Plant Archives Vol. 20, Supplement 2, 2020 pp. 2571-2579 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EXTRACTION OF SOYBEAN PEROXIDASE AND ITS APPLICATION IN THE TREATMENT OF CONTAMINATED SUBSTANCES

Nidhi Mehta[1], Asmita Sehgal[1], Maina Mohammed Idriss [1]
[1]School of Bioengineering and Biosciences, Lovely Professional University, (Phagwara), India
Corresponding Author: nidhimht533@gmail.com

Abstract

The current work was focused on the extraction of soybean peroxidase from soybean seeds. After carrying out the extraction, the purification of the soybean peroxidase is necessary for the removal of contaminants with the help of ammonium sulfate salt precipitation. This purified enzyme is then used to degrade phenol of different concentration phenol along with different concentrations of hydrogen peroxide as well as soybean peroxidase. And when no hydrogen peroxidase was added there was a very slow degradation of phenol, which proves that hydrogen peroxide works as a catalyst. Soybean peroxidase can target both organic and aqueous contaminants. The phenol degradation with the help of the soybean peroxidase is an efficient procedure that can be used on large scale. This is an inexpensive and an eco-friendly alternative for the treatment of wastewater with great future prospects.

***Keywords*:** Soybean peroxidase, Enzyme, Phenol, Hydrogen peroxide, Wastewater, Catalyst.

Introduction

Glycine max or Soybean is an edible bean which belongs to East Asia with number of uses. The Soybean contains a good amount of phytic acid, dietary minerals and vitamin B. Soybean oil is used in food industry as well as other industries (Kumar and Dwivedi, 2018a; Kumar *et al*. (2018b); Kumar *et al*., 2018c; Kumar and Dwivedi, 2018d; Kumar and Purnima *et al*., 2018e). The unfermented beans are used for the production of soy milk for the production of tofu. It is a most important source of protein. Soybeans develops from seeds into mature plants Dubey *et al.* (2018a). Soybean grows successfully in the summer weather and the optimum temperature is from 20^{o} C-30^{o} C. and can grow in a wide range of soils with good organic content (Dubey *et al.,* 2018b). They also perform nitrogen fixation which is a method of converting air nitrogen into ammonia with the help of microorganisms present into the soil. And soybean uses *Rhizobium.* It generally takes 80-120 days to get fully prepared (Nidhi *et al.,* 2018). The biggest producers for Soybean are United States, Brazil and Argentina (Kaur *et al.,* 2018). In India the biggest contributors in Soybean are Maharashtra and Madhya Pradesh. The origin of soybean is in Southeast Asia and was firstly grown by Chinese farmers.

Contents found in 100g of soybean seeds	Amount present
Water	63%
Fiber	6g
Carbohydrates	9.9g
Calories	173
Sugar	3g
Protein	16.6g
Fats	9g

Types of fats	Amount present
Saturated fats	1.3g
Mono-saturated fats	1.98g
Poly-unsaturated fats	5.06g
OMEGA-3	0.6g
OMEGA-6	4.47g

Vitamins and minerals

Molybdenum
Vitamin K1
Vitamin B9
Vitamin B1
Phosphorus
Copper
Manganese
Isoflavones
Saponins
Phytic acid

Thus Soybean is the best source of nutrition and among them protein is the prominent one. It also helps in the reduction of osteoporosis in the women who have undergone menopause. But if taken in large amounts can cause problems like diarrhea, thyroid problems as it suppresses the thyroid function, soy allergy.

Peroxidase catalyzes the redox reaction between hydrogen peroxide, an electron acceptor, and variety of other substrates in which either oxygen, or water, or both are liberated.

$$2H_2O_2. \xrightarrow{\text{PEROXIDASE}} 2H_2O + O_2$$

These enzymes are heat stable and are tend to found in plants as well as animals. These enzymes are classified into three classes on the basis of the homology of the amino acids and their capacity to bind to metals. Class 1 has intracellular peroxidases, Class 2 have secretory fungal peroxidase and Class 3 have secretory plant peroxidase. Soybean peroxidase belongs to Class 3.

The main and the strong application of the soybean peroxidase is the removal of phenol from wastewater. Phenol and its derivatives are mostly considered as pollutant because of its presence in the maximum of the industrial waste waters as petroleum refineries, coal factories, paper industry (Kumar and 2019f; Kumar and Siddique *et al*., 2019g; Siddique and Kumar, 2018h; Siddique *et al*., 2018i; Pathak *et al*., 2017j; Prakash and Kumar, 2017k; Kumar and Mandal, 2014L). And phenol is a hazardous for environment and as well as the

living organisms even at low concentration. Exposure to the high concentrations of phenol can cause liver damage, paralysis, and its short term effects are headaches, burning eyes, respiratory irritation and its chronic effects includes muscle pain, weakness, weight loss and fatigue.

Soybean peroxidase can be extracted from the either plant of the plant be it its leaves or roots or seeds or even seed hulls. It catalyzes the polymerization and thus precipitation of the aqueous phenol along with hydrogen peroxide. It possesses higher stability and activity under a wide range of pH.

