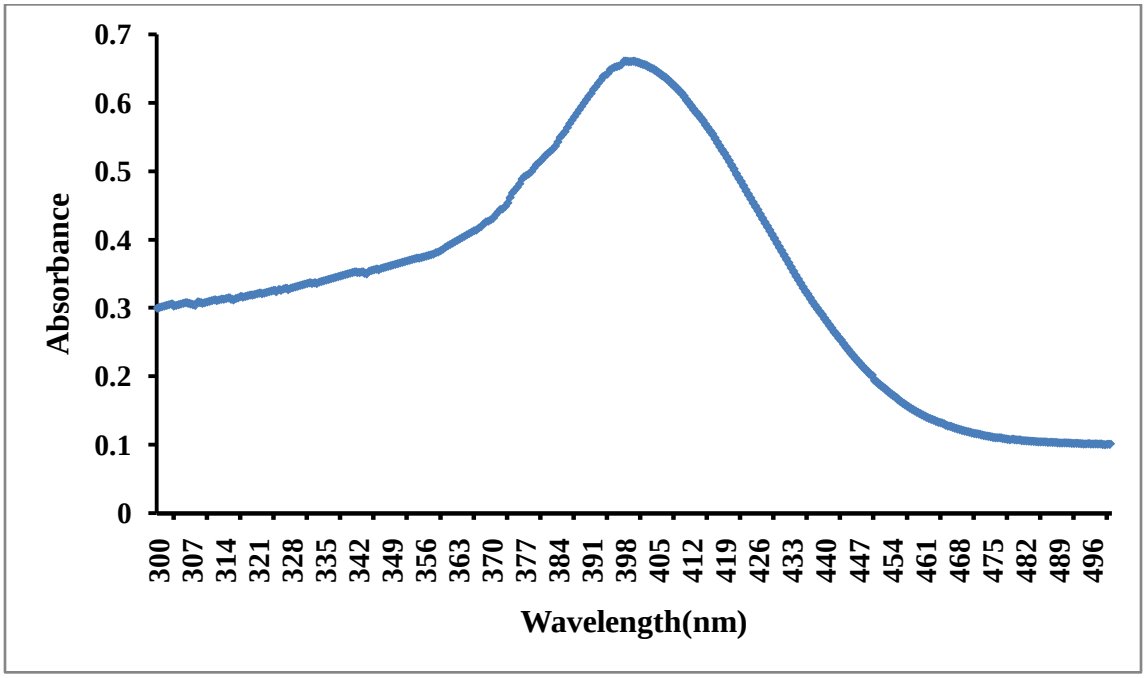

Figure 8. Plots of absorbance as a function of wavelength for para-nitrophenol (PNP)

5.1.1 Detection of incubation time

Experiments were performed to study the time of incubation for optimum enzyme release activity. Samples were incubated from 1 to 8 hours to know the stable incubation time

(Figure 9). It can be seen that after 3 hours of incubation there was no change in absorbance of the solution. All subsequent experiments were run with 3 hours incubation period.

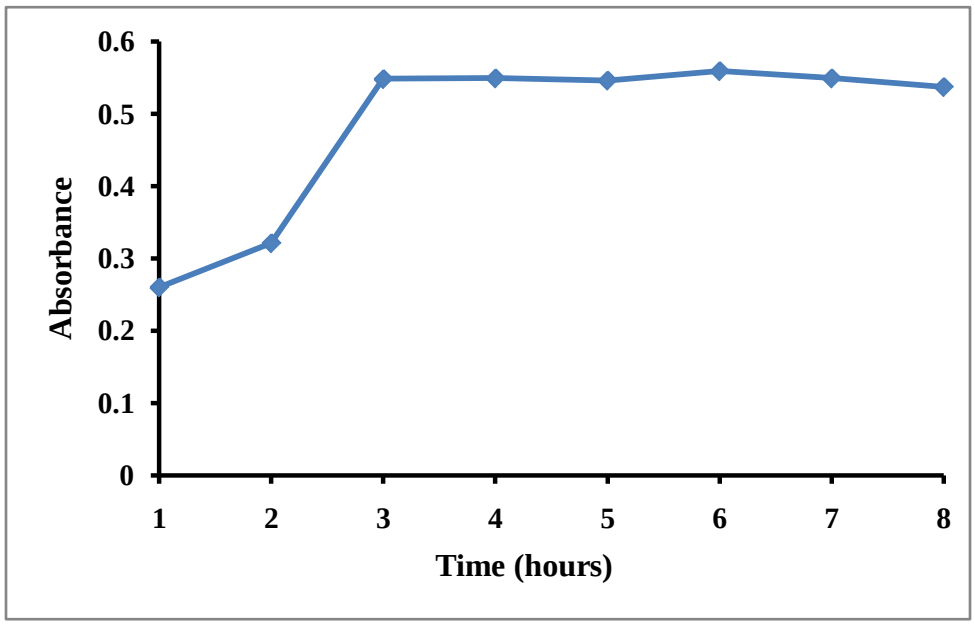

Figure 9. Absorbance vs time plots to optimize concentration of *Chlorella vulgaris* at different incubation periods

5.1.2 Effect of pH

Effect of pH on the algal response were tested with various buffer solutions of pH from 4 to 11 by adding concentrated hydrochloric acid and ammonia, as per requirement. The results are shown in Figure 10. Response of the biosensor was maximum in the pH range 8 to 9 which was selected for further studies. Absorbance values were less in the pH ranges 4 to 7 and 10 to 11.

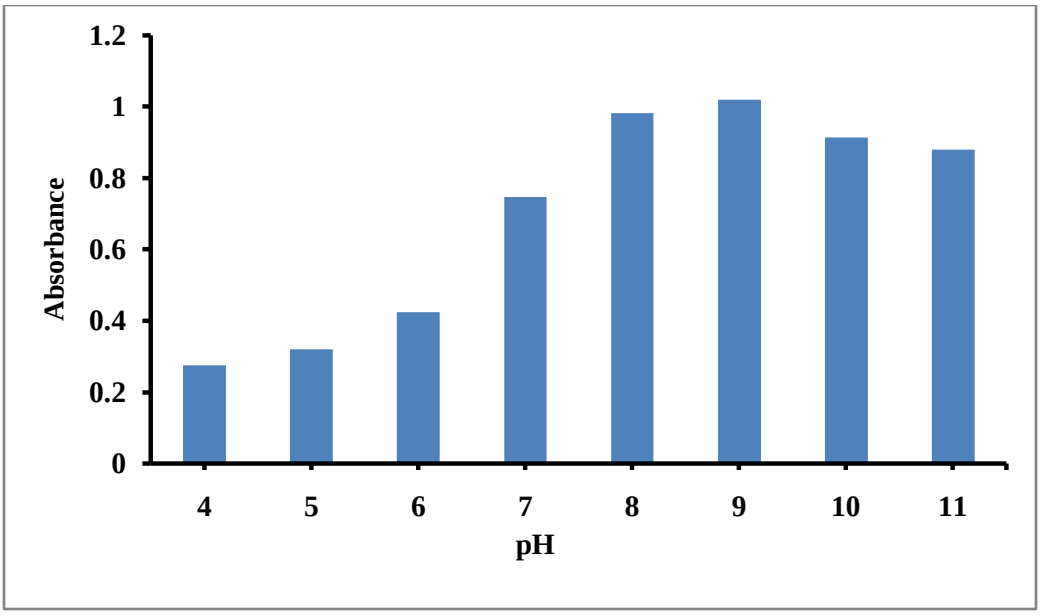

Figure 10. Absorbance as a function of pH of PNP in the presence of *Chlorella vulgaris* after 3 hours of incubation period

