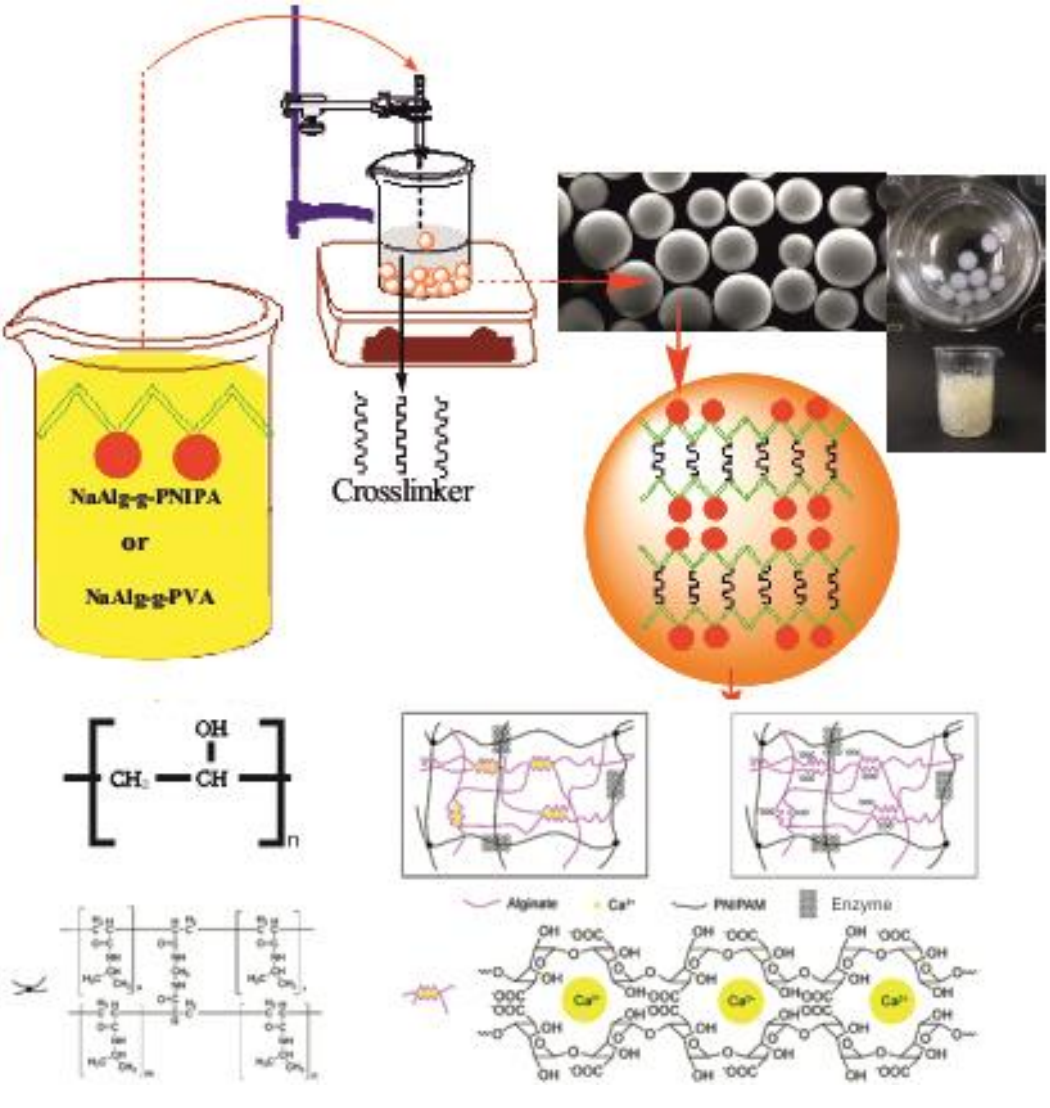
Project Title	**Detoxification And Decolorization Of Industrial Waste by Using Natural Enzyme**
Category	**Environment**
Name of Participant(s)	**Zeynep Leyla AKIN, Boran ALABAY, Bengisu KAPLAN**
Organization Name	**ALFA GROUP EDUCATIONAL INSTITUTIONS**
Country	**TURKEY**

Abstract

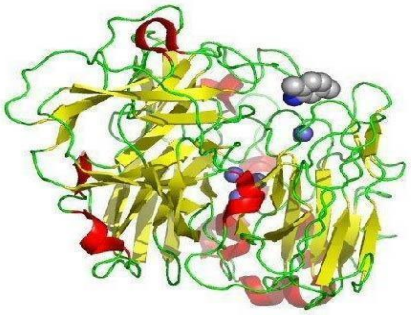
AN ALTERNATİVE AND ECONOMİCAL METHOD FOR THE DETOXİCİZATİON AND DECOLORİZATİON OF INDUSTRİAL WASTE

Purpose: To carry out detoxification and decolorization of industrial waste by using Laccase immobilized hydrogel beads which activated by carbodiimide, hydroxysuccininimide and carbodiimide and hydroxysuccininimide.

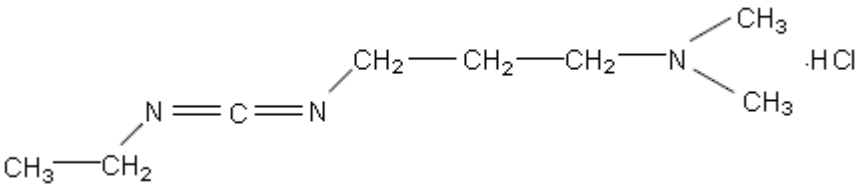


1. Used Chemicals:

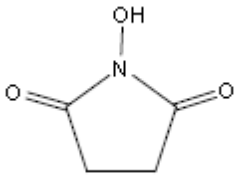
- Laccase enzyme as a biological catalyst(obtained from Trametes versicolor typed fungus)



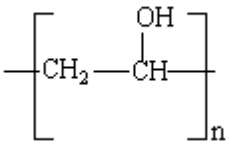
- 1-Etyl-3-(3-Dimetylaminopropyl) Karbodiimide Hydrochloride



- N-Hydroxysuccinimide

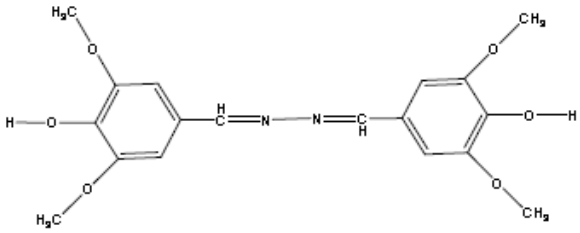


- Polyvinylalcohol (PVA)



- Calcium chloride,$CaCl_2$

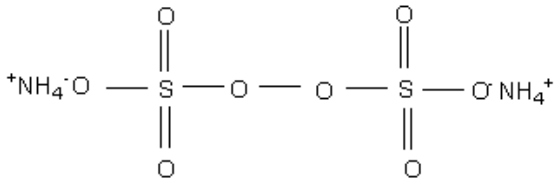
- 4-Hydroxy-3,5-dimetoxybenzaldehide azin (Syringaldazine)



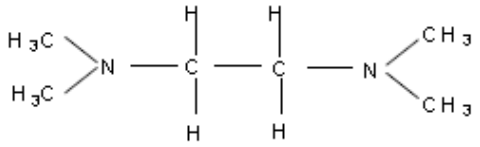
- N-izopropyl acrylamide

$H_2C=CH-CO-NH-CH(CH_3)_2$

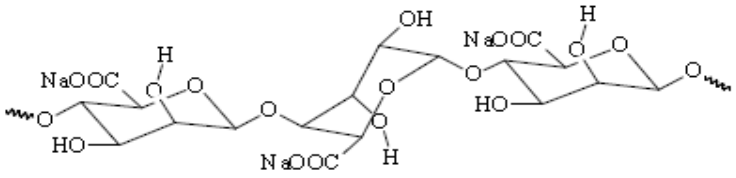
- Amonium sulphate



- Ethylalcohol,C_2H_5OH
- Phosforic acid, H_3PO_4
- Citric acid, $C_6H_8O_7$
- Sodyum hidroksit, NaOH
- N,N,N'N'-Tetrametyletilendiamine (TEMED)



- Sodium alginate



2. Procedures:

A. Mechanism

Syringaldazine was accepted as a model of industial waste(phenolic compounds). Interactions between laccase and syringaldazine were observed. Phenolic compounds(industrial waste) can be oxidized, detoxified and decolorizated by Laccaz enzym(obtained from Trametes versicolor typed fungus) activated and immobilized.

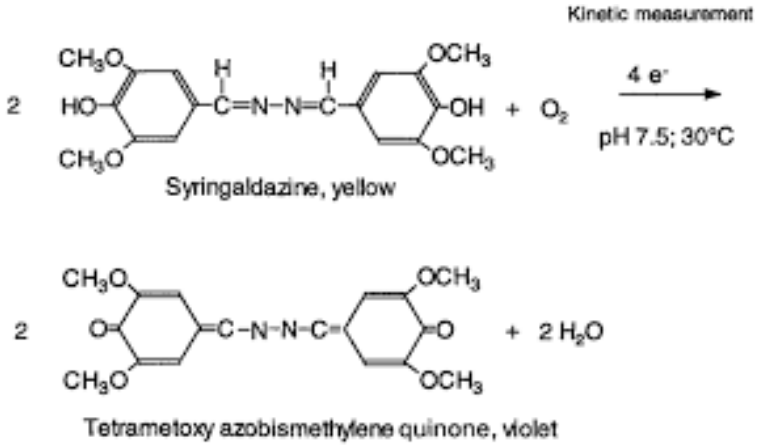


Figure 2.1. Oxidization reaction of Syringaldazine (a model of industrial waste)