Soybean peroxidase is preferred for the removal of phenols from phenols from waste water over horseradish peroxidase because it is inexpensive and has a better temperature tolerance and has higher redox potentiall. Soybean peroxidase is used along with hydrogen peroxide for the degradation of phenol in the waste water (Kumar *et al*., 2014m; Kumar *et al*., 2014n; Kumar, 2013o; Kumar and Dwivedi, 2015p; Gogia *et al*., 2014q). Hydrogen peroxide (H_2O_2) is a clear viscous liquid. It is used as a bleaching agent, and is an active component in the detergents and is also used as disinfectant and in the cosmetics and it is also used for the sewage treatment. When in pure form it is a very hazardous to use because of its unstable form, there is a risk of explosion. So generally it is used as a water solution. It is highly oxidative in nature so when used in higher concentrations can be harmful to eyes and can cause burns to the skin. Therefore for most of the laboratory works 30% hydrogen peroxide is used. But if used in higher concentrations precautions should be made (Kumar, 2014r; Kumar *et al*., 2012s; Mishra *et al*., 2012t; Kumar *et al*., 2011u; Kumar *et al*., 2011v). For the purification of extracted soybean peroxidase we will use salting out method with the help of ammonium sulfate. Salting out method is the method in which we use high concentrations of salt for the precipitation of the protein. Prakash & Kumar, (2018). This method works as when the salt is added to the given salt it breaks into its positive and negative charges, thus the dissociated charges starts to bind with the respective charges of the given solvent, as the negative charged molecule of the salt will interact with the positive charge of the solvent and thus the positive charge of the salt will interact with the negative charge of the solvent. Ammonium sulfate salt precipitation is usually used for the purification of enzymes in the laboratories because of its high solubility in the water. It also stabilizes the structure of the protein and is in expensive, thus easily available. It is a quick method for protein purification so it is used in the initial steps of protein purification and can also be used for purification of proteins in the bulk. Though ammonium sulfate precipitation is the ideal way for the purification of the protein but sometimes along with the desired proteins it can also precipitate the contaminants, therefore other methods of purification of protein can be used such as chromatography (ion-exchange or size- exclusion).

During the extraction of the soybean peroxidase it is important to heat the enzyme to deactivate the work of the catalase enzyme present in the peroxidase enzyme and quickly cooled by placing it on the ice (Haldhar *et al.,* 2019)

The hydrogen peroxidase is working as a catalyst for the degradation of phenol along with soybean peroxidase (Kumar and Pathak, 2016w; Pathak *et al*., 2016x; Kumar *et al*., 2018y; Kumar *et al*., 2018z; Kumar *et al*., 2018aa; Kumar and Kumar, *et al*., 2018bb; Kumar *et al*., 2018cc). A catalyst is an enzyme that fastens up the rate of reaction without getting consuming up. And to show that the hydrogen peroxidase is acting as the catalyst we have taken a sample of phenol only with soybean peroxidase. And the result ought to be that there is degradation of protein but on a much slower rate.

Material and Methods

The work that has been done proves that the soybean peroxidase that has been obtained from the soybean seeds can degrade the phenol in the wastewater. To check its efficiency for the different concentrations of the phenol we have taken 5g, 10g, 15g, 20g and 25 g of phenol respectively in the 500 ml of distilled water. To increase the efficiency of the reaction a catalyst has been taken in account and that is hydrogen peroxide. The different sets have been taken for the efficient check of the soybean peroxidase. For 5g, 10g, 15g, 20g and 25 g of phenol in 500 ml of distilled water, we have taken 0.1ml, 0.2ml and 0.3 ml of hydrogen peroxide respectively along with 0.2ml, 0.4ml, 0.8ml and 1.6 ml of soybean peroxidase respectively. After mixing all these substances together we have kept them at aside for 45 minutes and then after 45 minutes absorbance is checked at 540 nm by using UV spectrophotometer. The different absorbances show the degradation of phenol by soybean peroxidase.

(A) Extraction of soybean peroxidase from soybean seeds

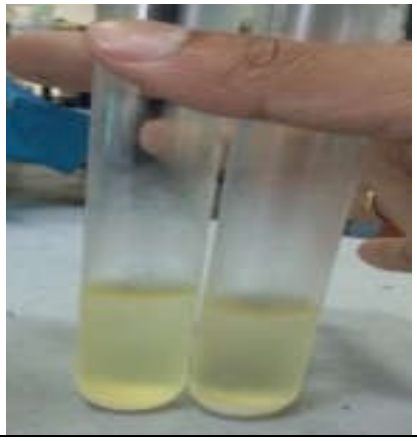
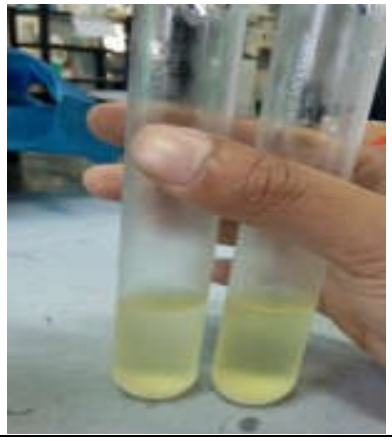
Take 50g of soybean seeds and wash them properly. And soak them in distilled water for 24 hours. Removed the water and crush the soaked seeds with 200ml of distilled water until crushed properly. Extracted enzyme from soaked soybean seeds for different hours.

For 24 hours: The freshly ground soybean seeds are taken and put them in the centrifuge tubes and centrifuged it for 15 minutes at 10,000 rpm. After collection the supernatant and discarded the pellet. Supernatant filtered the with the help of filter paper .

For 48 hours: Stored the ground seeds in the refrigerator for another 24hours.This mixture taken and put into centrifuge tubes and centrifuged it for 15 minutes at 10,000 rpm. Supernatant collected the and the pellet discarded. Supernatant filtered the with the help of filter paper .