5.1.3 Effect of biomaterial concentration

To know optimum amount of the biomaterial for maximum performance of the biosensor, experiments were conducted to study inhibition of atrazine (10^{-5}M - 10^{-3}M) in the presence of different concentrations of the biomaterial in the range 0.1 mL to 0.5 mL (solution containing million cells per mL). Results are given in Figure 11 and it is seen that inhibition (%) of biomaterial is maximum in 10^{-3}M atrazine concentration as compared to with 10^{-4}M and 10^{-5}M solutions. For 10^{-3} M atrazine solution maximum inhibition (%) occured at 0.2 mL/10 mL of the biomaterial amount. Beyond and less than this concentration inhibition (%) of biomaterial decreased. Hence, 0.2 mL biomaterial can be taken as optimized concentration of the biomaterial.

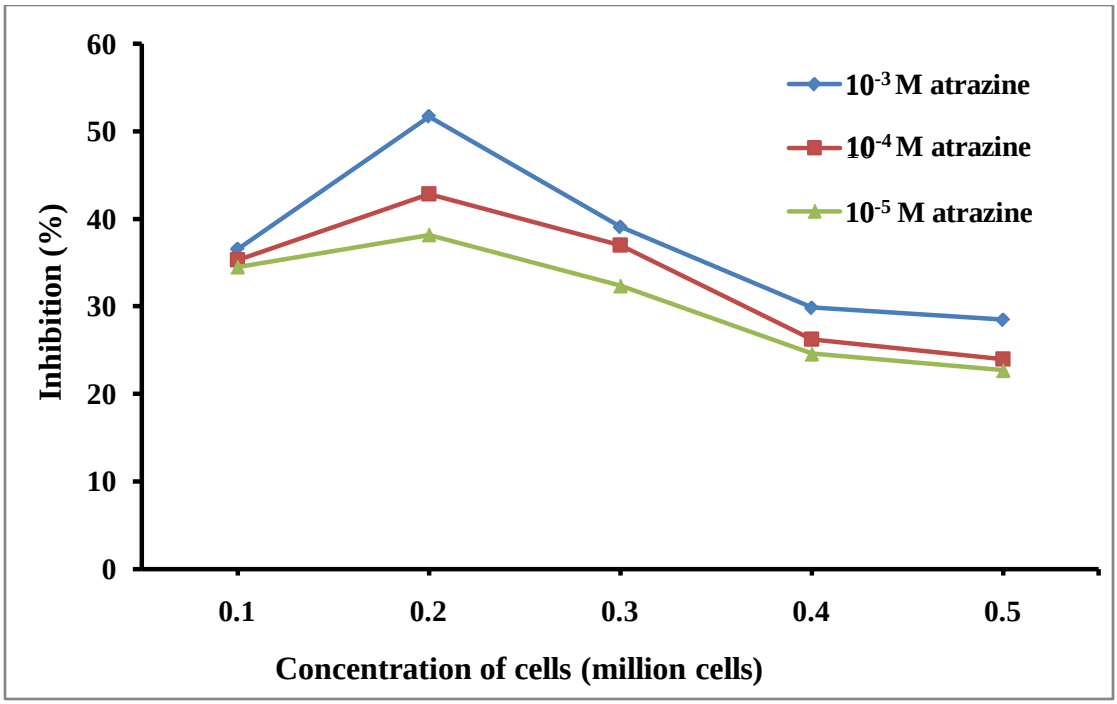

Figure 11. Plots of inhibition of atrazine as a function of concentration of PNP at different concentrations of *Chlorella vulgaris* after 3 hours incubation

5.1.4 Optimization of substrate concentration

Enzyme release activity was optimized with different concentrations of the substrate p-NPP (0.2 mg/10 mL) from 0.1 mL to 1.0 mL. Results in Figure 12 show that the biosensor response was maximum for 0.4 mL substrate concentration and after which the absorbance remained constant. Hence, 0.4 mL subsrate concentration was taken as optimized value for further study.

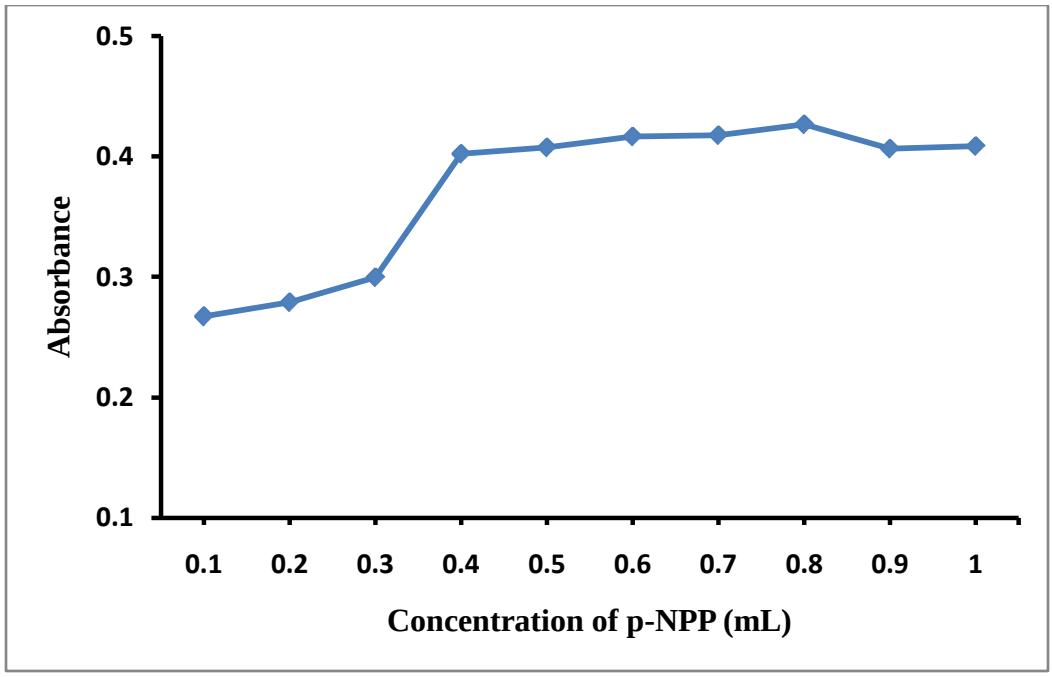

Figure 12. Absorbance vs concentration plots of PNP at different concentrations of p-NPP (0.02 mg/mL)

5.2 Optimization of pesticide amount

5.2.1 Detection of herbicides

Toxicity of herbicides i.e., atrazine, metribuzin and glyphosate on enzyme release activity was studied by this experiment. All herbicides (1 mL/10 mL) was taken 10^{-4} M equimolar concentration with optimized biomaterial 0.2 mL (immobilized and free state) and optimized substrate 0.4 mL for 3 hours. Inhibition of enzyme activity by different herbicides both in immobilized state and free state is shown in Figure 13. It can be seen from the figure that biomaterial when taken in immobilized form experiences more inhibition than in the free state. This observation was same for all the three herbicides.