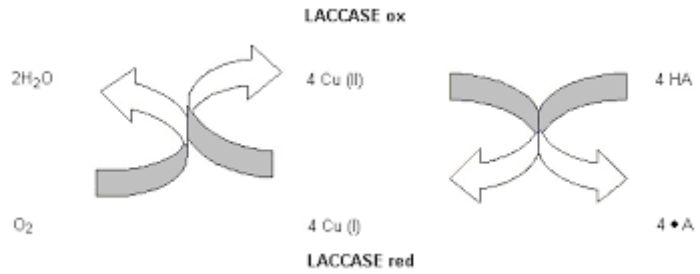


Figure 2.2. The Convertion of Laccase enzyme as biological catalyst

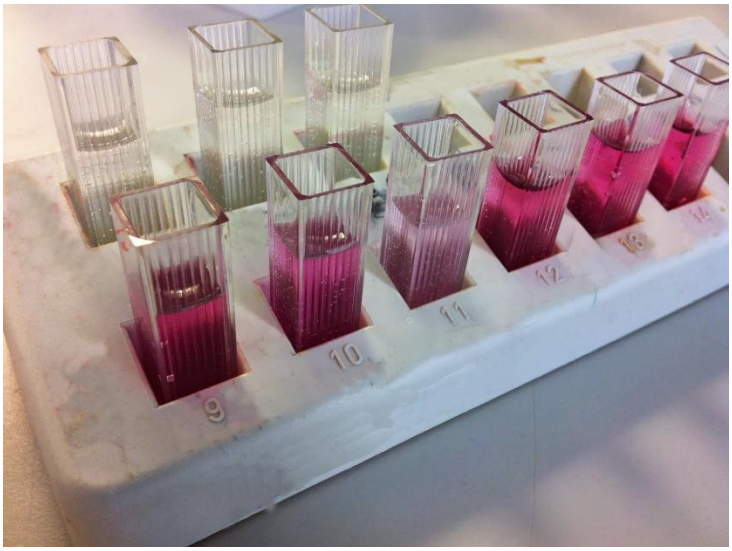


Figure 2.3. The Decolorization of Syringaldazine

B. Enzyme immobilization by using carbodiimide, hydroxysuccininimide and carbodiimide and hydroxysuccininimide

Sodium alginate (1% by mass, 0.5 g) was dissolved in 50 mL of purified water. and 10 mL of solution and 5 mL of PVA solution was mixed. Later obtained solution was added drop by drop into calcium chloride solution and water-insoluble Ca-alginate polymeric spheres were obtained due to sodium-calcium exchange. The surface activation was carried out with carbodiimide as follows. The Ca-alginate polymeric spheres were added to the carbodiimide, Hydroxysuccininimide and Carbodiimide and Hydroxysuccininimide solution (0.5 mM), followed by stirring at room temperature with a magnetic stirrer for 4 hours to allow overnight activation to complete. The enzyme solution (0.05 g laccase was prepared by dissolving in 20 mL (0.04 M pH = 6.5) phosphate buffer) was added to the Carbodiimide, Hydroxysuccininimide and Carbodiimide and Hydroxysuccininimide bound spheres and the enzyme was immobilized by stirring with magnetic stirrer for four hours. Enzymes adsorbed onto the surface were removed by washing with deionized water. It was then stored in distilled water at 4 ° C for use. The same procedure was repeated by replacing 5 mL of PVA with 1 mL of P (NIPA) solution.

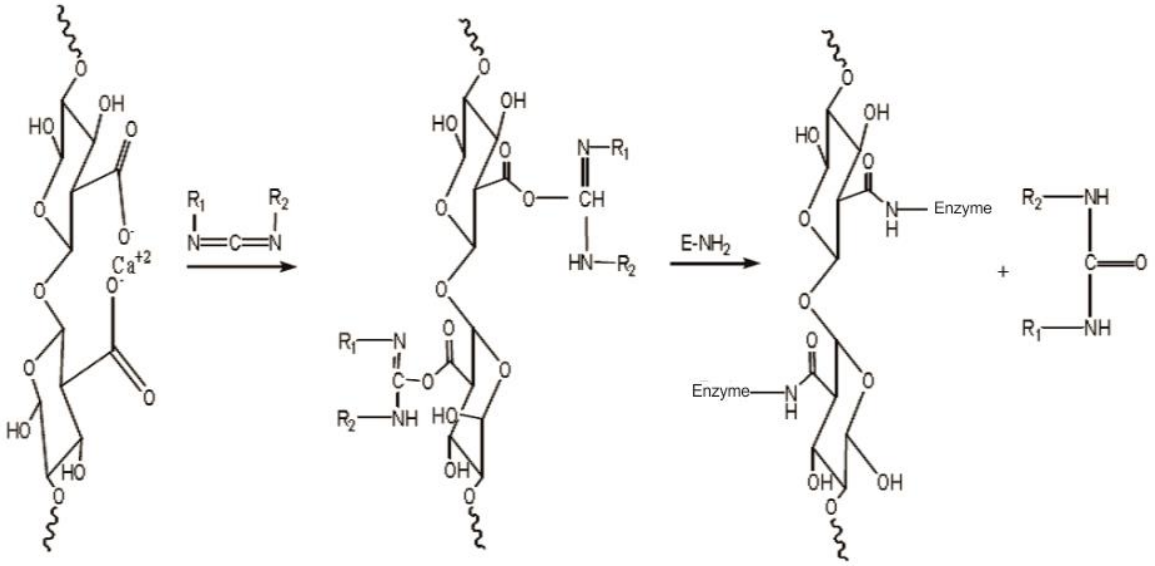


Figure 2.3. Carboxyl group bearing matrices can be activated by using carbodiimide to immobilize en zymes

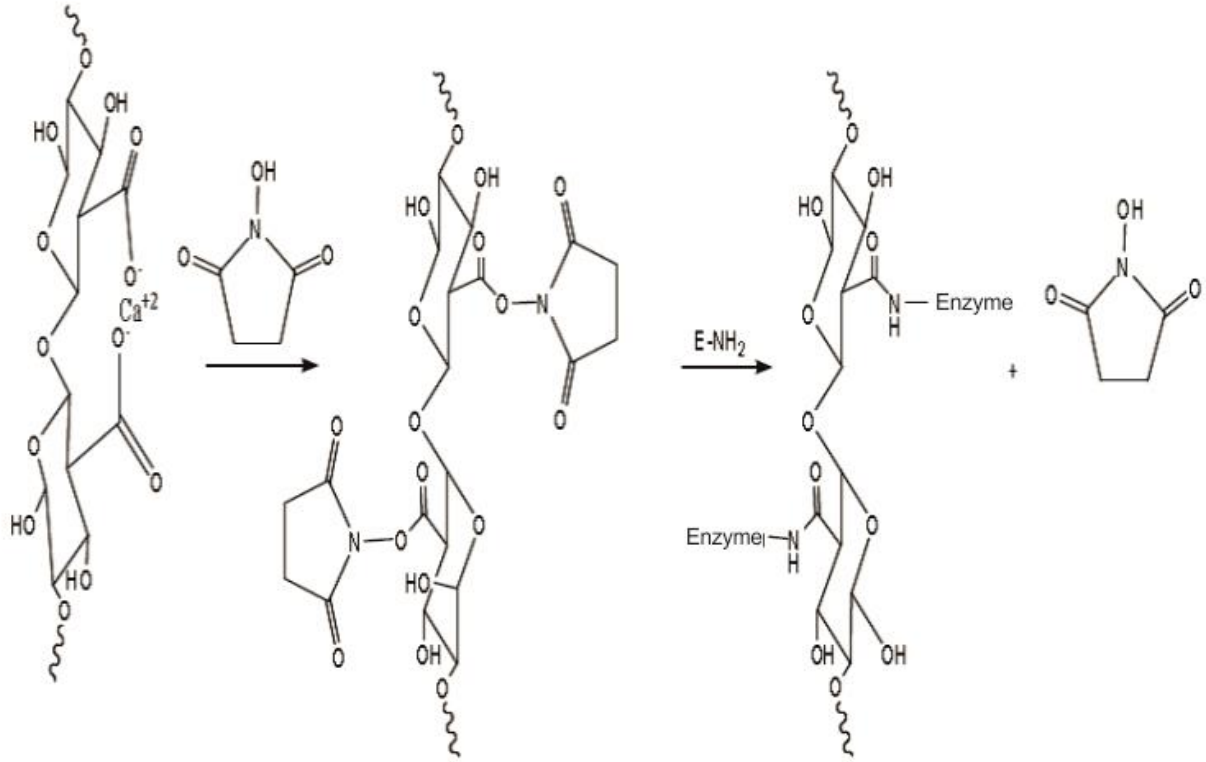


Figure 2.4. Carboxyl group bearing matrices can be activated by using Hydroxysuccininimide to immobilize enzymes

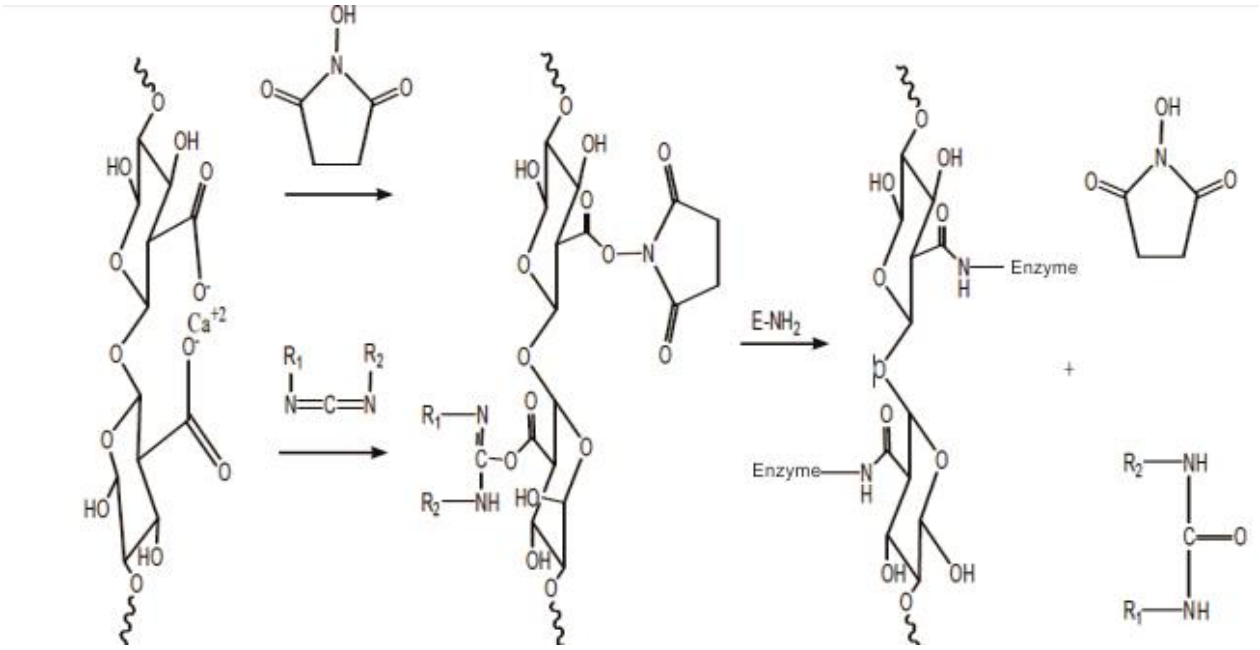
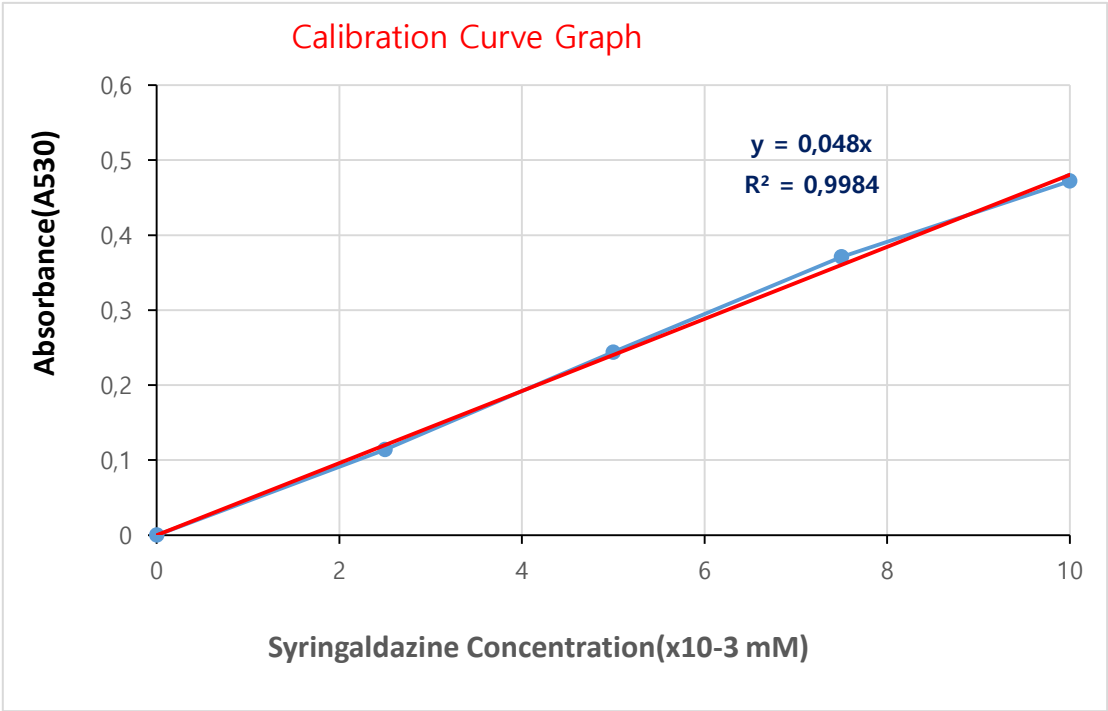