For 72 hours: Stored the ground seeds in the refrigerator for another 24hours. Took this mixture and put into centrifuge tubes and centrifuged for 15 minutes at 10,000 rpm. Supernatant collected the and the pellet discarded. Supernatant filtered the with the help of filter paper

Different soaked soybean seeds were taken for the extraction of peroxidase to check if there is any change in the color or its enzymatic activity. But there was no such difference seen either in the activity or the color.

		
After 24 hours	After 48 hours	After 72 hours

(B) Purification of the extracted enzyme

The purification of the soybean peroxidase is necessary for the removal of contaminants. Ammonium sulfate salt precipitation is usually used for the purification of enzymes in the laboratories because of its high solubility in the water. It also stabilizes the structure of the protein and is in expensive, thus easily available. To purify the extracted Soybean Peroxidase we will use Solid Ammonium Sulfate. To make 50% saturation, we will take 10 ml of enzyme and will add 3.01 grams of ammonium sulfate. Now centrifuge this mixture at 10,000 rpm for 15 minutes at 4ºC. Then incubate the centrifuged mixture for 4 hours at 4ºC. Now take the supernatant and make it 85% saturated. For this we will add 2.37 grams of ammonium sulfate in 11.6 ml of the supernatant. Now centrifuge this mixture for 15 minutes at 10,000 rpm +at 4ºC. Now to desalt the enzyme we will dialyze it against distilled water at 4ºC.

(C) Estimation for the degradation of phenol

We will take different concentrations of phenol along with different concentrations of hydrogen peroxide as well as soybean peroxidase.

For 5g of Phenol IN 500 ml of Distilled Water: We will take different concentrations of H_2O_2 and soybean peroxidase. IN 0.1 ml of H_2O_2, the concentration of soybean peroxidase will be 0.2, 0.4, 0.8 and 1.6 respectively. IN 0.2 ml of H_2O_2, the concentration of soybean peroxidase will be 0.2, 0.4, 0.8 and 1.6 respectively. IN 0.3 ml of H_2O_2, the concentration of soybean peroxidase will be 0.2, 0.4, 0.8 and 1.6 respectively. For the other concentrations also the same procedure was repeated.

For 5g of Phenol IN 500 ml of distilled water but without hydrogen peroxide: We will take only the different concentrations of soybean peroxidase and that will be 0.2, 0.4, 0.8 and 1.6 respectively. Now mix all the components (phenol, H_2O_2 and soybean peroxidase) well and keep aside for 45 minutes. Now take the absorbance on a UV Spectrophotometer at 540 nm wavelength.

***Note**: H_2O_2 used 30% (standard laboratory usage).

(D) Spectrophotometric analysis: After mixing all these substances together we have kept them at aside for 45 minutes and then we check their absorbances at 540 nm by using UV spectrophotometer. Different absorbances show the degradation of phenol by soybean peroxidase (Prasann *et al.,* 2018).

Results

We got different results for the different sets of soybean peroxidase with hydrogen peroxide for the degradation of phenol in the synthetic wastewater. As we keep on increasing the amount of phenol the effectiveness of the soybean peroxidase decreases. And when no hydrogen peroxidase was added there was a very slow degradation of phenol, which proves that hydrogen peroxide works as a catalyst.

Absorbance at 540 nm on a UV spectrophotometer:

FOR 5g PHENOL

Concentration of soybean peroxidase	H_2O_2 (0.1ml)	H_2O_2 (0.2ml)	H_2O_2 (0.3ml)
0.2	0.059	0.022	0.04
0.4	0.054	0.015	0.035
0.8	0.05	0.19	0.34
1.6	0.046	0.02	0.03

FOR 10g PHENOL

Concentration of soybean Peroxidase	H_2O_2 (0.1ml)	H_2O_2 (0.2ml)	H_2O_2 (0.3ml)
0.2	0.062	0.135	0.146
0.4	0.059	0.073	0.080
0.8	0.31	0.073	0.65
1.6	0.046	0.046	0.046

FOR 15g OF PHENOL:

Concentration of soybean Peroxidase	H_2O_2 (0.1ml)	H_2O_2 (0.2ml)	H_2O_2 (0.3ml)
0.2	0.225	0.210	0.146
0.4	0.155	0.135	0.084
0.8	0.110	0.105	0.065
1.6	0.125	0.084	0.056

FOR 20g OF PHENOL:

Concentration of soybean eroxidase	H_2O_2 (0.1ml)	H_2O_2 (0.2ml)	H_2O_2 (0.3ml)
0.2	0.350	0.315	0.315
0.4	0.255	0.205	0.215
0.8	0.265	0.185	0.190
1.6	0.195	0.168	0.165

FOR 25g OF PHENOL:

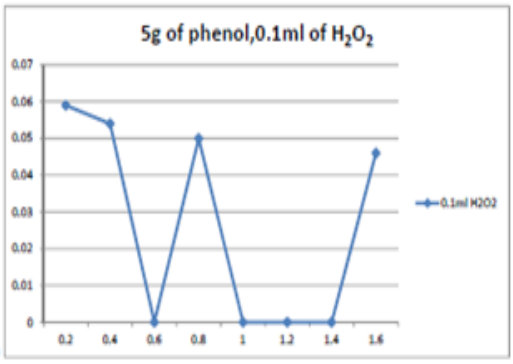
Concentration of soybean peroxidase	H_2O_2 (0.1ml)	H_2O_2 (0.2ml)	H_2O_2 (0.3ml)
0.2	0.568	0.560	0.566
0.4	0.545	0.540	0.548
0.8	0.500	0.495	0.520
1.6	0.480	0.485	0.490