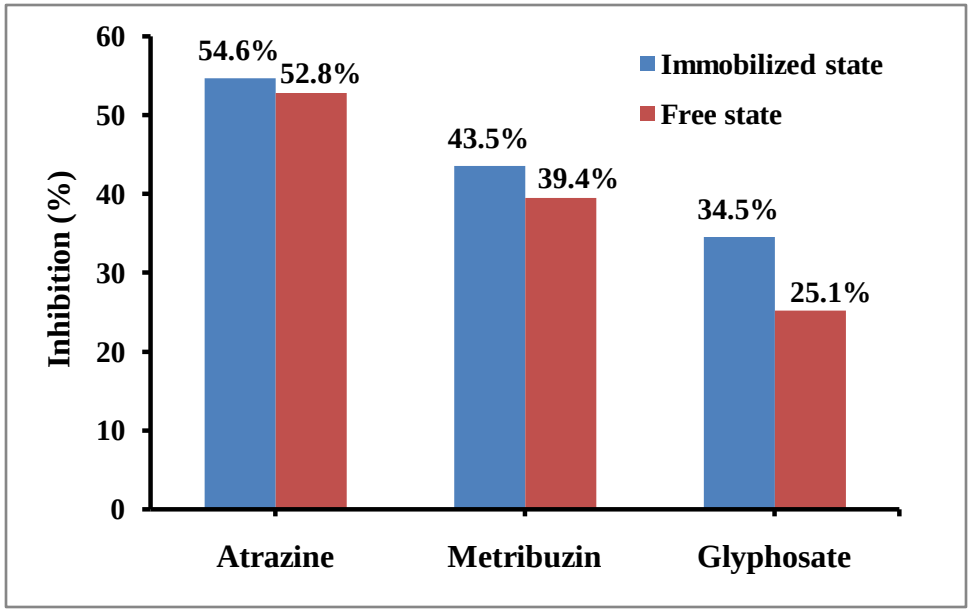

Figure 13. Extent of inhibition to the release of enzyme activity by different herbicides (10^{-4}M) in immobilized and free states

5.2.2 Measurement of inhibition (%) by atrazine at different concentration

Inhibition (%) by atrazine was studied for different concentration 10^{-10} M to 10^{-3}M in immobilized and free states. The inhibition increased with increase in concentration of the immobilized and free states but atrazine inhibition was maximum in immobilized state as shown in Figure 14.

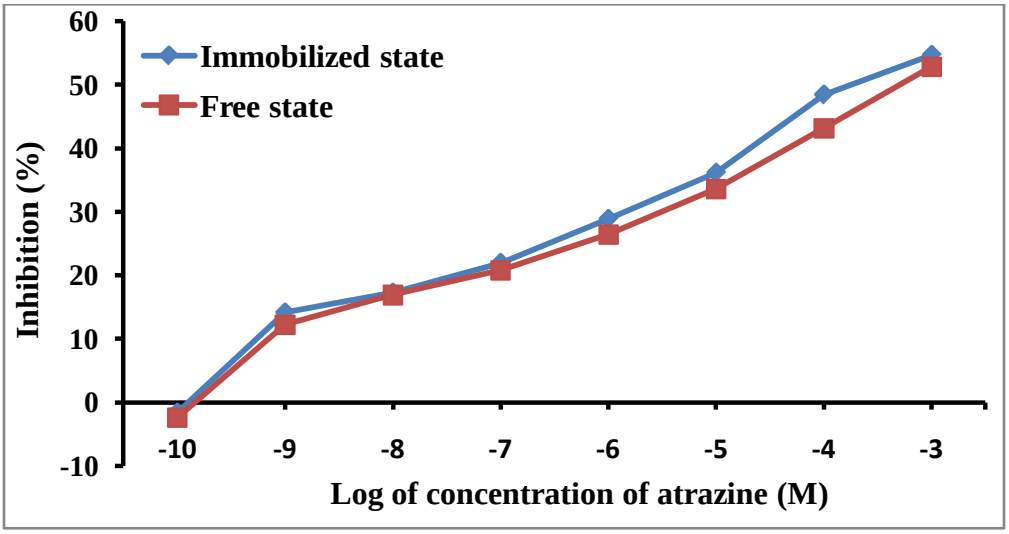

Figure 14. Inhibition of enzyme activity by atrazine at different concentrations

5.2.3 Toxicity of metribuzin at different concentrations

Enzyme release activity was studied in presence of metribuzin at different concentrations 10^{-10} M to 10^{-3} M in free and immobilized states of the biomaterial. Metribuzin inhibition (%) increased more with increase in concentration in the immobilized state as compared with free state of the biomaterial, as shown in Figure 15. Inhibition (%) by metribuzin in the free state was observed in the concentration range 10^{-7}M to 10^{-3}M while in immobilized state in the range 10^{-8} M to 10^{-3} M.

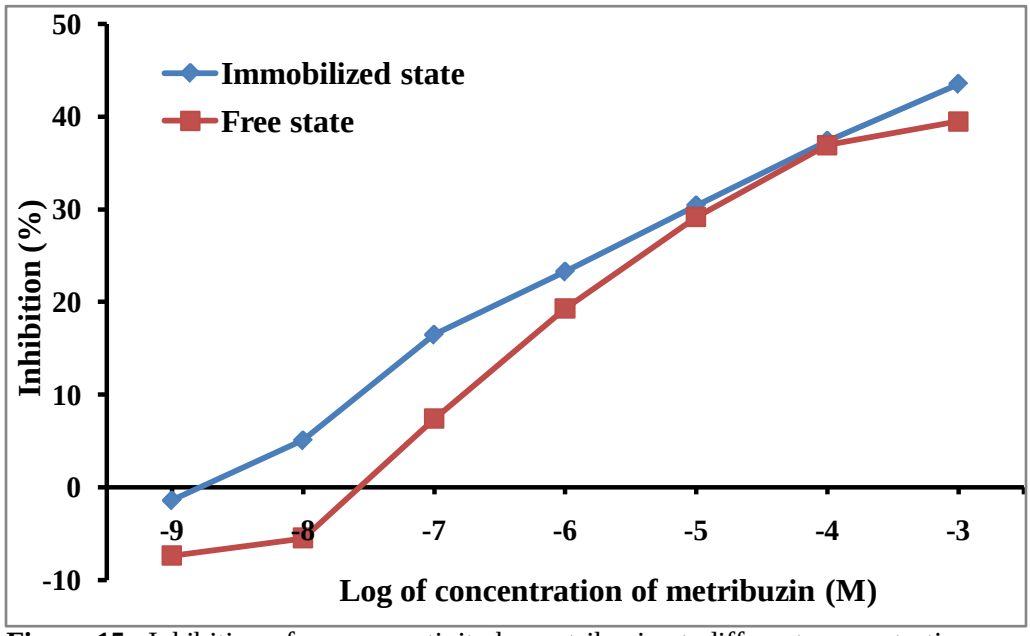

Figure 15. Inhibition of enzyme activity by metribuzin at different concentration.