Figure 2.5. Carboxyl group bearing matrices can be activated by using Carbodiimide and Hydroxysuccininimide to immobilize enzymes

C. Preparation of Syringaldazine Calibration Curve

To prepare the syringaldazine calibration curve, syringaldazine solutions were prepared at different concentrations (0.100 mM, 0.075 mM, 0.050 mM, 0.025 mM), 9 mL citrate buffer (0.04 M, pH: 5.3) and 1 mL syringaldazin prepared at different concentrations. solution was added. 0.1 mL of Laccase enzyme (0.01 mg / mL) was added to the solutions and the reaction flasks were kept in a shaking water bath at 25 ° C for 10 minutes and then absorbed at 530 nm wavelength using UV visible region spectrophotometer (SHIMADZU UV / Vis1800) at 12 minutes. read. The absorbance values corresponding to syringaldazine concentrations were plotted and a calibration chart was prepared.



D. Activity determination of immobilized enzyme

Carbodiimide-activated PVA-CaAlj for the determination of activity of immobilized laccase,hydroxysuccinimide activated PVA-CaAlj, carbodiimide and hydroxysuccinimide activated PVA-CaAlj, carbodiimide activated P (NIPA) -CaAlj, hydroxysuccinimide activated P(NIPA) -CaAlj, carbodiimide and hydroxylsuccinimide activated P(NIPA) -CaAlj polymer beads were prepared by immobilized laccase enzyme on which 9 mL of citrate buffer (0.04 M, pH: 5.3), 1 mL syringaldazine solution (0.1 mM) was added. The mixture was shaken in a shaking water bath at 25 ° C for 10 minutes and absorbance values were measured at 12 minutes and 530nm. And activity was calculated as following equations.

$$V = \frac{C}{t} = \frac{A_{530nm}}{t} \times \frac{C}{A_{530nm}}$$

$$\frac{C}{A_{530nm}} = \frac{1}{Slope}$$

V:Activation, C: Concentration, A: Absorbance

E. Effect of Substrate Concentration on Enzyme Activity

The relations between enzyme and substrate were determined as following equations.

$$E + S \underset{k2}{\overset{k1}{\rightleftharpoons}} ES \xrightarrow{k3} E + P$$

$$V_0 = \frac{V_{mak} \times S}{K_m + S}$$

$$K_m = \frac{k_2 + k_3}{k_1}$$

$$\frac{1}{V} = \frac{K_m}{V_{mak}} \times \frac{1}{S} + \frac{1}{V_{mak}}$$

$$Slope = \frac{Km}{V_{Mak}}$$

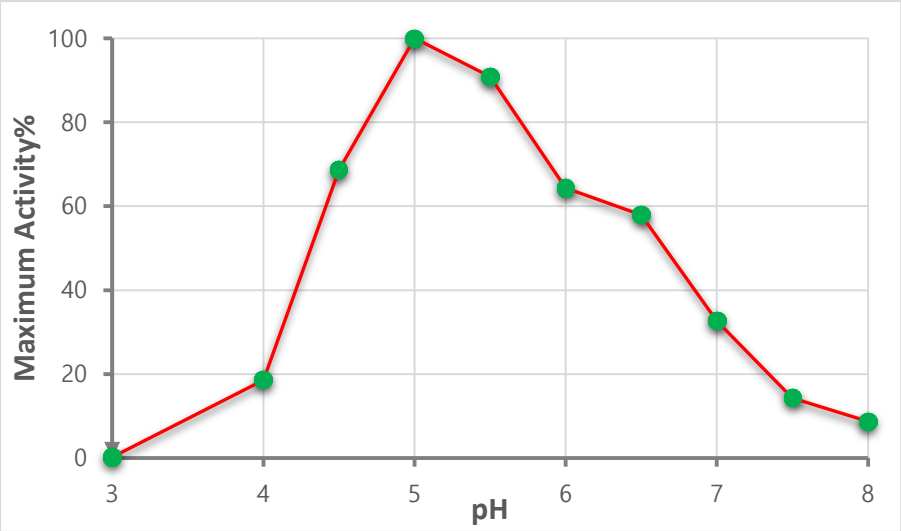
E:Enzyme, S:Substrate Concentration, P:Products, k: rate constant, K:Michaelis Menten constant, V_{mak}:Maximum rate or activation, V: rate or activation

3.Data

Laccase Activity in polymeric hdrogel beads were observed by using some parameters like pH, temperature, substrate concentration, storage time and reusage numbers

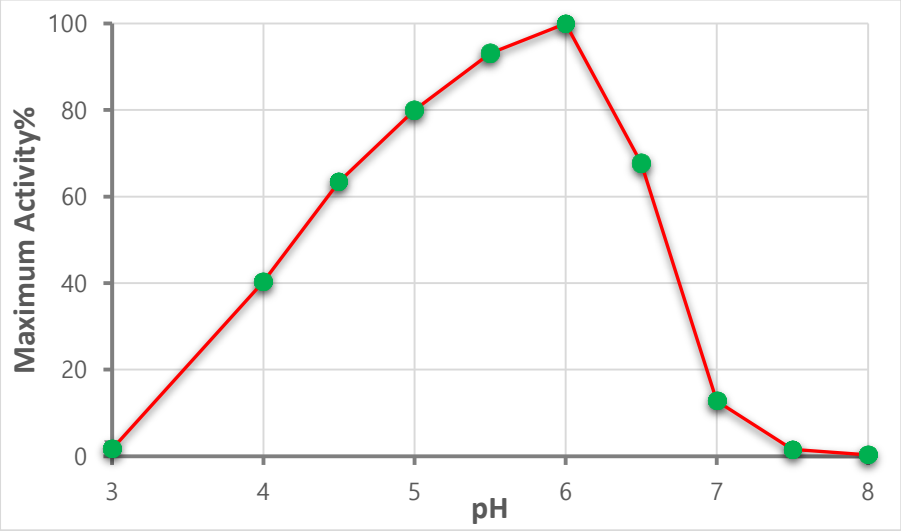
3.1.pH effect

a) pH Effect on Free Laccase Activity



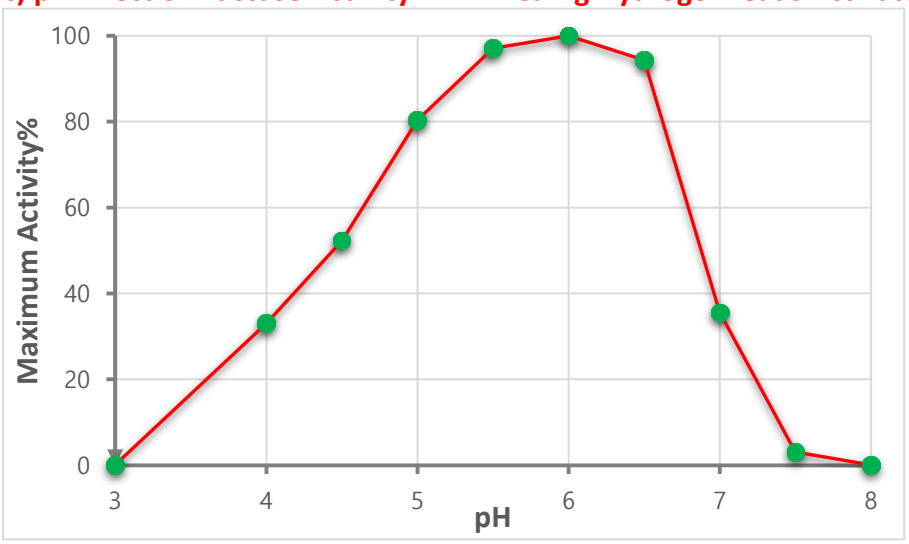
Maximum activation was observed at pH 5.

b) pH Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide



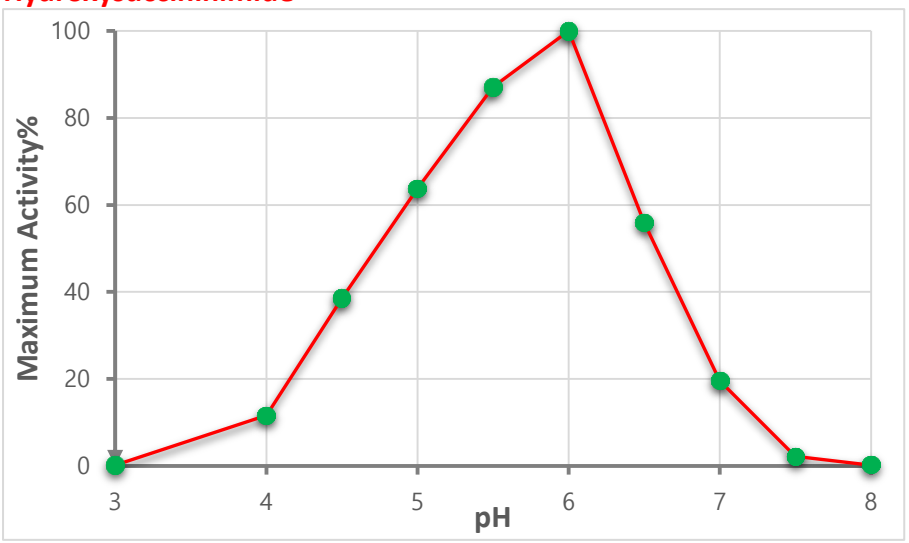
Maximum activation was observed at pH 6.