One sample is without hydrogen peroxidase to check its effect as a catalyst along with soybean peroxidase:

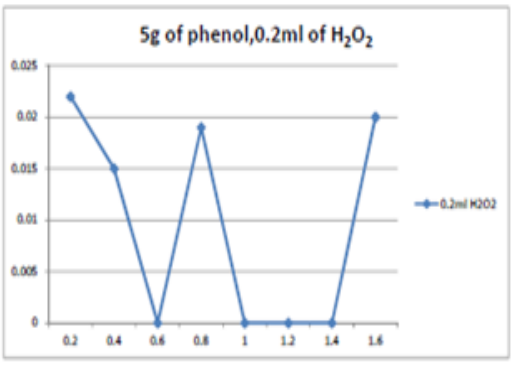
Concentration of soybean peroxidase	NO H_2O_2
0.2	0.196
0.4	0.173
0.8	0.153
1.6	0.120

The results are as follows in the graph form and their significance has also been shown:

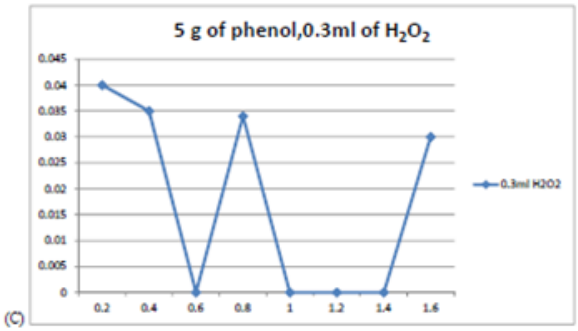
FOR 5g PHENOL:



(A)

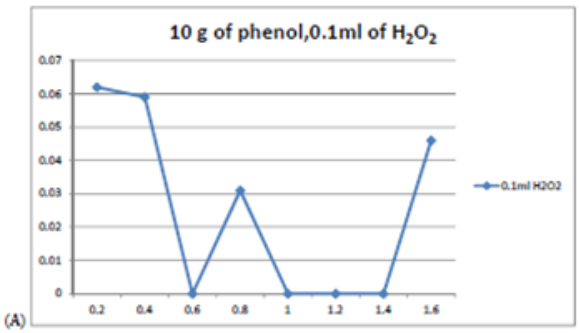


(B)



(C)

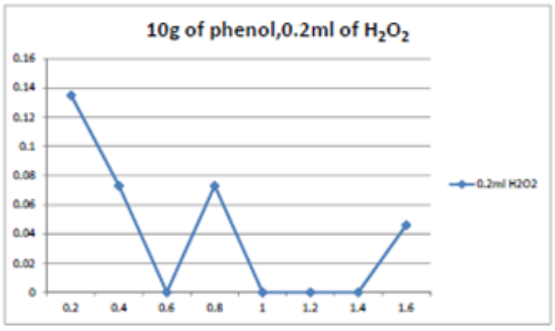
FOR 10g PHENOL:



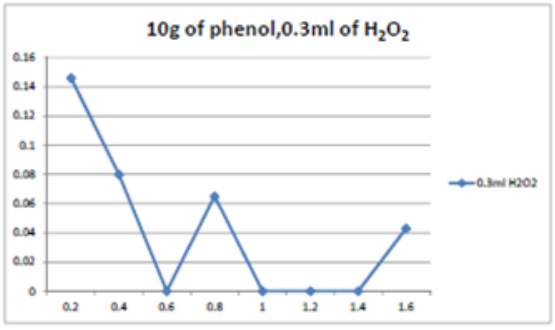
(A)

+

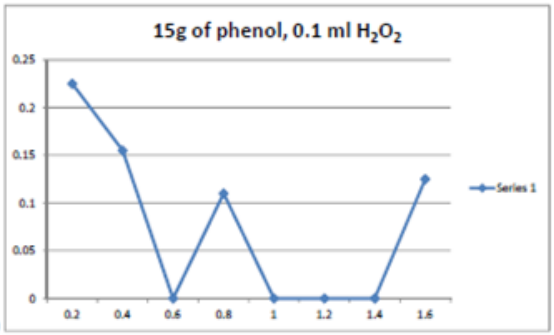
FOR 15g PHENOL:



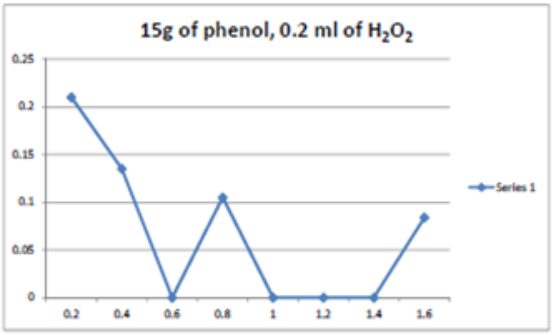
(B)



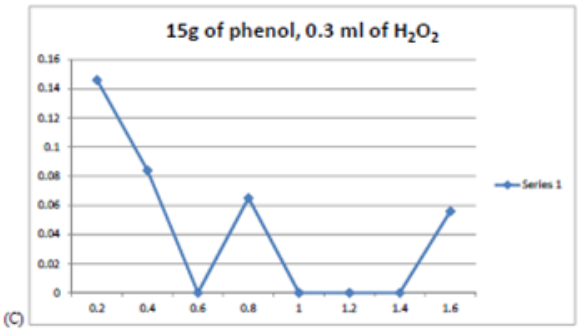
(C)