5.2.4 Effect of glyphosate on enzyme activity

Experiment were conducted to study the effect of glyphosate at different concentrations on biomaterial in free and immobilized states. Glyphosate showed inhibition (%) upto 10^{-6} to 10^{-3}M concentration in free state but inhibition (%) increased with immobilized biomaterial upto 10^{-7} to 10^{-3}M. Result are given in Figure 16.

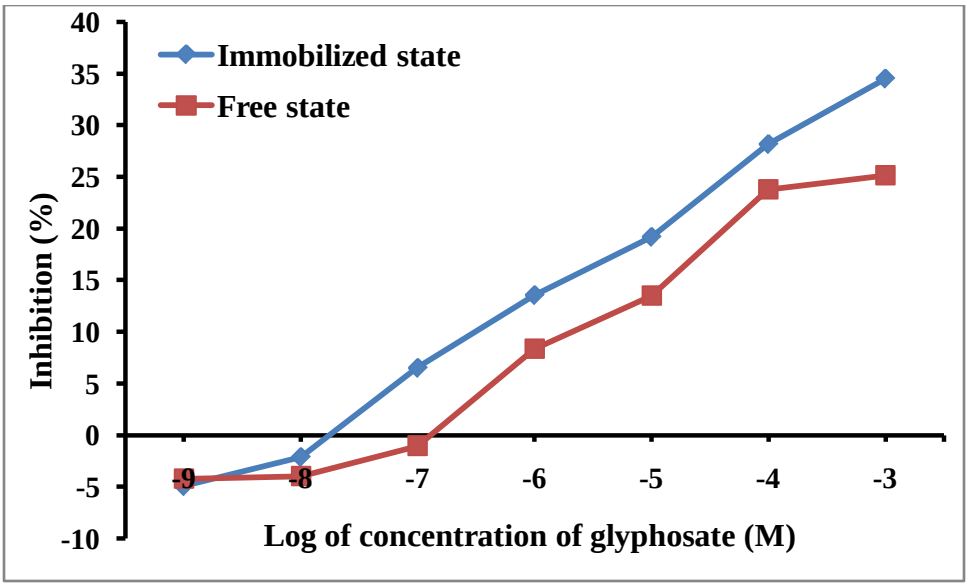

Figure 16. Effect of concentration of glyphosate on *Chlorella vulgaries* in the immobilized and free states

5.3 Detection of organo-phosphorous pesticides

Figure 17 shows toxicity of organo-phosphorous pesticides on enzymatic activities. Acephate, chlorpyrifos, triazophos and malathion each of 10^{-4} M inhibited the alkaline phosphatase activity by 23.2%, 20.3%, 16.1% and 0% respectively, in free state while immobilized state the inhibition (%) decreased as 36.9%, 26.5%, 20.3% and 0%, respectively. Acephate was more toxic to alkaline phosphatase than chlorpyrifos and triazophos but malathion shows no toxicity on alkaline phosphatase.

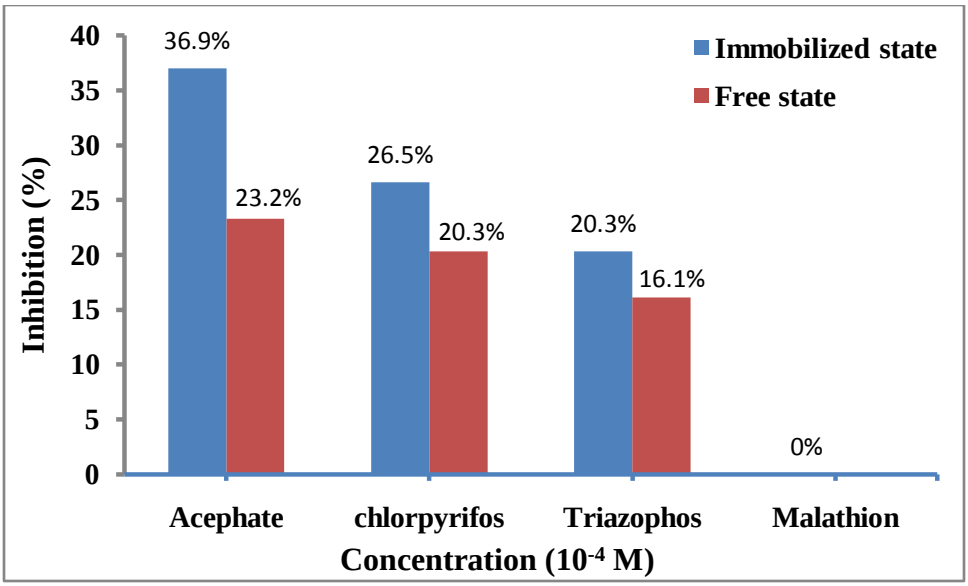

Figure 17. Inhibition of organo-phosphorous pesticides in immobilized as well as free states

5.3.1 Toxicity of acephate

Acephate showed inhibition (%) with different concentration from 10^{-10} to 10^{-3}M in the immobilized and free states given in Figure 18. Acephate inhibition (%) increases with increase in concentration. Acephate showed inhibition (%) from 10^{-9} to 10^{-3}M in immobilized state and in free state the inhibition (%) occured upto 10^{-8} to 10^{-3}M.

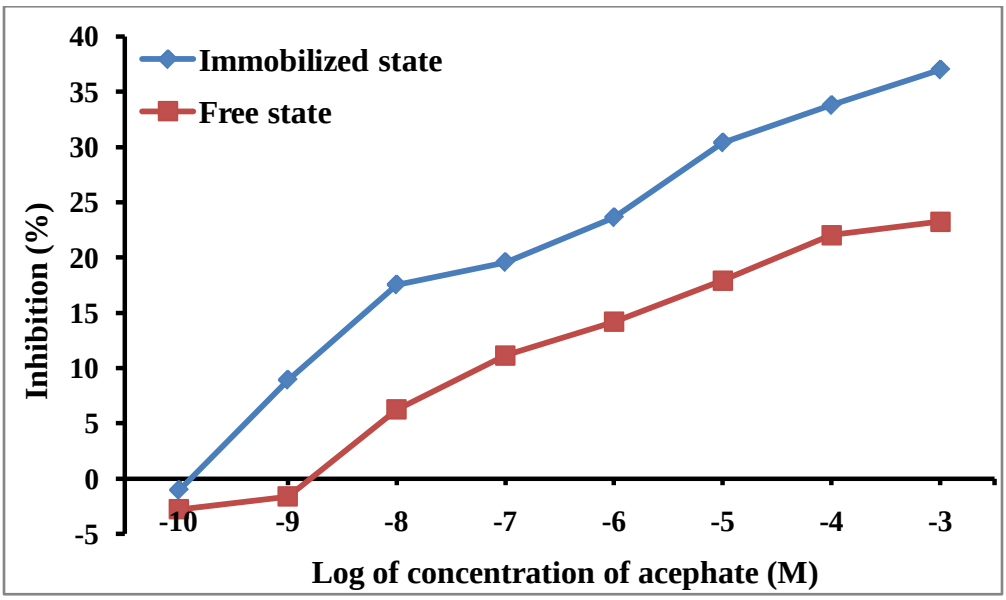

Figure 18. Inhibition of enzyme activity by acephate at different concentrations in free and immobilized states

5.3.2 Detection of chlorpyrifos

In this experiment studied the inhibition of alkaline phosphatase enzyme by chlorpyrifos with different concentrations from 10^{-9} to 10^{-3}M. Toxicity of chlorpyrifos increases with increase of concentrations. Chlorpyrifos inhibited the alkaline phosphatase upto 10^{-7} to 10^{-3}M in free and immobilized state but inhibition in immbolized states is maximum as compared to with free states algal material. Result are given in Figure 19.