c) pH Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Hydroxysuccininimide



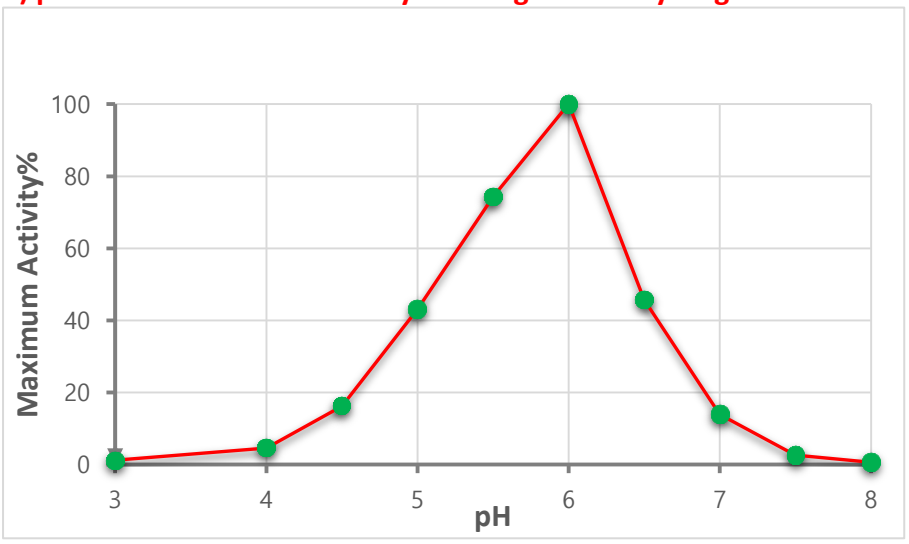
Maximum activation was observed at pH 6.

d) pH Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide and Hydroxysuccininimide



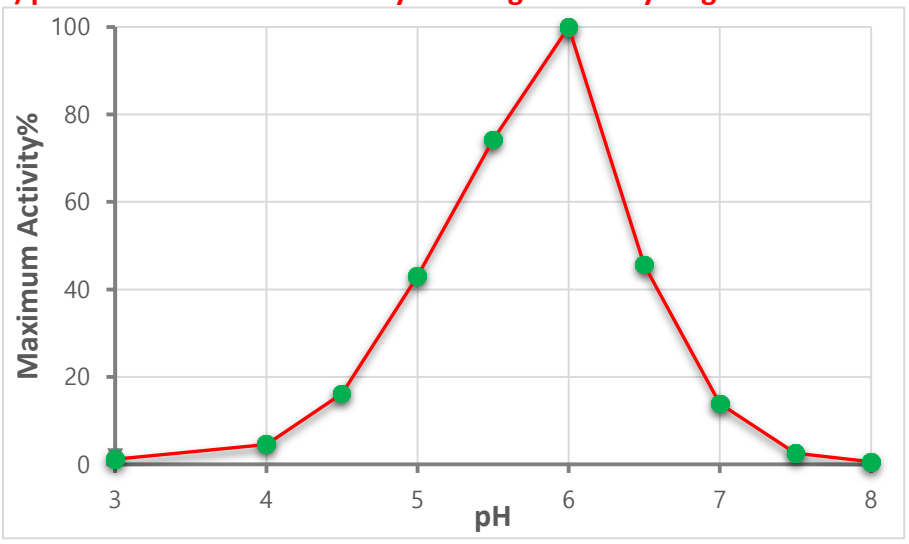
Maximum activation was observed at pH 6.

e) pH Effect on Laccase Activity in CaAlg -PNIPA Hydrogel Beads Activated by Carbodiimide



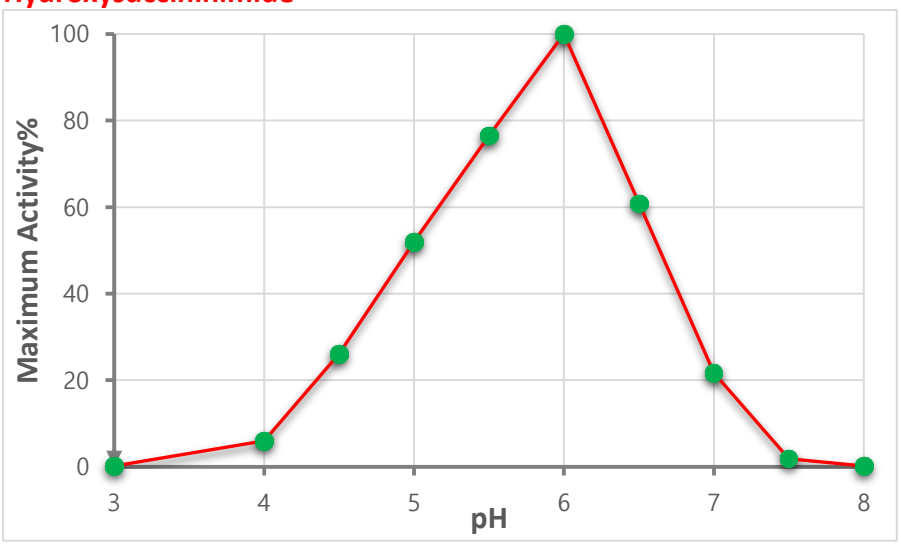
Maximum activation was observed at pH 6.

f) pH Effect on Laccase Activity in CaAlg-PNIPA Hydrogel Beads activated by Hydroxysuccininimide



Maximum activation was observed at pH 6.

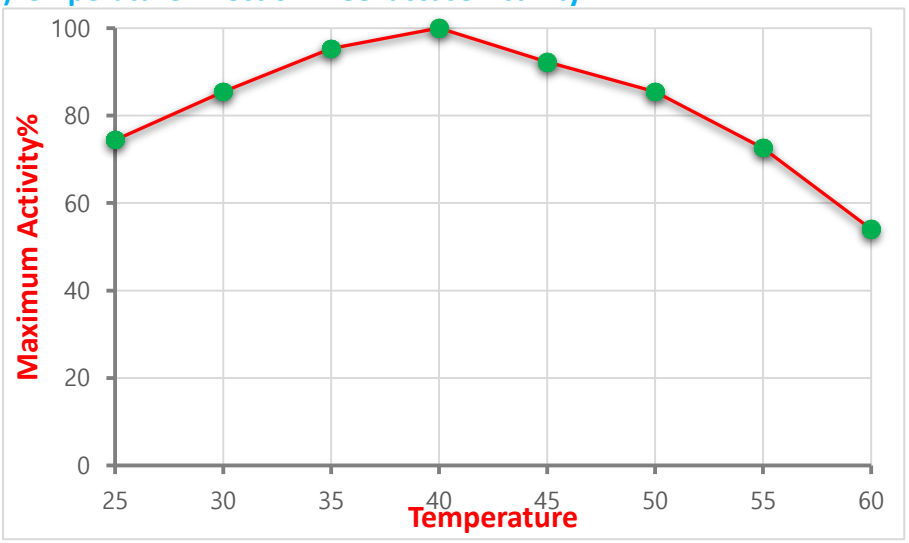
g) pH Effect on Laccase Activity in CaAlg-PNIPA Hydrogel Beads Activated by Carbodiimide and Hydroxysuccininimide



Maximum activation was observed at pH 6.

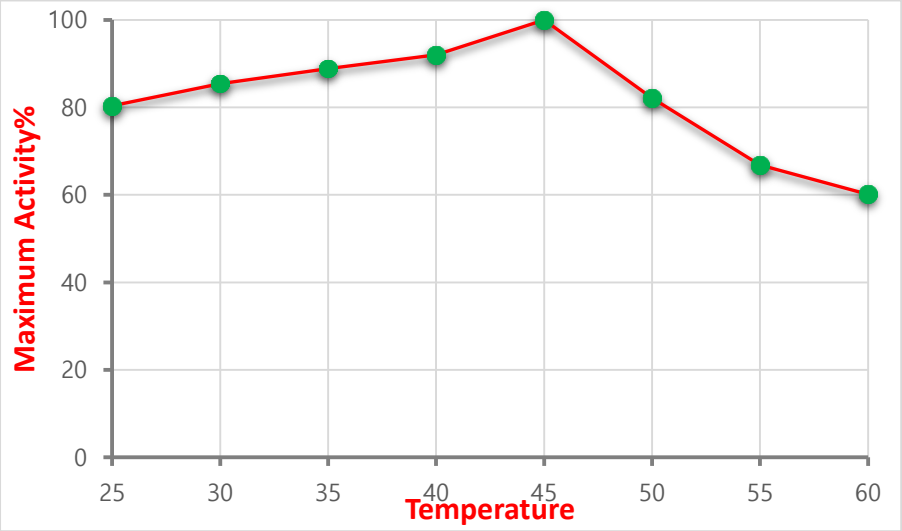
3.2.Temperature effect

a)Temperature Effect on Free laccase Activity



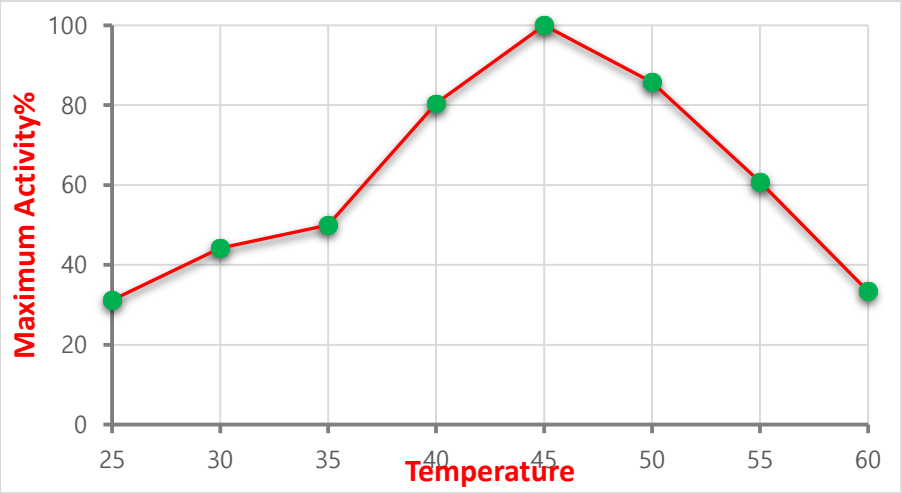
Maximum activation was observed at 40°C.