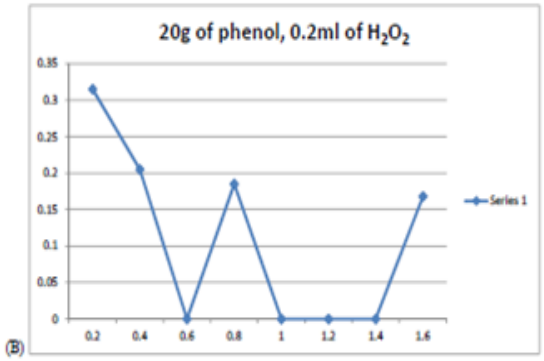
(A)



(B)

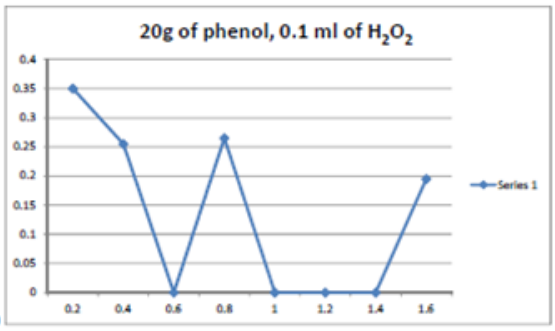


(C)

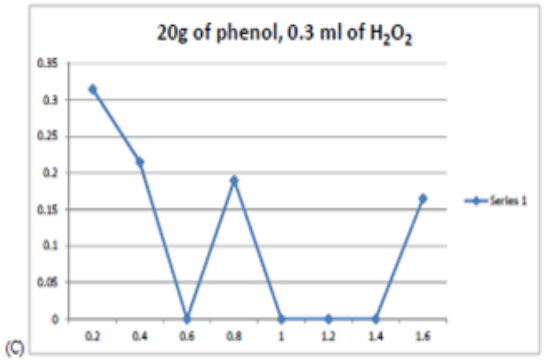


(B)

FOR 20g PHENOL:

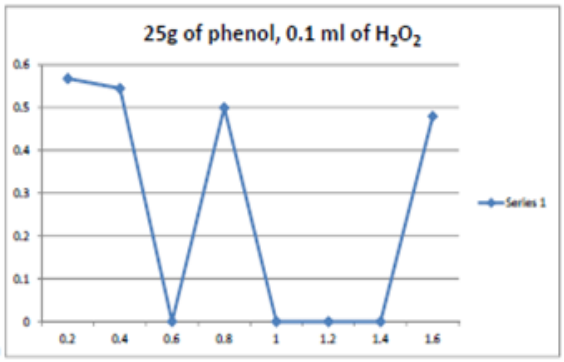


(A)

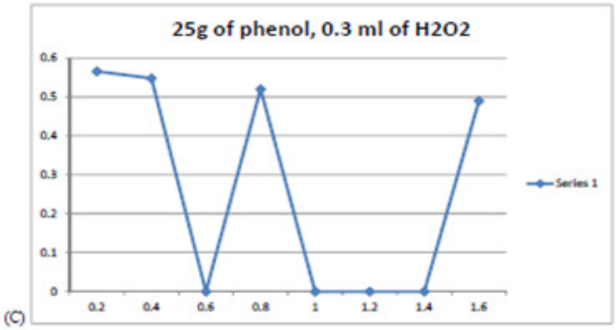


(C)

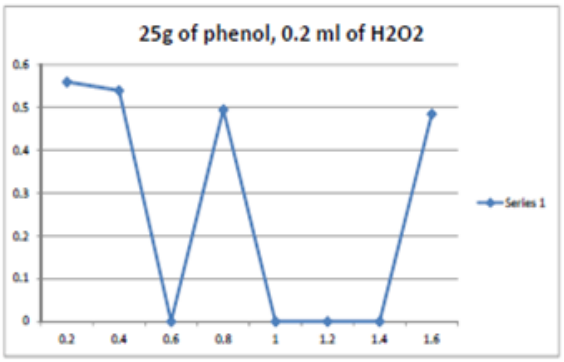
FOR 25g PHENOL:



(A)

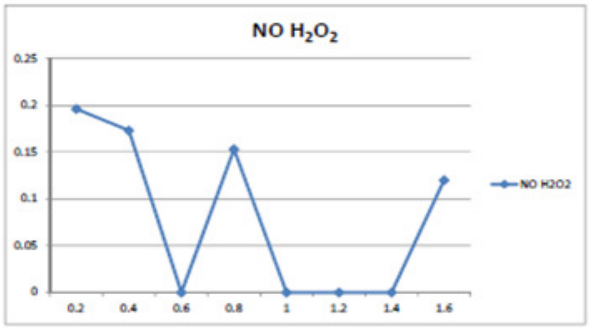


(C)



(B)

FOR NO PRESENCE OF H_2O_2 AND 5g OF PHENOL:



There is a huge difference between the readings of soybean peroxidase with hydrogen peroxide and soybean peroxidase without any hydrogen peroxidase. Soybean peroxidase is capable to degrade the phenol on its own but on a much slower rate. Therefore it can be said that hydrogen peroxidase do work as a catalyst for

Discussion

For all the readings the graph that we have plotted is similar. As we keep on increasing the amount of phenol into the water with the constant amount of soybean peroxidase and hydrogen peroxide, it seems that it is not that effective as it is when there are lower amount of phenol into the water (Churasia *et al.*, 2016). That is effective but not that much.