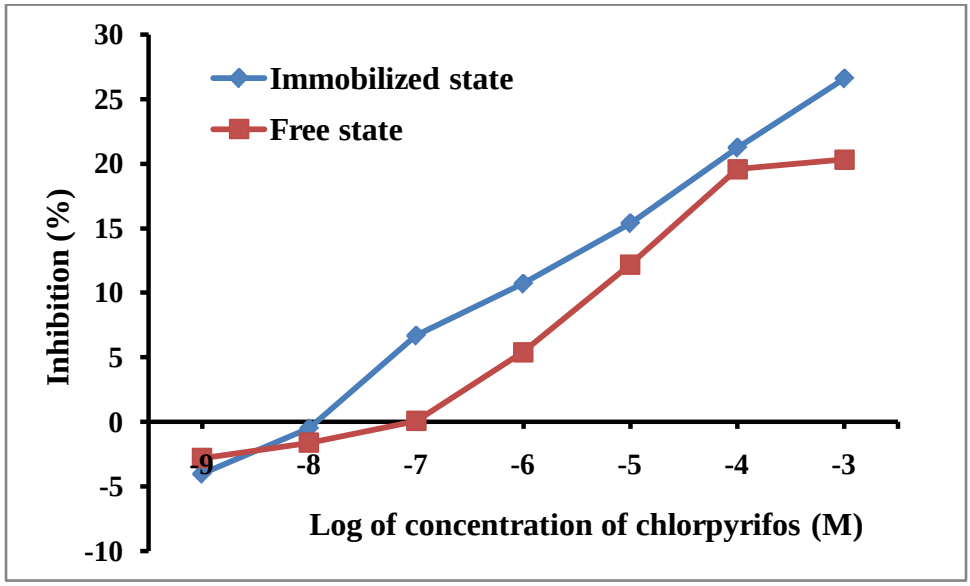

Figure 19. Inhibition (%) of alkaline phosphatase at different concentrations of chlorpyrifos in immobilized and free states

5.3.3 Optimization of triazophos

Effect of triazophos on enzyme activity of *Chlorella vulgaris* was studied at different concentration from 10^{-9} to 10^{-3}M. Triazophos inhibition increases with increase in concentration both in immobilized and free states . But inhibition (%) was maximum in immobilized state than in the free state. Triazophos showed effect on enzyme activity upto 10^{-7} to 10^{-3}M and results are given in Figure 20.

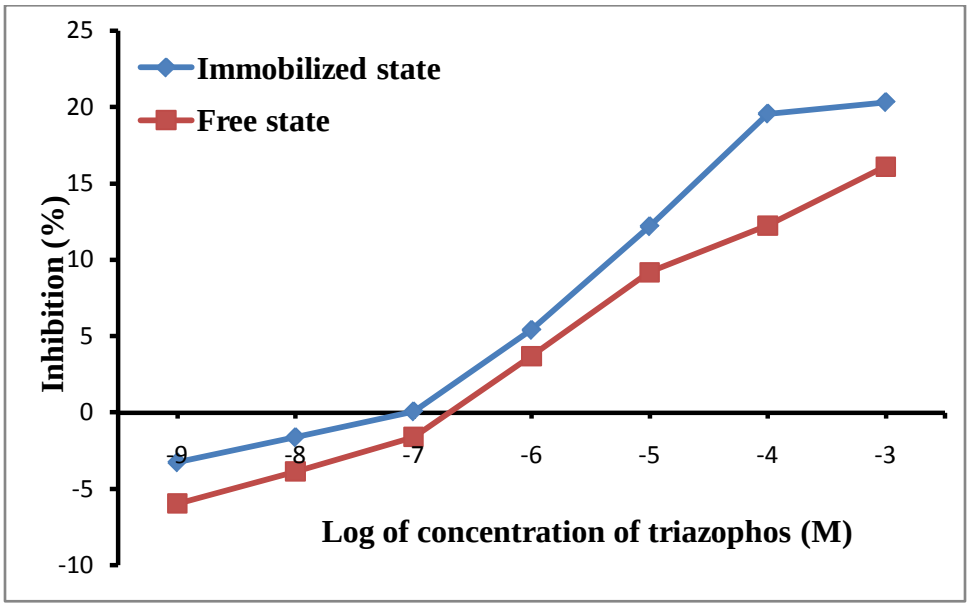

Figure 20. Plots showing toxicity of triazophos at different concentrations

5.4 Interference effect of herbicides

5.4.1 Effect of metribuzin and glyphosate with atrazine

Experiments were conducted to study the effect of interference on metribuzin and glyphosate with different concentrations of atrazine from 2×10^{-10} to 2×10^{-3}M/mL. Equal amount of metribuzin and glyphosate (0.5mL of 2×10^{-4}M each) were added to a flask containing 0.2 mL (2×10^{-10} M to 2×10^{-3} M) of atrazine and algae biomaterial (*Chlorella vulgaris*) (0.2 mL), 0.3 mL $MgCl_2$ used as enzyme activator and substrate p-NPP (0.4 mL), buffered at pH 8.7 with Tris-HCl buffer. The total volume was made upto 10.0 mL with buffer solution. The reaction mixture was stirred for 3 hours. Results shown in Figures 21 and 22.

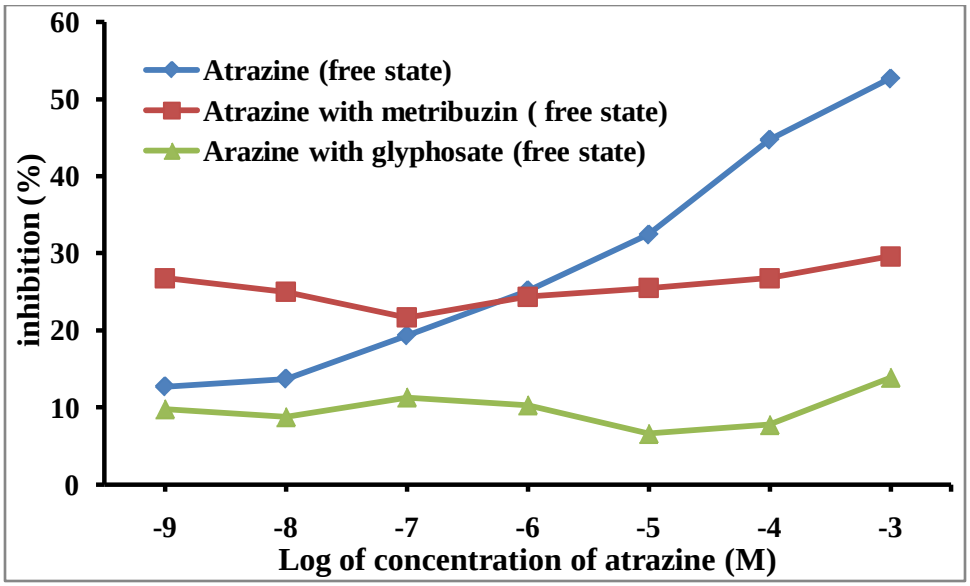

Figure 21. Plots showing interference effect of herbicides on biomaterial in free state