b)Temperature Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide



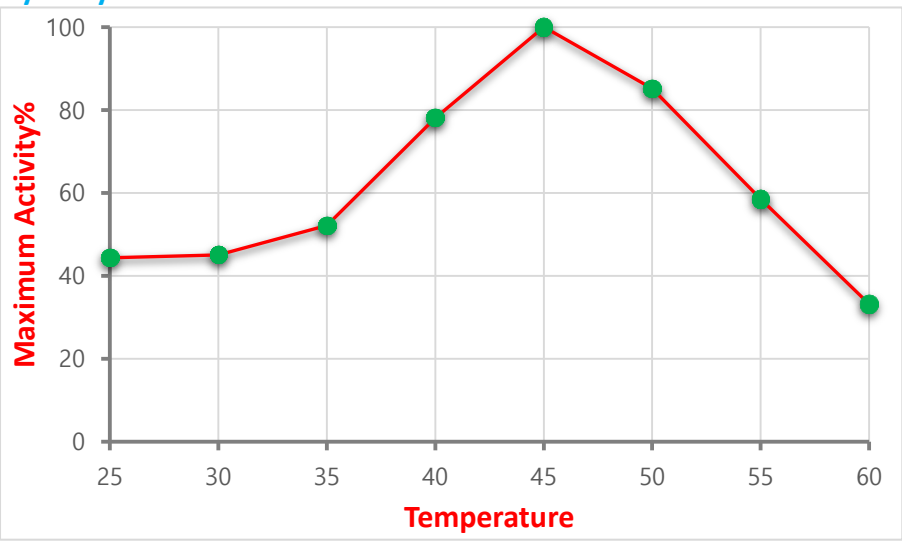
Maximum activation was observed at 45°C.

c)Temperature Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by hydroxysuccininimide



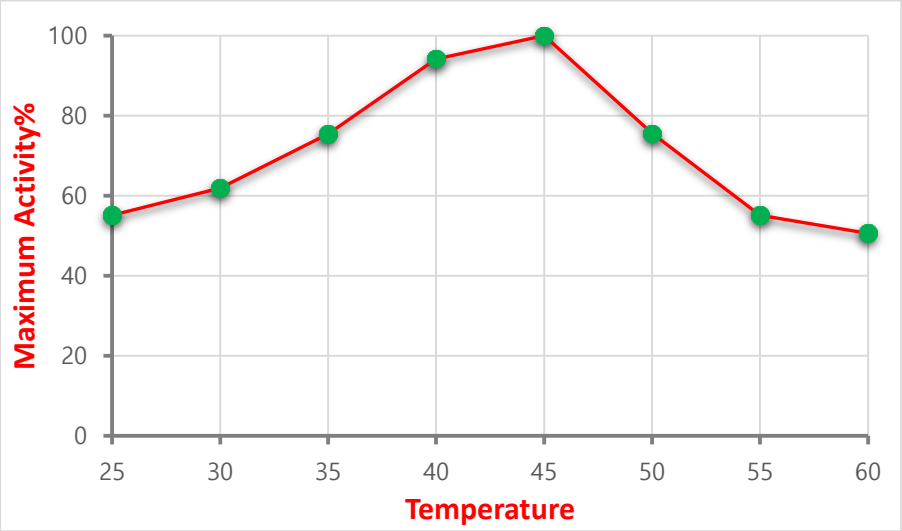
Maximum activation was observed at 45°C.

d)Temperature Effect on Activity of PVA-CaAlg Hydrogel Beads Activated by Carbodiimide and Hydroxysuccininimide



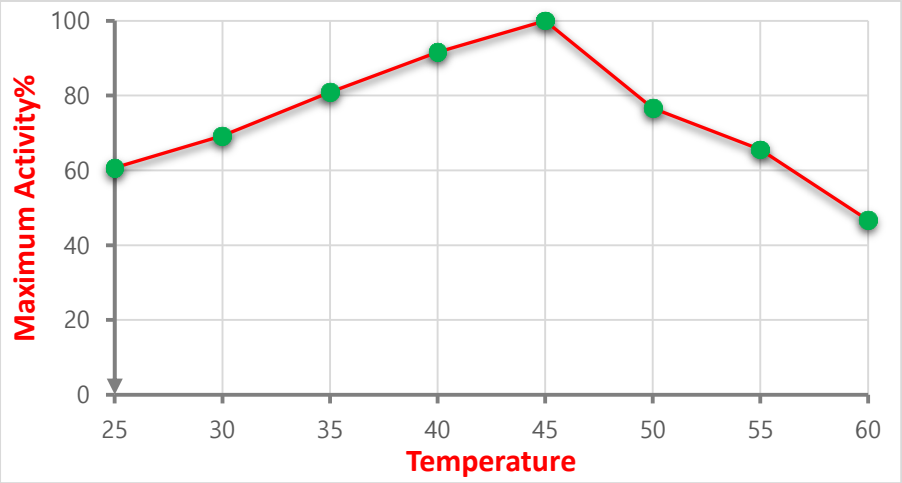
Maximum activation was observed at 45°C.

e)Temperature Effect on Laccase Activity of PNIPA-CaAlg Hydrogel Beads Activated by Carbodiimide



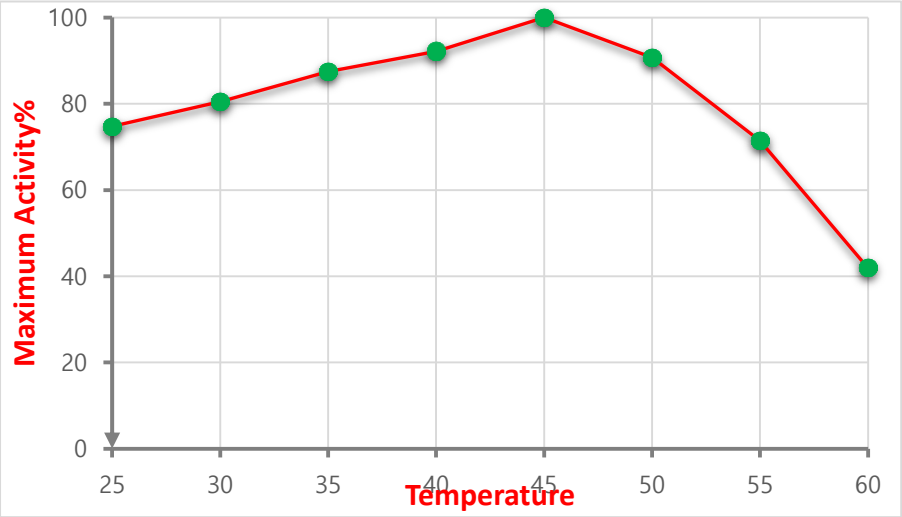
Maximum activation was observed at 45°C.

f)Temperature Effect on Activity of PNIPA-CaAlg Hydrogel Beads Activated by Hydroxysuccininimide



Maximum activation was observed at 45°C.

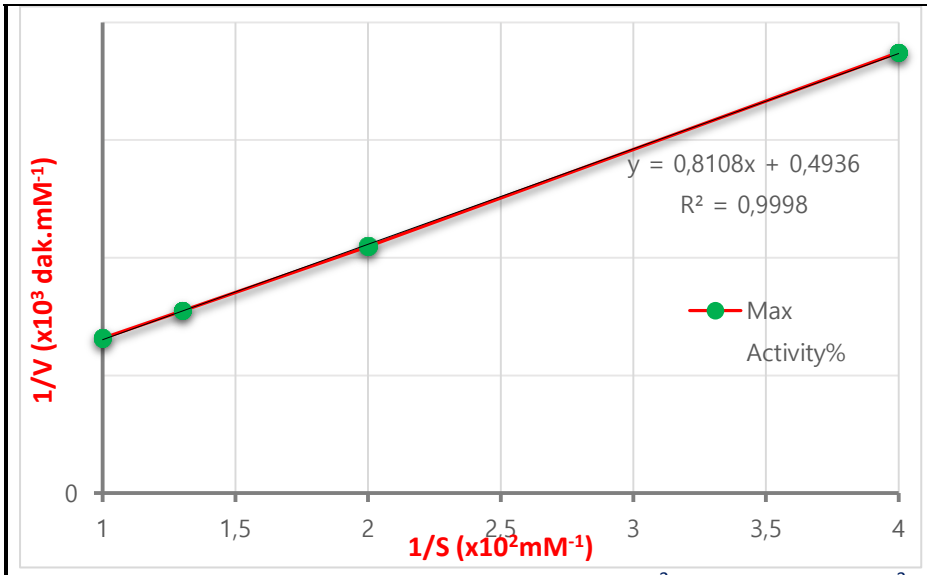
g)Temperature Effect on Activity of PNIPA-CaAlg Hydrogel Beads Activated by Carbodiimide and Hydroxysuccininimide



Maximum activation was observed at 45°C.

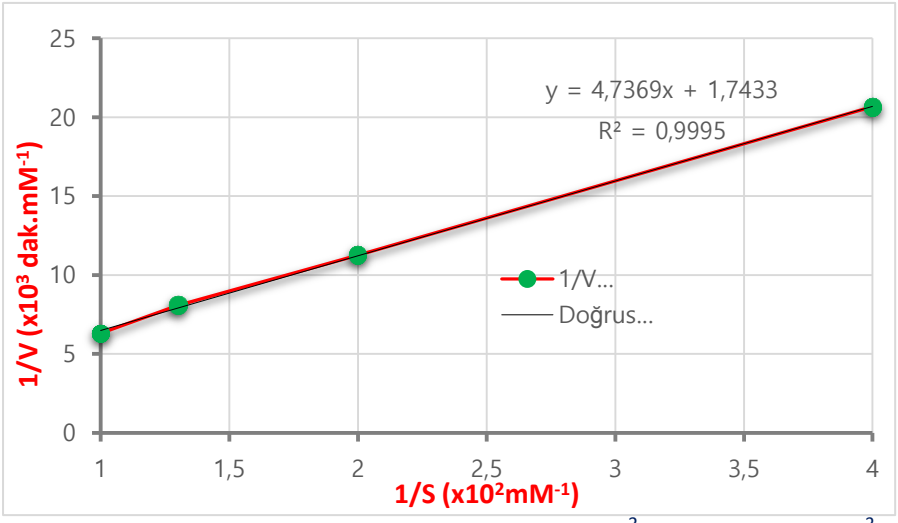
3.3.Substrate Concentration effect

a)Substrate Concentration Effect on Free Laccase Activity



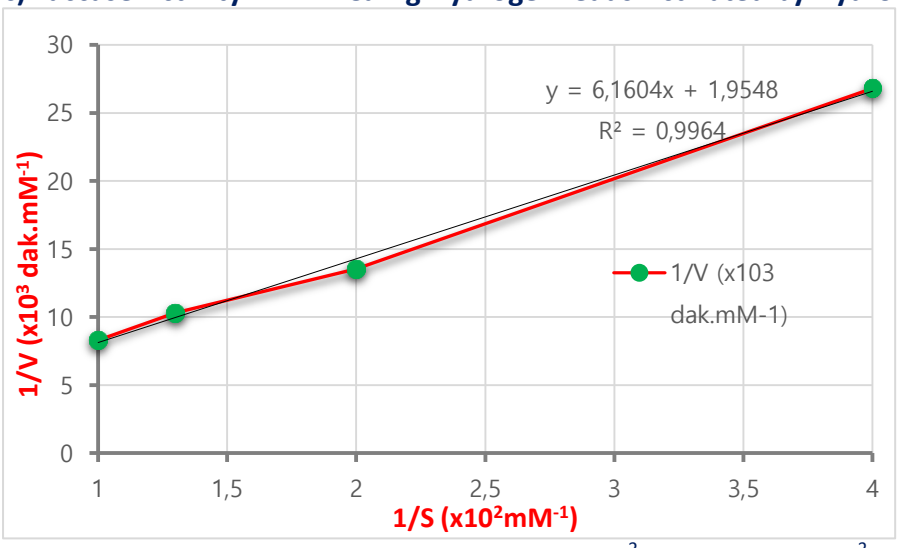
Km and Vmax values were calculated as 16,98x10^{-3}mM and 2,09 x10^{-3}mM.dak^{-1}

b) Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide



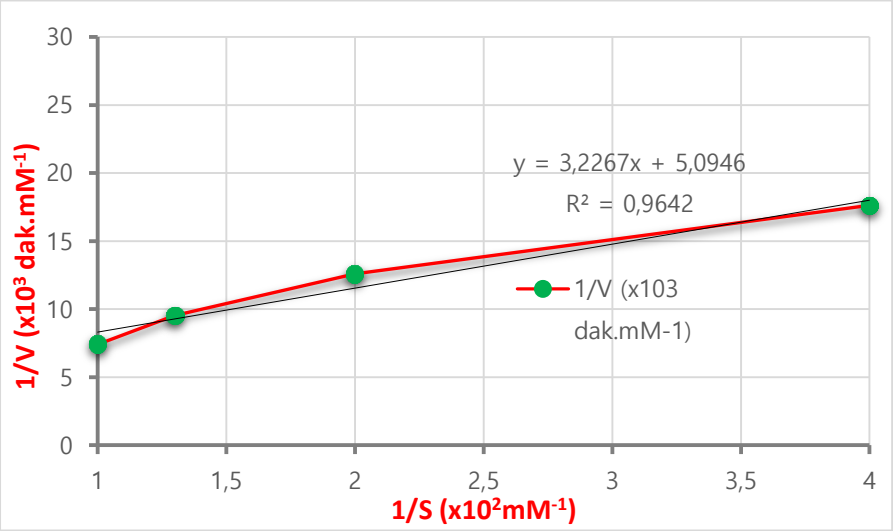
Km and Vmax values were calculated as 28,8x10^{-3} mM and 6.00x10^{-3}mM.dak^{-1}

c) Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Hydroxysuccinimide



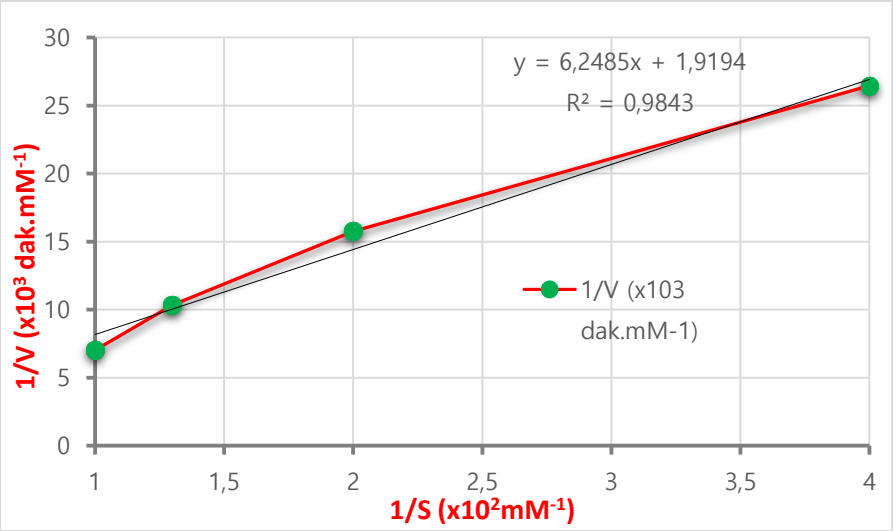
Km and Vmax values were calculated as 32,7x10^{-3}mM and 5,30x10^{-3}mM.dak^{-1}

d) Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide and Hydroxysuccinimide



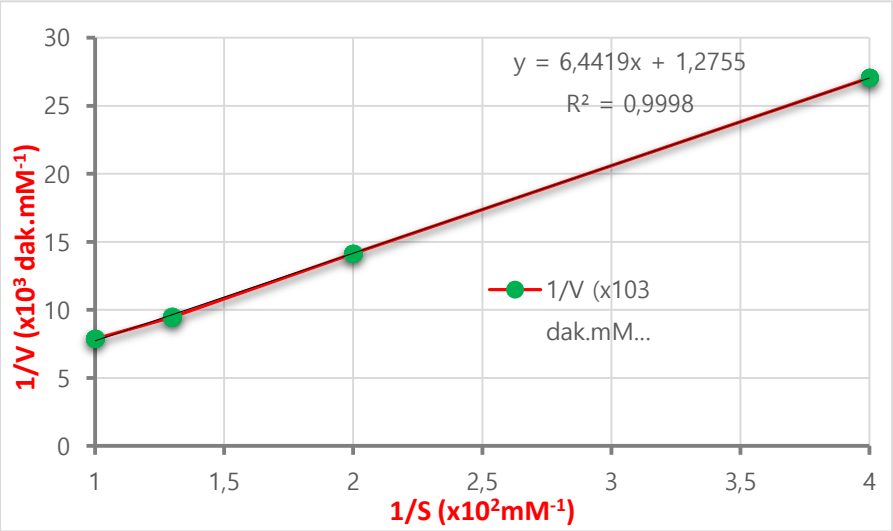
Km and Vmax values were calculated as 48,35x10^{-3}mM and 8,07x10^{-3} mM.dak^{-1}

e) Laccase Activity in PNIPA-CaAlg Hydrogel Beads Activated by Carbodiimide



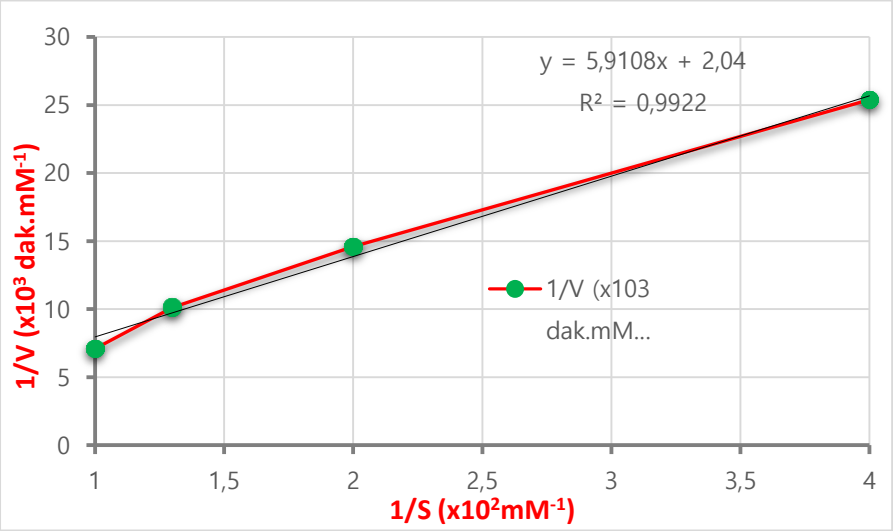
Km and Vmax values were calculated as 37x10^{-3} mM and 5,90x10^{-3}mM.dak^{-1}

f)Laccase Activity in PNIPA-CaAlg Hydrogel Beads Activated by Hydroxysuccinimide



Km and Vmax values were calculated as 55,5x10^{-3} mM and 8,58x10^{-3}mM.dak^{-1}

g)Laccase Activity in PNIPA-CaAlg Hydrogel Beads Activated by Carbodiimide and Hydroxysuccinimide



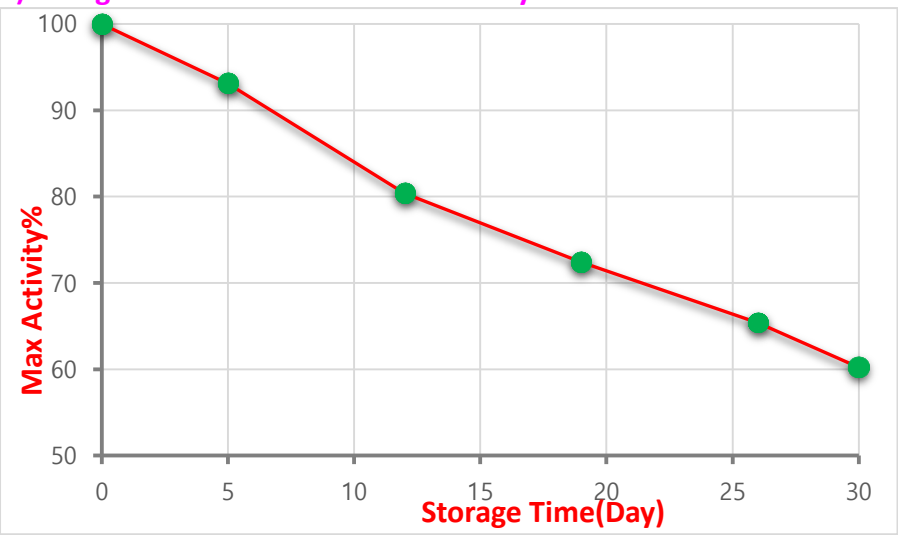
Km and Vmax values were calculated as 30,8 x 10^{-3} and 5,19x10^{-3} mM.dak^{-1}

It was observed enzyme activity and substrate concentration were directly proportional.

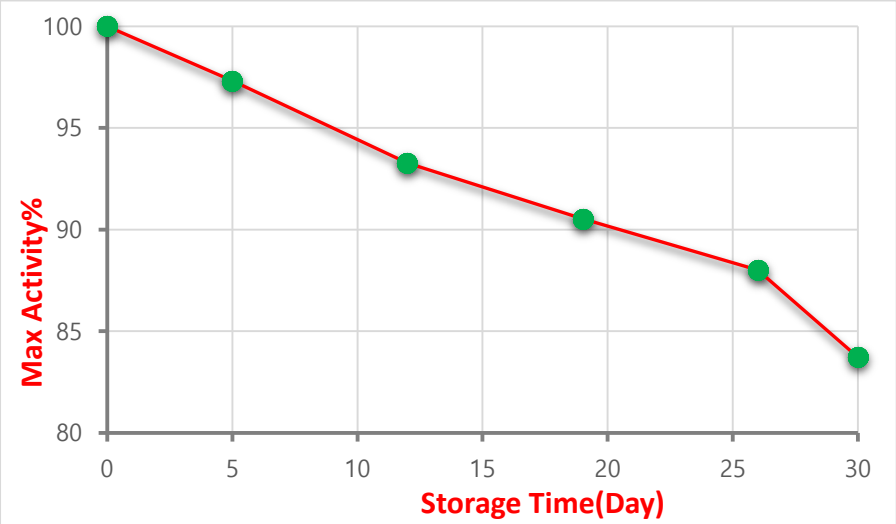
K_m and substrate affinity of enzyme are inversely proportional.