And when we took the reading for the degradation of phenol without the presence of hydrogen peroxide, the degradation was much slower

Significance test:

For 5 g

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	abs1	.05225	4	.005560	.002780
	abs2	.06175	4	.085551	.042775
Pair 2	abs1	.05225	4	.005560	.002780
	abs3	.11125	4	.152555	.076277
Pair 3	abs2	.06175	4	.085551	.042775
	abs3	.11125	4	.152555	.076277

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	abs1 & abs2	4	-.265	.735
Pair 2	abs1 & abs3	4	-.244	.756
Pair 3	abs2 & abs3	4	.999	.001

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	abs1 - abs2	-.009500	.087188	.043594	-.148235	.129235	-.218	3	.841
Pair 2	abs1 - abs3	-.059000	.154006	.077003	-.304059	.186059	-.766	3	.499
Pair 3	abs2 - abs3	-.049500	.067139	.033570	-.156333	.057333	-1.475	3	.237

For 10 gms

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	abs1 - abs2	.037500	.136710	.068355	-.180036	.255036	.549	3	.621
Pair 2	abs1 - abs3	-.111250	.156621	.078311	-.360470	.137970	-1.421	3	.251
Pair 3	abs2 - abs3	-.148750	.285536	.142768	-.603102	.305602	-1.042	3	.374

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	abs1	.11925	4	.127356	.063678
	abs2	.08175	4	.037713	.018856
Pair 2	abs1	.11925	4	.127356	.063678
	abs3	.23050	4	.282731	.141366
Pair 3	abs2	.08175	4	.037713	.018856
	abs3	.23050	4	.282731	.141366

For 15 gms

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	1	.15375	4	.051051	.025526
	2	.13350	4	.055127	.027564
Pair 2	1	.15375	4	.051051	.025526
	3	.08775	4	.040549	.020275
Pair 3	2	.13350	4	.055127	.027564
	3	.08775	4	.040549	.020275

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	1 & 2	4	.962	.038
Pair 2	1 & 3	4	.975	.025
Pair 3	2 & 3	4	.995	.005

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	1 - 2	.020250	.015174	.007587	-.003895	.044395	2.669	3	.076
Pair 2	1 - 3	.066000	.014652	.007326	.042686	.089314	9.009	3	.003
Pair 3	2 - 3	.045750	.015370	.007685	.021292	.070208	5.953	3	.009

For 20 gms

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	1	.26625	4	.063819	.031910
	2	.21825	4	.066249	.033124
Pair 2	1	.26625	4	.063819	.031910
	3	.22125	4	.065749	.032874
Pair 3	2	.21825	4	.066249	.033124
	3	.22125	4	.065749	.032874

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	1 & 2	4	.936	.064
Pair 2	1 & 3	4	.951	.049
Pair 3	2 & 3	4	.996	.004

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	1 - 2	.048000	.023367	.011683	.010818	.085182	4.108	3	.026
Pair 2	1 - 3	.045000	.020412	.010206	.012519	.077481	4.409	3	.022
Pair 3	2 - 3	-.003000	.005715	.002858	-.012095	.006095	-1.050	3	.371

For 25 gms

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	1	.52325	4	.040360	.020180
	2	.52000	4	.035824	.017912
Pair 2	1	.52325	4	.040360	.020180
	3	.53100	4	.033247	.016623
Pair 3	2	.52000	4	.035824	.017912
	3	.53100	4	.033247	.016623

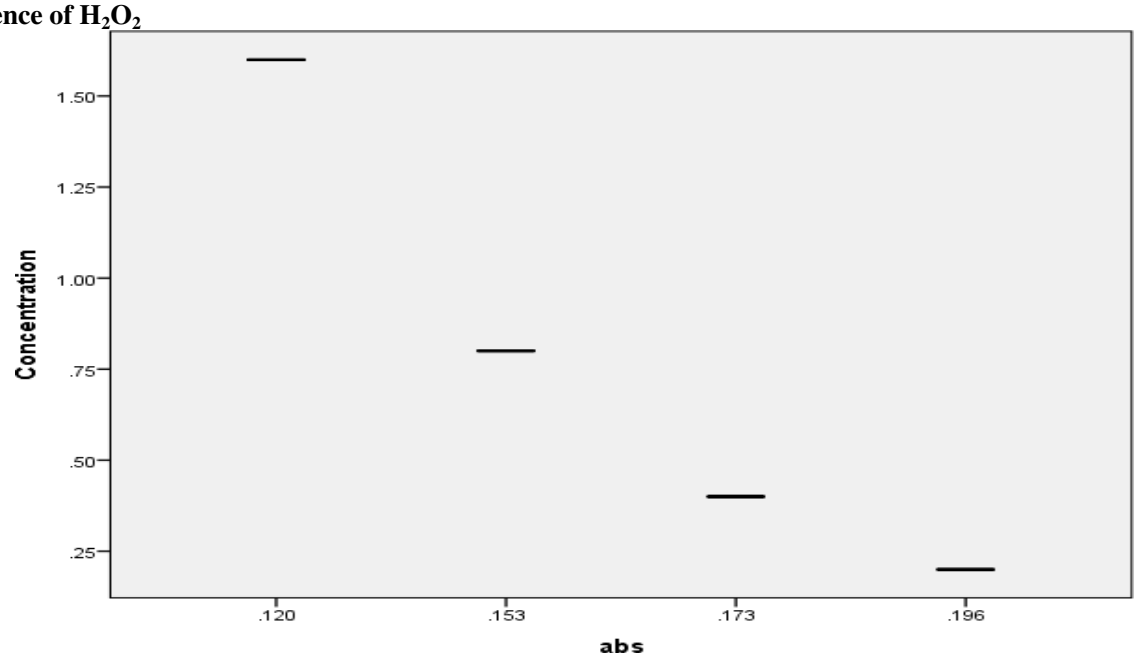
Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	1 & 2	4	.996	.004
Pair 2	1 & 3	4	.985	.015
Pair 3	2 & 3	4	.966	.034