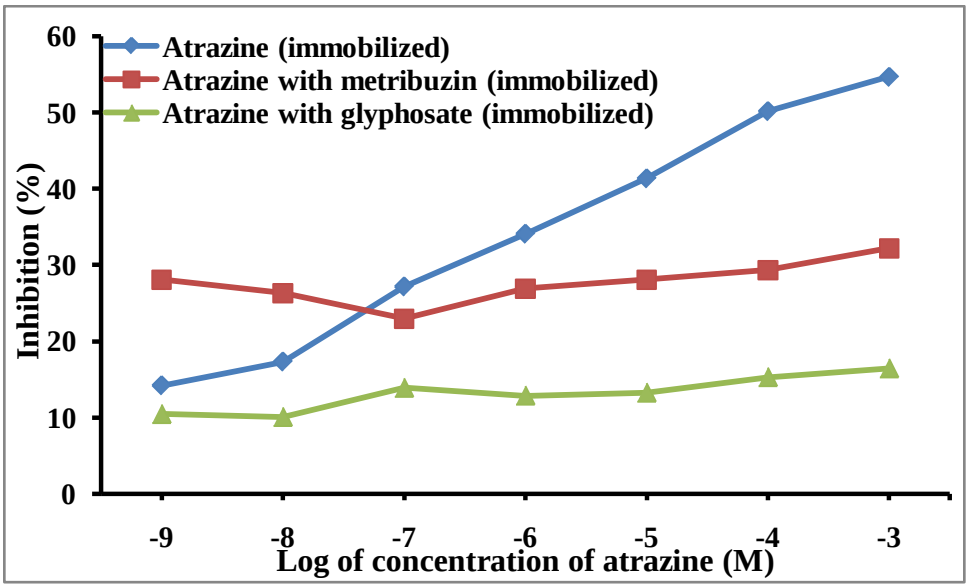

Figure 22. Plots showing interference effect of herbicides on biomaterial in immobilized state

5.5 Interference study of pesticides

5.5.1 Acephate with chlorpyrifos and triazophos

Interference of chlorpyrifos (2×10^{-4}M/0.5 mL) and triazophos (2×10^{-4}M/0.5 mL) with different concentrations of acephate from 2×10^{-9} to 2×10^{-3}M were studied .Result are given

in Figure 23 (free state) and 24 (immobilized state), respectively. From the figures it can be seen that chlorpyrifos and triazophos did not show much interference with acephate in immobilized and free states.

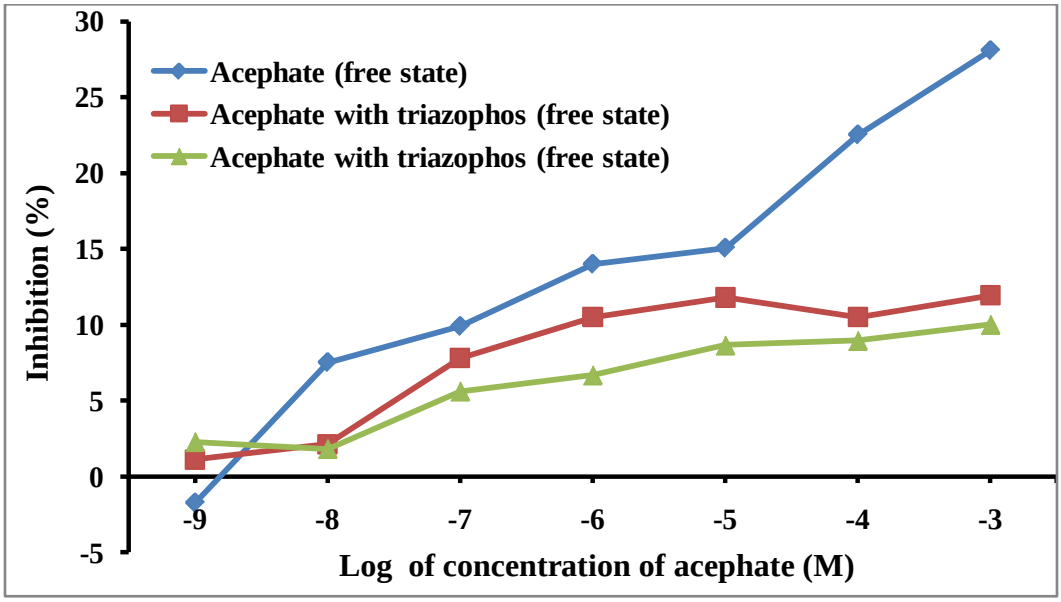

Figure 23. Plots showing interference effect of pesticides on biomaterial in free state

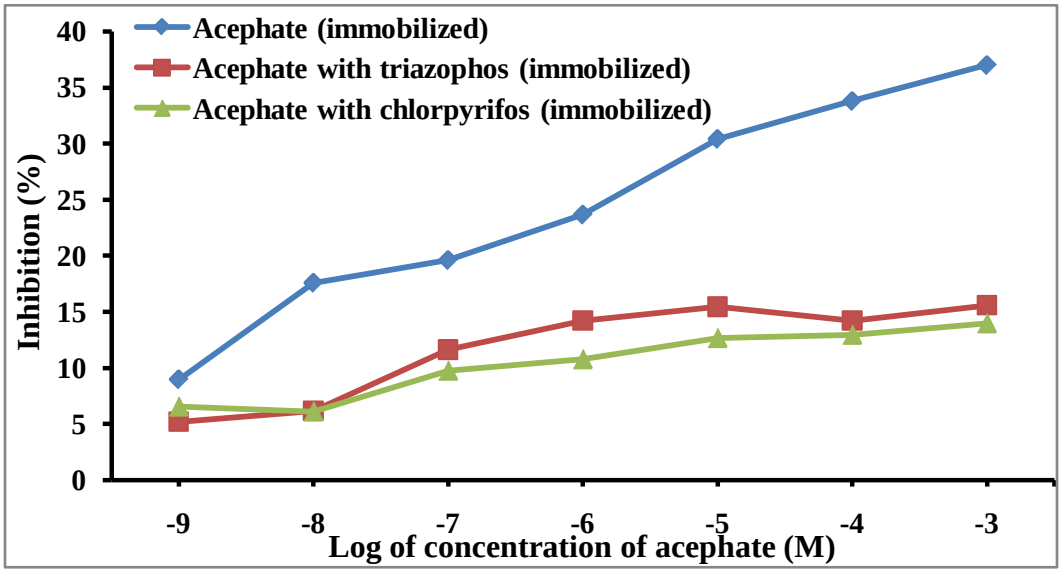

Figure 24. Plots showed the interference effect of pesticides to immobilized biomaterial

5.5.2 Interference study of acephate with malathion, chlorpyrifos and triazophos

Effect of interference of chlorpyrifos (2×10^{-4}M/0.5 mL), triazophos (2×10^{-4}M/0.5 mL) and malathion (2×10^{-4}M/0.5 mL) on different concentrations of acephate from 2×10^{-10}-2×10^{-3}M

were studied. Results are shown in Figures 25 (free state) and 26 (immobilized state). Malathion shows interference with acephate and decreased the inhibition (%) as compared with the interference of malathion with chlorpyrifos and triazophos in both free and immobilized states.