3.4. Storage Time effect

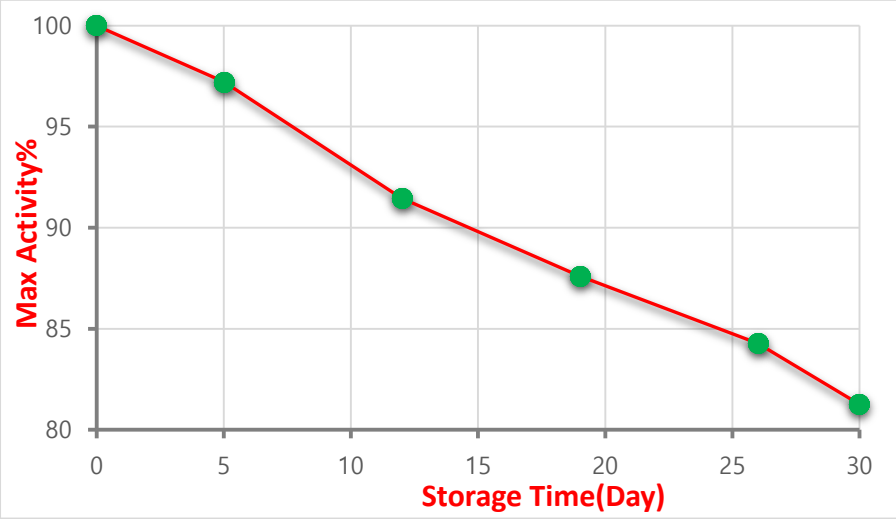
a)Storage Effect on Free Laccase Activity



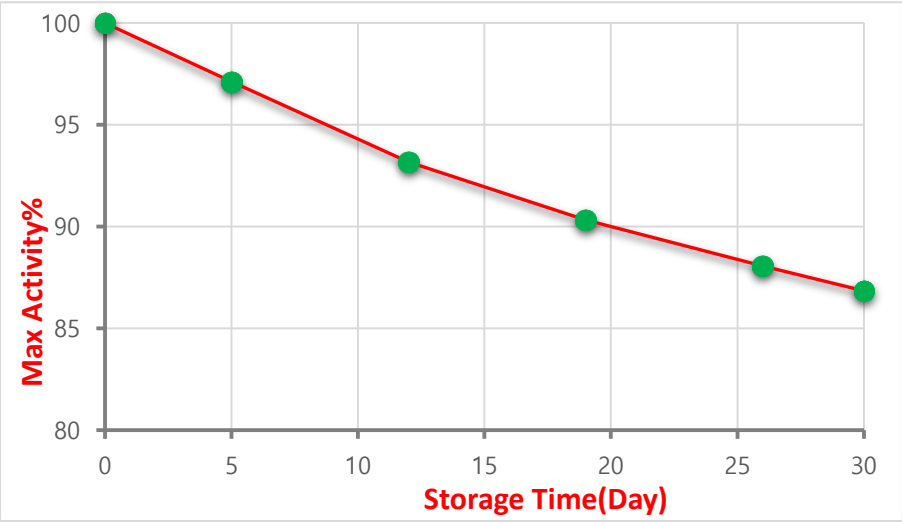
b)Storage Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide



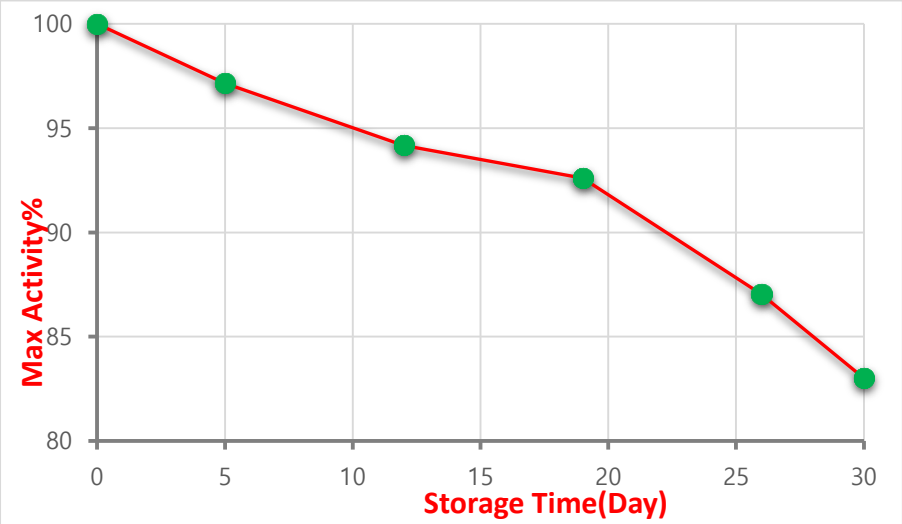
c)Storage Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Succinimide



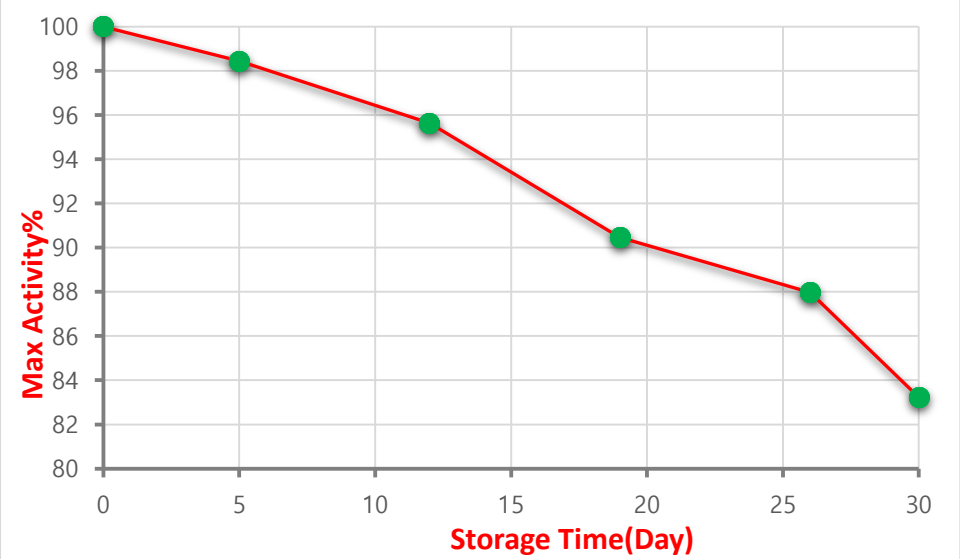
d)Storage Effect on Laccase Activity in PVA-CaAlg Hydrogel Beads Activated by Carbodiimide and Succinimide



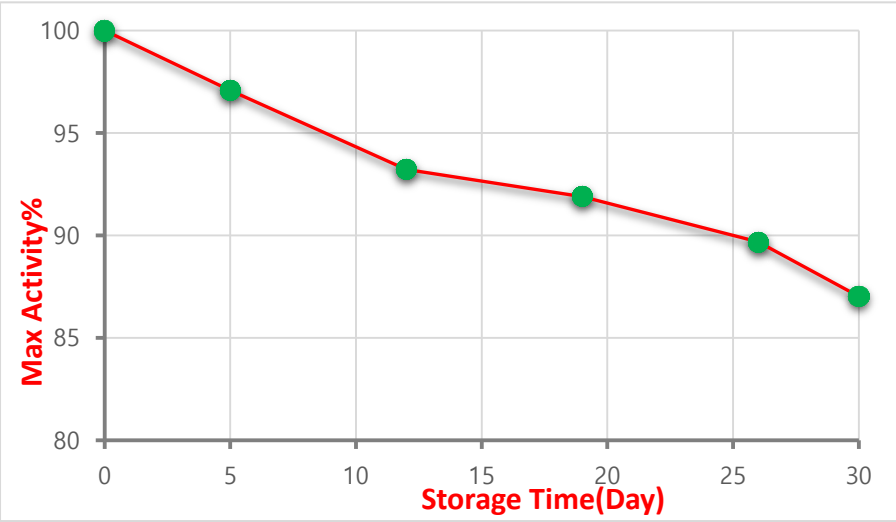
e)Storage Effect on Laccase Activity of CaAlg-PNIPA Beads Activated by Carbodiimide



f)Storage Effect on Laccase Activity in CaAlg-PNIPA Hydrogel Beads Activated by Succinimide

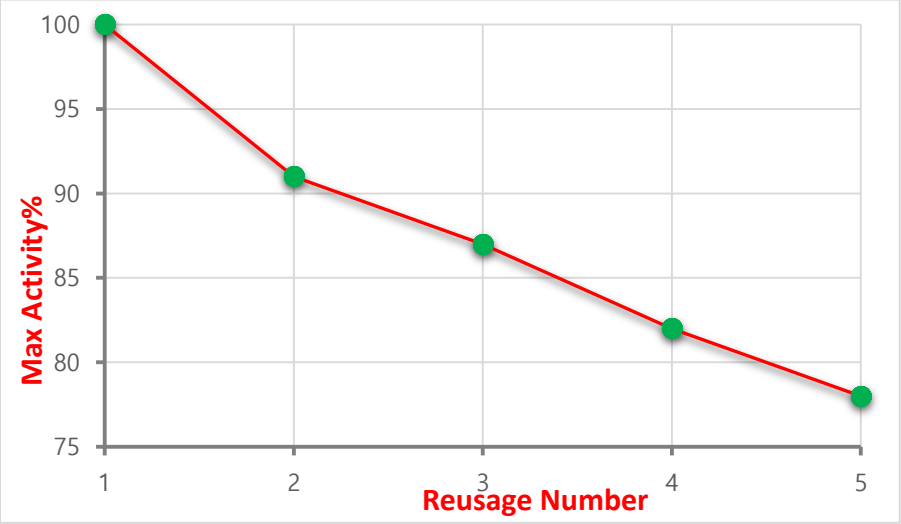


g)Storage Effect on Laccase Activity in CaAlg-PNIPA Hydrogel beads Activated by Carbodiimide and Succinimide