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	1 - 2	.003250	.005679	.002839	-.005786	.012286	1.145	3	.335
Pair 2	1 - 3	-.007750	.009535	.004768	-.022922	.007422	-1.626	3	.203
Pair 3	2 - 3	-.011000	.009416	.004708	-.025983	.003983	-2.336	3	.102

For no presence of H_2O_2



No change in the degradation of phenol when there is any presence of H_2O_2

Conclusions

The soybean peroxidase from the soybean seeds has pro ved to be an effective and cheap method for the degradation of phenol in the wastewater. The use of hydrogen peroxidase is as a catalyst. When we tried to degrade the phenol without the use of hydrogen peroxide it took a long time for the degradation and not much change was seen. Hence the working of hydrogen peroxide as a catalyst. For the higher amount of synthetic phenolic water the constant amount of soybean peroxidase is not as effective as it was for the lower phenol concentration. Hence, from all the results we have obtained we can say that the application of soybean peroxidase is effective for the degradation and it is less expensive.

These results report that efficient purification is essential for the application of soybean peroxidase or such enzymes in the processes used for industrial wastewater treatment including bioremediation.

References

Asian Journal of Chemistry (2013). Vol. 25: 115887-5890.

Flock, C.; Bassi, A. and Gijzen, M. (1999). Study on removal of aqueous phenol and 2-chlorophenol with purified soybean peroxidase and raw soybean hulls. Journal Chemical Technology Biotechnology, 74: 303-309

Ankudey, E.l.G. and Woode, M. (2014). Assessment and characterization of Peroxidase activity in Locally grown Bean varieties from Ghana. International Journal of Scientific and Research Publications, vol. 4(6).

Alyas, F.; Zia, M.A. and Rehman, K. (2002). Extraction and Purificatiom of Peroxidase from Soybean Seeds. Pak J, Agri. Sci., Vol. 39(4).

Ghaemmaghani, F.; Alemzadeh, I. and Motamed, S. (2010). Seed coat Soybean Peroxidase: Extraction and Biocatalytic properties Determination. Iranian Journal of Chemical Engineering Vol. 7(2).

Habib, F.; Rehman, K.; Zia, M.A.; Rehman, Z. and Saeed, M.K. (2003). Peroxidase: Purification from Soybean seeds. Pakistan Journal of Biological Sciences, 6(2): 130-132.

International Journal of Advanced Scientific and Technical Reasearch (2015).

Johnson (1991). Biocatalytic oxidation using soybean and other legume peroxidases.

Al-Ansari, M.M.; Saha, B.; Mazloum, S.; Taylor, K.E.; Bewtra, J.K. and Biswas, N. (2011). Soybean Peroxidase Applications In Wastewater Treatment. Nova science Publishers, Inc.

Kolhe, P.M.; Ingle, S.T. and Wagh, N.D. (2015). Removal of Phenol at Higher Concentration from Aqueous medium using crude Peroxidase catalysed reaction coupled with Hydrogen Peroxide. Int J Adv Scientific and Tech Res., 5(3): 70-78.

Pokora (1993). Soybean Peroxidase treatment of contaminated substances. U.S. Pat. No. 5, 178, 762.

Jiang, X. and Zheng, H. (2013). Removal of Bisphenol A by soybean Hulls Peroxidase. Asian Journal of Chemistry, 25(11): 5887-5890.

Churasia, A.; Singh, J. and Kumar, A. (2016). Production of biodiesel from soybean oil biomass as renewable energy source. Journal of environmental biology, 37(6): 1303.

Dubey, N.; Avinashe, H.A. and Shrivastava, A.N. (2018a). Principal component analysis in advanced genotypes of soybean (*Glycine max* (L.) Merrill) over seasons. Plant Archives, 18(1): 501-506.

Dubey, N.; Avinashe, H.A. and Shrivastava, A.N. (2018b). Assessment of genetic diversity in soybean (Glycine max (L.) Merrill) genotypes. Research on Crops, 19(2): 271-275.

Nidhi, D.; Avinashe, H.A. and Shrivastava, A.N. (2018). Genetic parameters and character association studies of soybean genotypes in Madhya Pradesh region. Annals of Biology, 34(2): 207-211.

Kaur, P.; Waghmare, R.; Kumar, V.; Rasane, P.; Kaur, S. and Gat, Y. (2018). Recent advances in utilization of flaxseed as potential source for value addition. OCL, 25(3): A304.

Haldhar, R.; Prasad, D. and Bhardwaj, N. (2019). Extraction and experimental studies of Citrus aurantifolia as an economical and green corrosion inhibitor for mild steel in acidic media. Journal of Adhesion Science and Technology, 33(11): 1169-1183.

Kaur, P.; Pandey, D.K.; Gupta, R.C. and Dey, A. (2019). Simultaneous microwave assisted extraction and

HPTLC quantification of mangiferin, amarogentin, and swertiamarin inestern Himalayas. Industrial crops and products, 132: 449-459.

Kumar, P.P *et al*., (2018e). Impact of Polyamines and Mycorrhiza on Chlorophyll Substance of Maize Grown under Cadmium Toxicity, International Journal of Current Microbiology and Applied Sciences, 7(10): 1635-1639.