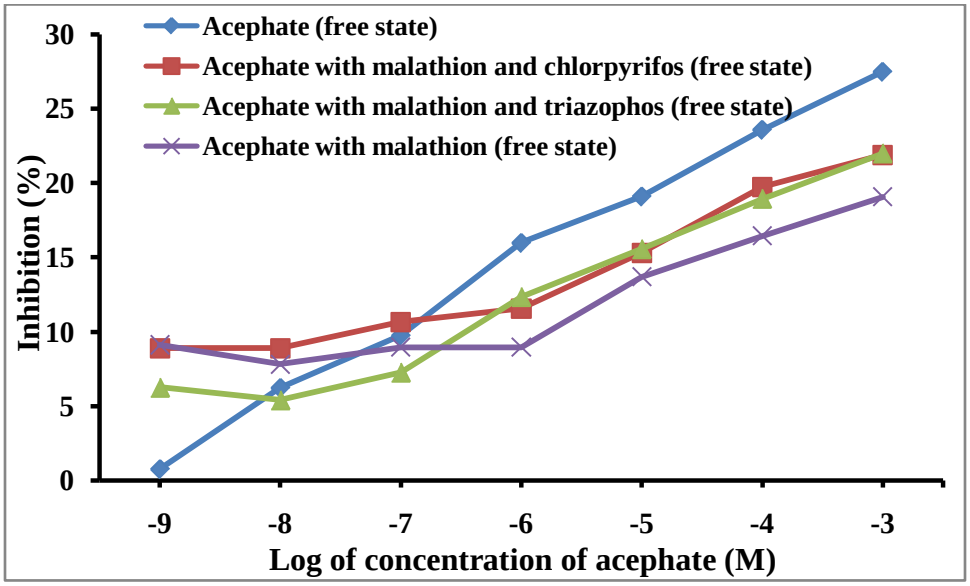

Figure 25. Plots of interference of malathion with chlorpyrifos and triazophos on acephate in free state

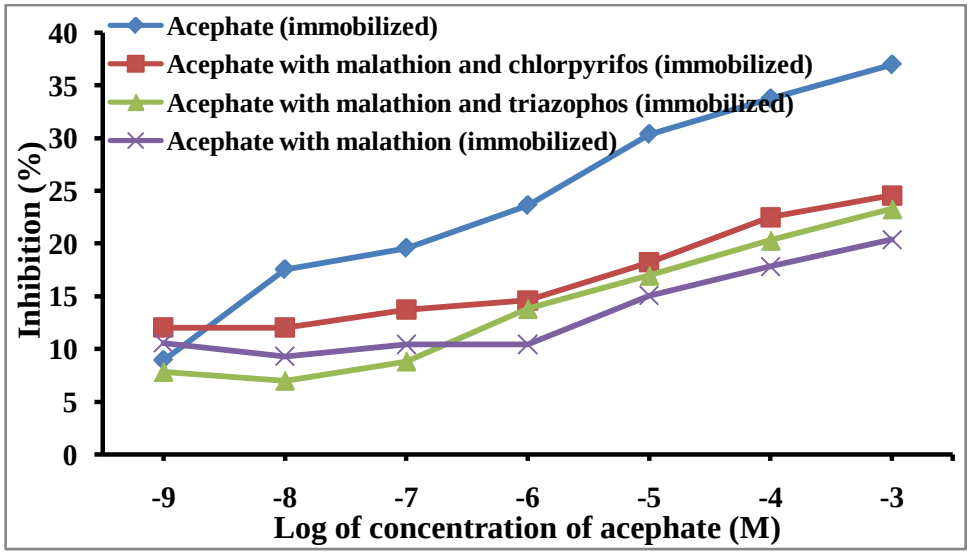

Figure 26. Plots of interference of malathion with chlorpyrifos and triazophos in immobilized state

5.6 Interference study of herbicides with pesticides

5.6.1 Effect of atrazine with acephate

Optimization of interference of atrazine (2×10^{-4}M/0.5 mL) with different concentrations of acephate from 2×10^{-10} to 2×10^{-3}M/ mL.Results are shown in Figures 27 (free state) and Figure 28 (immobilized state). Atrazine shows interference with acephate and increased the inhibition (%) in immobilized states as compared with free state.

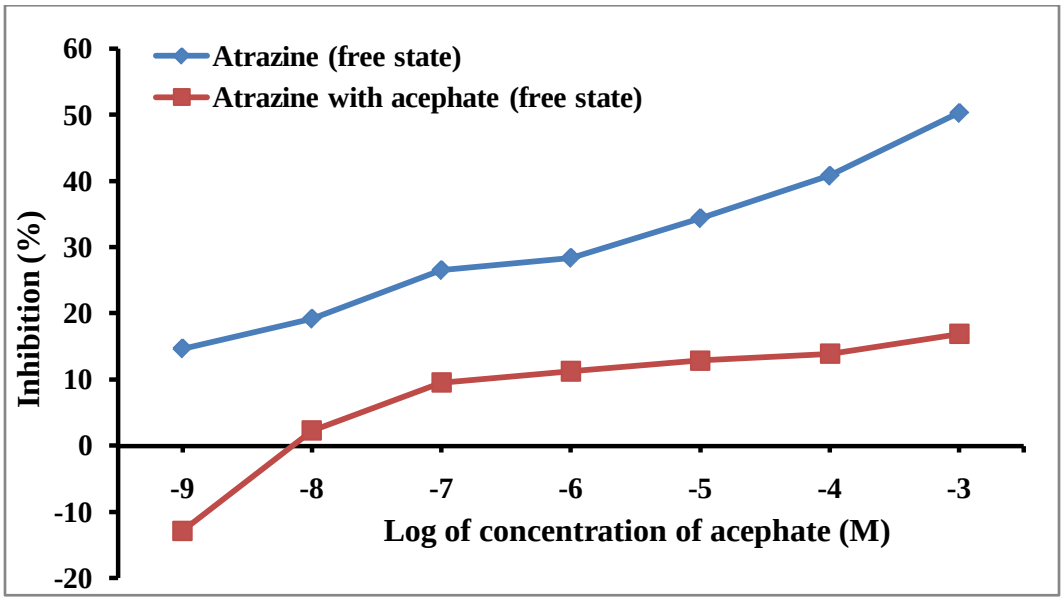

Figure 27. Plots showing the interference effect of atrazine on acephate in free state