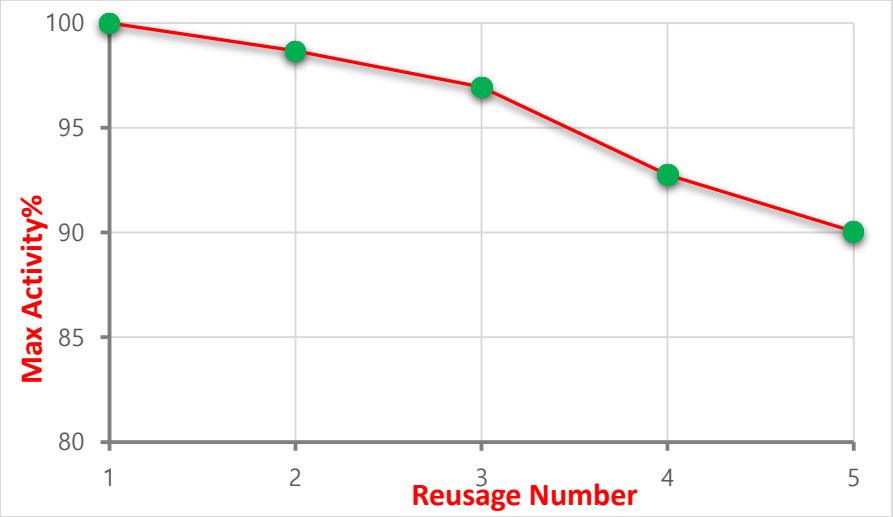


3.5.Reusage Number

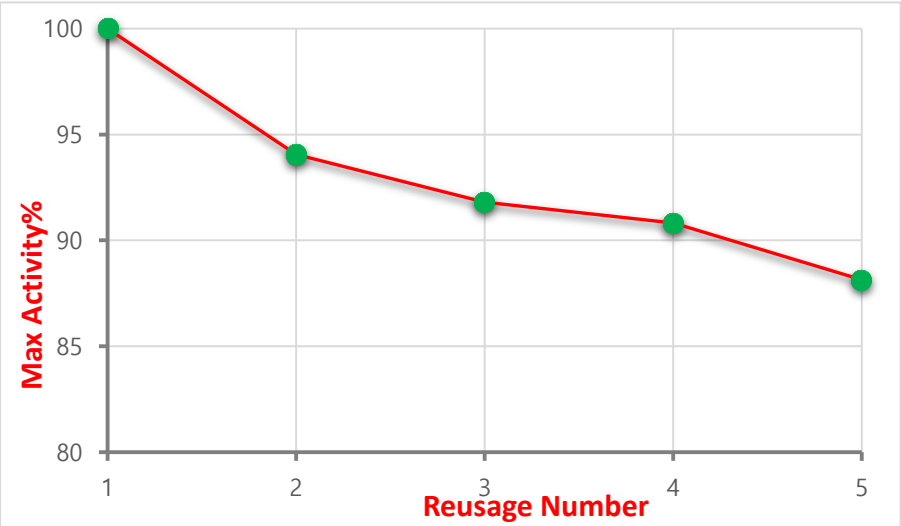
a)Effect of Reusage Number on Free Laccase Activity



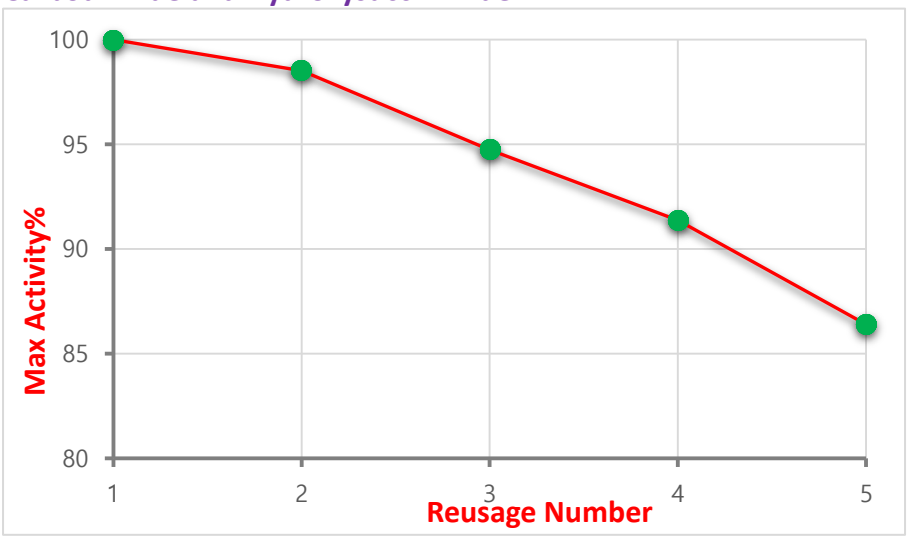
b)Effect of Usage Number on Laccase Activity in PVA-CaAlg Hydrogel beads Activated by Carbodiimide



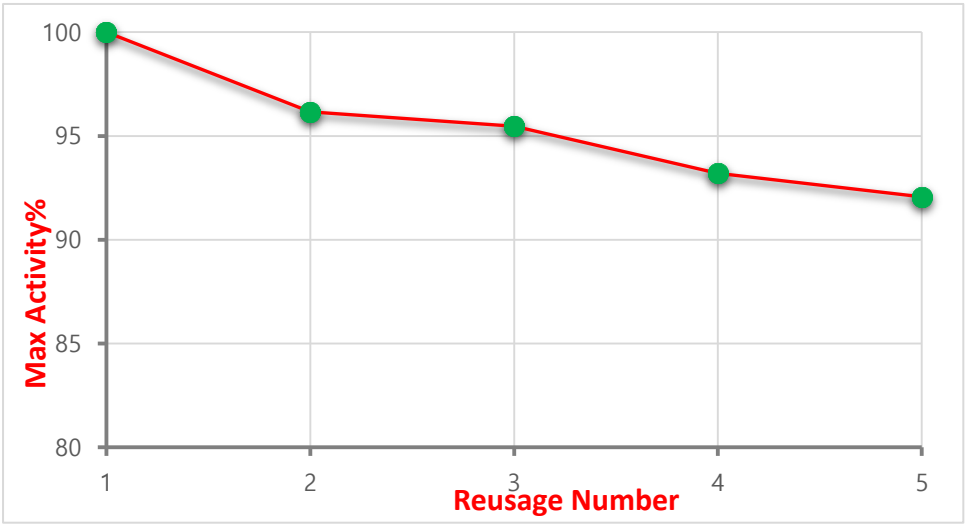
c)Effect of Reusage Number on Laccase Activity in PVA-CaAlg Hydrogel beads Activated by Hydroxysuccinimide



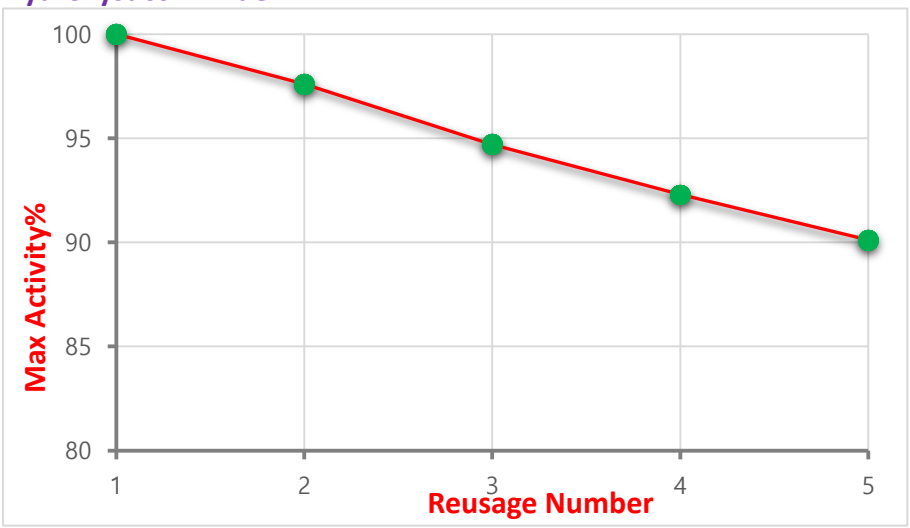
d)Effect of Reusage Number onto Laccase Activity in PVA-CaAlg Hydrogel beads Activated by Carbodiimide and Hydroxysuccinimide



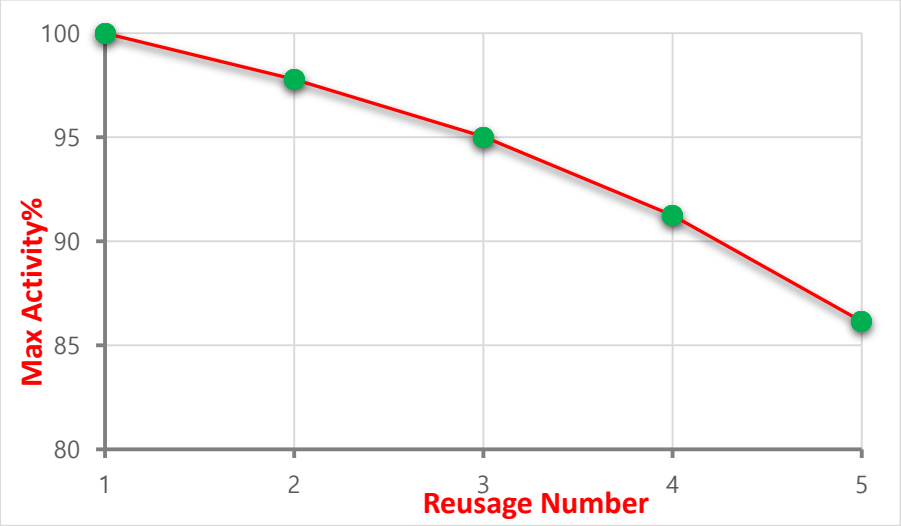
e)Effect of Reusage Number on Laccase Activity in CaAlg-PNIPA Hydrogel beads Activated by Carbodiimide



f)Effect of Reusage Number on Laccase Activity in CaAlg-PNIPA Hydrogel beads Activated by Hydroxysuccinimide



g)Effect of Reusage Number on Laccase Activity in CaAlg-PNIPA Hydrogel beads Activated by Carbodiimide and Hydroxysuccinimide



Data Summary:

Laccase Activity% based on Kind of Hydrogel Bead	Optimum pH	Optimum Temparature	Maximum Laccase activity% due to reuse (5 times)	Maximum Laccase activity% based on storage time (30 days)	Km (mM)	Vm $mM.min^{-1}$
Laccase Free(no hydrogel beads	5	40	---	60%	$16,98x10^{-3}$	$2,09\ x10^{-3}$
Ca alg.+ pva + carb.	6	45	90	83,7%	$28,8x10^{-3}$	$6.00x10^{-3}$
Ca alg. + pva + hydr.	6	45	88	81,2%	$32,7x10^{-3}$	$5,30x10^{-3}$
Ca alg.+ Pva + carb. + hydr.	6	45	86	86,8%	$48,35x10^{-3}$	$8,07x10^{-3}$
Ca alg.+ p-nipa+ carb.	6	45	92	83%	$37x10^{-3}$	$5,90x10^{-3}$
Ca alg.+ p-nipa + hydr.	6	45	90	83,2%	$55,5x10^{-3}$	$8,58x10^{-3}$
Ca-alg.+ p-nipa + carb. + hydr.	6	45	86	87%	$30,8\ x\ 10^{-3}$	$5,19x10^{-3}$

4.Conclusion

1. Maximum activity% of Laccase enzyme on hydrogel beads was observed pH 6
2. Maximum activity% of Laccase enzyme on hydrogel beads was observed 45°C
3. It was observed enzyme activity and substrate concentration were directly proportional.
4. It was observed that the enzyme activity% on the hydrogel beads maintained around 80% for 30 days for 30 days.
5. Maximum activity% of Laccase enzyme on hydrogel beads was observed 80% and above for 5 reusage.
6. Laccase immobilized hydrogel beads which activated by carbodiimide, hydroxysuccinimide and both of them; can be used as an economic and alternative method for detoxicization and decolorization of industrial waste.
7. Our refining systems can be acceptable as the sustainable refining system.
8. Our results are consistent with the results of the studies in the Literatures.