Kumar, P. and Pathak, S. (2019f). Responsiveness index of sorghum (Sorghum bicolor (l.) Moench) grown under cadmium contaminated soil treated with putrescine and mycorrhiza, Bangladesh J. Bot., 48 (1).

Kumar, P.; Siddique, A. *et al*., (2019g). Role of Polyamines and Endo-mycorrhiza on Leaf Morphology of Sorghum Grown under Cadmium Toxicity, Biological Forum – An International Journal. 11(1): 01-05.

Siddique, A. and Kumar, P. (2018h). Physiological and Biochemical basis of Pre-sowing soaking seed treatments-An overview, Plant Archive, 18(2): 1933-1937.

Siddique, A.; Kandpal, G. and Kumar P. (2018i). Proline accumulation and its defensive role under Diverse Stress condition in Plants: An Overview, Journal of Pure and Applied Microbiology, 12(3): 1655-1659.

Pathak, S.; Kumar, P.; Mishra, P.K. and Kumar, M. (2017j). Mycorrhiza assisted approach for bioremediation with special reference to biosorption, Pollution Research, 36(2).

Prakash, A. and P. Kumar, (2017k). Evaluation of heavy metal scavenging competence by in-vivo grown Ricinus communis L. using atomic absorption spectrophotometer, Pollution Research, 37(2): 148-151.

Kumar, P. and Mandal, B. (2014L). Combating heavy metals toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants, vegetos, 26(2): 416-425.

Kumar, P.; Mandal, B. and Dwivedi, P. (2014m). Phytoremediation for defending heavy metal stress in weed flora, International Journal of Agriculture, Environment & Biotechnology, 6(4): 587-595.

Kumar, P.; Kumar, P.K. and Singh, S. (2014n). Heavy metal analysis in the root, shoot and a leaf of *Psidium guajava* L. by using atomic absorption spectrophotometer, Pollution Research, 33(4): 135-138.

Kumar, P. (2013o). Cultivation of traditional crops: an overlooked answer. Agriculture Update, 8(3): 504-508.

Kumar, P. and Dwivedi, P. (2015p). Role of polyamines for mitigation of cadmium toxicity in sorghum crop, Journal of Scientific Research, B.H.U., 59: 121-148.

Gogia, N.; Kumar, P.; Singh, J.; Rani, A.S. and Kumar, P. (2014q). Cloning and molecular characterization of an active gene from garlic (*Allium sativum* L.), International Journal of Agriculture, Environment and Biotechnology, 7(1): 1-10.

Kumar, P. (2014r). Studies on cadmium, lead, chromium, and nickel scavenging capacity by *in-vivo* grown Musa paradisiacal. using atomic absorption spectroscopy, Journal of Functional and Environmental Botany, 4(1): 22-25.

Kumar, P.; Dwivedi, P. and Singh, P. (2012s). Role of polyamine in combating heavy metal stress in stevia rebaudiana Bertoni plants under in vitro condition, International Journal of Agriculture, Environment and Biotechnology, 5(3): 185-187.

Mishra, P.K.; Maurya, B.R. and Kumar, P. (2012t). Studies on the biochemical composition of *Parthenium hysterophorus* L. in different season, Journal of Functional and Environmental Botany, 2(2): 1-6.

Kumar, P.; Mandal, B. and Dwivedi, P. (2011u). Heavy metal scavenging capacity of *Mentha spicata* and *Allium cepa,* Medicinal Plant-International Journal of Phytomedicines and Related Industries. 3(4): 315-318.

Kumar, P.; Mandal, B. and Dwivedi, P. (2011v). Screening plant species for their capacity of scavenging heavy metals from soils and sludges. Journal of Applied Horticulture, 13(2): 144-146.

Kumar, P. and Pathak, S. (2016w). Heavy metal contagion in seed: its delivery, distribution, and uptake, Journal of the Kalash Sciences, An International Journal, 4(2): 65-66.

Pathak, S.; Kumar, P.; Mishra, P.K. and Kumar, M. (2016x). Plant-based remediation of arsenic-contaminated soil with special reference to sorghum- a sustainable approach for a cure, Journal of the Kalash Sciences, An International Journal, 4(2): 61-65.

Kumar, P.; Harsavardhn, M. *et al.,* (2018y). Effect of Chlorophyll a/b ratio in Cadmium Contaminated Maize Leaves Treated with Putrescine and mycorrhiza, Annals of Biology, 34(3): 281-283.

Kumar, P.; Yumnam, J. *et al*. (2018z). Cadmium Induced Changes in Germination of Maize Seed Treated with Mycorrhiza, Annals of Agri-Bio Research, 23(2): 169-170.

Kumar, P.; Pandey, A.K. *et al*. (2018aa). Phytoextraction of Lead, Chromium, Cadmium, and Nickel by Tagetes Plant Grown at Hazardous Waste site, Annals of Biology, 34(3): 287-289.

Kumar, P.; Kumar, S. *et al*., (2018bb). Evaluation of Plant Height and Leaf Length of Sorghum Grown Under Different Sources of Nutrition, Annals of Biology, 34(3): 284-286.

Kumar, P.; Krishna, V. *et al*. (2018cc). Assessment of Scavenging Competence for Cadmium, Lead, Chromium and Nickel Metals by in vivo Grown *Zea mays* L. using Atomic Absorption Spectrophotometer, Annals of Ari-Bio Research, 23(2): 166-168.