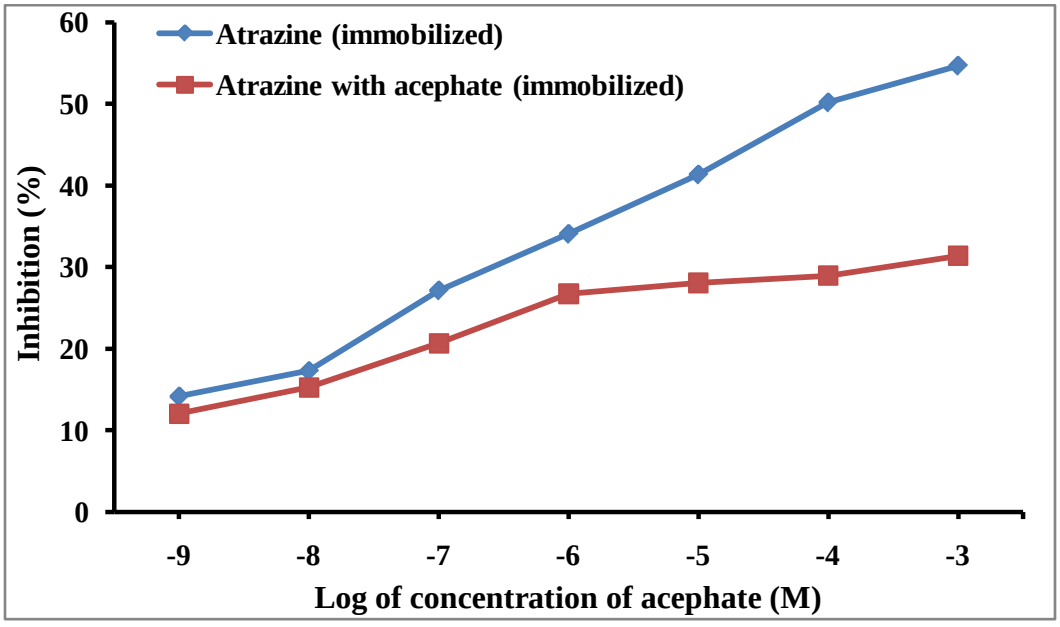

Figure 28. Plots showing the interference effect of atrazine on acephate in immobilized state

5.7 Interference study of pesticides with herbicides

5.7.1 Effect of acephate with atrazine

Interference study of acephate (2×10^{-4}M/0.5 mL) with different concentrations of atrazine from 2×10^{-10} to 2×10^{-3}M/ mL were studied. Results are shown in Figures 29 (free state) and Figure 30 (immobilized state). Acephate did not show much interference effect with atrazine in immobilized and free states .

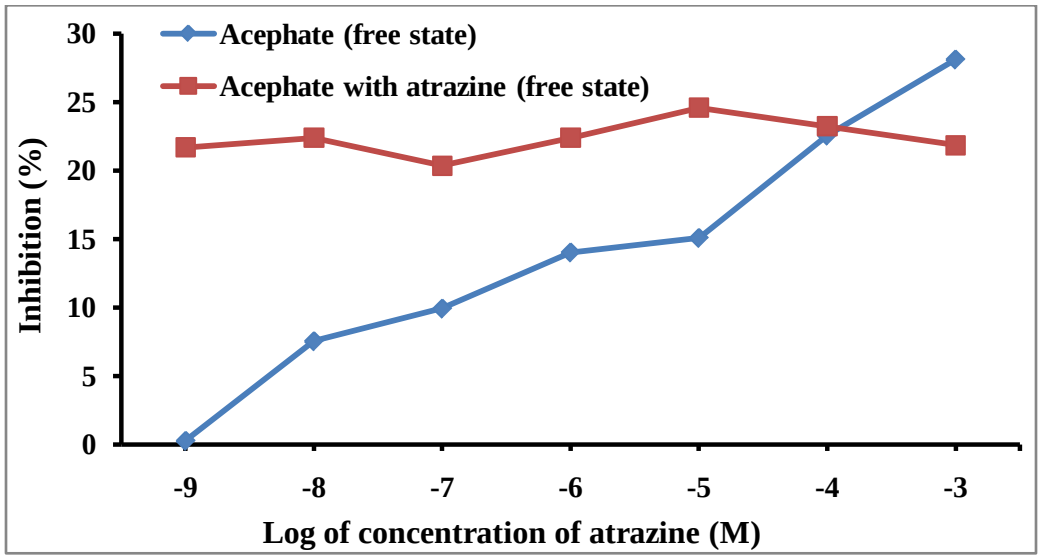

Figure 29. Plots showing the interference effect of acephate on atrazine in free state

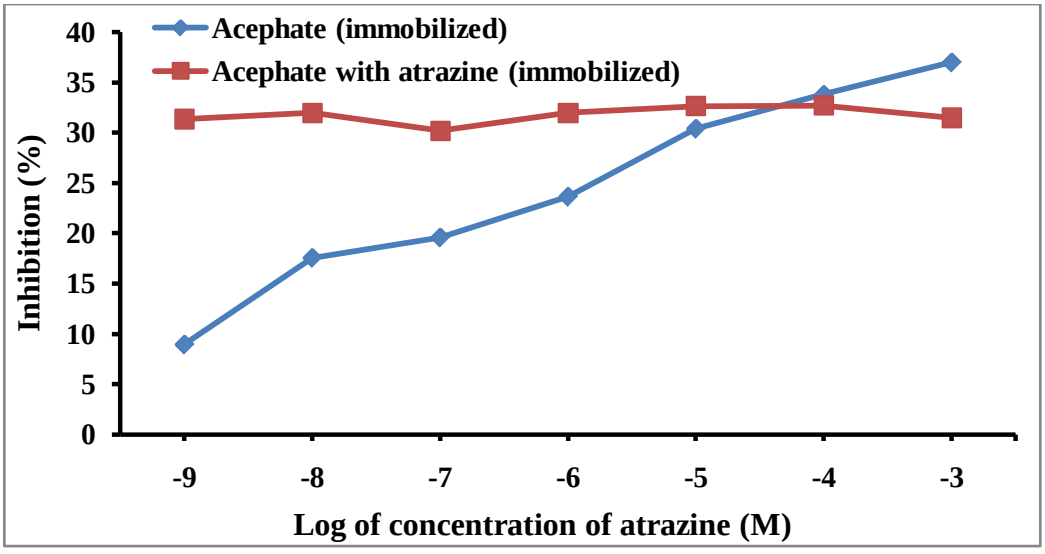

Figure 30. Plots showing the interference effect of acephate on atrazine in immobilized state

Conclusions:

A optical biosensor based on free and immobilized whole cell *Chlorella vulgaris* microalgae as a bioreceptor and UV-Vis spectrophotometer as a transducer has been developed and tested for alkaline phosphatase activity analysis. This biosensor developed for the detection of herbicides like atrazine, metribuzin, glyphosate and organophosphorus pesticides like acephate, chlorpyrifos, triazophos, malathion both in free and immobilized state biomaterial even at very low concentration. The inhibition of *Chorella vulgaris* microalgae alkaline phosphatase activities in presence of pesticides was measured. These biosensors were also used for the detection of interference of different pesticides and herbicides. Detection limit for herbicides like atrazine, metribuzin, glyphosate was found to be in free state 10^{-10} M, 10^{-8} M, 10^{-6} M and in immobilized state 10^{-10} M, 10^{-9} M, 10^{-7} M respectively. Detection limit for organophosphorus pesticides like acephate, chlorpyrifos, triazophos was found to be in free state 10^{-8} M, 10^{-7} M, 10^{-6} M and in immobilized state 10^{-9} M, 10^{-7} M, 10^{-7} M respectively. Malathion did not inhibit the alkaline phosphatase detected by this optical biosensor.

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