Optimization of the growth conditions for maximum production of the peroxidase by *Bacillus subtilis*

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Absitract : This working was achived in Section of Biology,faculty of Science, Kufa university which contain peroxidase separation and classically of route parameters to work greatest defer of peroxidase through *Bacillus subtilis* . Fermentation of solid-state of *Bacillus subtilis* was accepted for improved production of peroxidase using hydrogen peroxide as the substrate of enzyme , greatest activity of the enzyme was achieved in best growth environment , the best conditions were the isolated of *Bacillus subtilis* from soil and growth in synthetic medium , it gave elevated titer of peroxidase activity , the lactose as carbon source , yeast extract as nitrogen source , after 18 hr of incubation, incubation temperature 30 °C and pH = 7 .

Key word : peroxidase , *Bacillus subtilis*

Microbiology Classification QR (100- 130)

1- Introduction : *Bacillus subtilis* is Gram-positive bacteria belonging to the family Bacillaceae of the order Bacillales (1) . In the field of biotechnology , *Bacillus subtilis* is chief plus to microbiology as it is an essential microorganism because it has the capability to manufacture a diversity of helpful substances , it is therefore cultivated for manufacturing making , often commercially for uses in making proteins , in the food industries and in pharmaceuticals . several functional enzymes are produced using industrial fermentation of *Bacillus subtilis*, for example , protease and lipase (2) , Catalase and peroxidase (3) . *Bacillus subtilis* is cultured for the industrial production of many substances . Various strains of *Bacillus subtilis* are used in the industrial preparation of Mycosubtilin (4) , Bacillomycin D (5) , and Bacillysoin (6) .

significantly, peroxidases are broadly near in both gram positive and gram negative bacteria . In bacteria peroxidases are originate as intracellular enzymes and seemed to play vital roles in defense against H_2O_2 induced cell harm (7). Peroxidases are confidential as oxidoreductases and they are known the administrator EC number 1.11.1.7, in peroxidase enzyme the jump cofactor necessary for its activity is haem. Haem is a difficult between an iron ion and protoporphyrin molecules. peroxidase

catalyzes the move of oxygen from the hydrogen peroxide to an appropriate substrate and thus brings about oxidation of the substrate. Peroxidase employ H_2O_2 as electron acceptor for catalyzing different oxidative reactions (8) . Peroxidases are commonly establish in all over the plants, animals and microorganisms, symptomatic of their key role in biological systems. It oxidizes a huge amount of little molecular weight fragrant compounds similar to phenols, flavonoids, benzidine derivatives, and macromolecules of lignin or humic substances. Peroxidase has involved engineering consideration for the reason that of its efficacy as a catalyst in the crush and document, cloth and laundry industries, intended for the rotting of pollutants, or for employ as biosensors and further applications (9) . in favor of the peroxidase significance , the current work has been achived to peroxidase separation and optimization of route parameters to employment peroxidase greatest give way via *Bacillus subtilis.*

2 - Materials and Methods :

2- 1 : Samples collection and separation of bacteria

Bacillus subtilis remote from diverse soils, the total soil samples were 15 and which collected from different places in Najaf city , the *Bacillus subtilis* culture was isolated from the soil by the serial dilution method , Serial dilutions were prepared up to 10^{-7} and extend over nutrient agar plates which were incubated for 24 hrs at 37°C , sub-cultured on sterile nutrient agar slants and stored at 4°C for additional use. recognition of bacterial strain , the strain was examined for colony morphology, Gram's staining, motility test, sporulation etc. , diverse physiological tests similar to catalase test, Glucose fermentation test, Voges- Proskauer test, hydrolysis of casein, hydrolysis of gelatine etc., were performed as standard methods (10).

2-2 : Inoculum preparation and isolation of peroxidase

Pure culture of recently isolated *Bacillus subtilis* was maintained on nutrient agar slants at 4 °C, the growth (synthetic) medium (Table 1**)** was autoclaved for 15 min , after cooling the flasks, the bacterial colonies were inoculated into nutrient broth with first pH 7.0 at temperature 37°C incubated for 48 hrs on shaking incubator ,fifty ml were taken of broth and transferred it into centrifuge tubes. They were centrifuged at 6000 rpm for 10 min . Supernatant having crude peroxidase was composed and the pellet was discarded. The supernatant was used for enzyme determination (11) .

Table 1. Growth (synthetic) medium compositions of of *Bacillus subtilis* (11).

S/N	compositions	amount (g/L)
1	$C_6H_{12}O_6$	10
2	$FeSO_4$	0.01
3	sodium nitrate	2
4	$MgSO_4$. 7H2O	1
5	K_2HPO_4	1
6	sodium chloride	1.8
7	Distilled water Up to	1000 ml mark

2 – 3 : Assay of peroxidase

Peroxidase activity was assayed spectrophotometrically according to. (12) .

2-4 : Determination of protein: Protein content was calculated according to (13) figure (1) by training of standard concentratiuon which were used in standard curve .

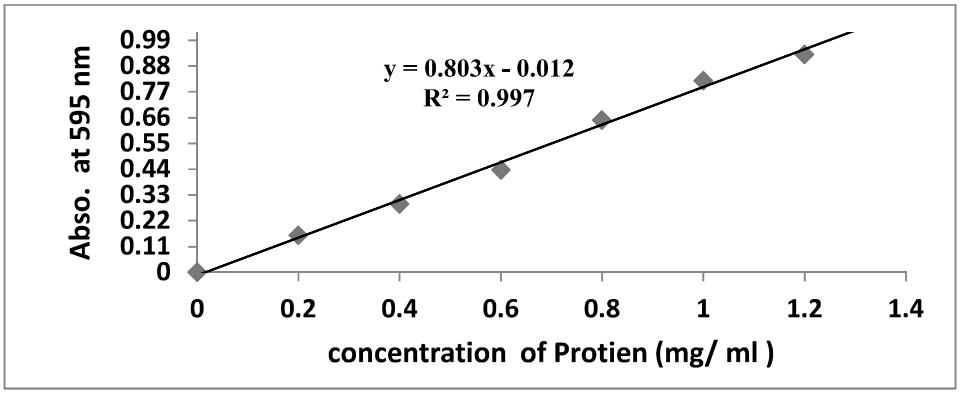

Figure (1) . The diverse concentration of bovine serum albumin at 595 nm for standard curve .

2-5 : Factors that influenced on creation of peroxidase (14) : they are many factors that involve on peroxidase production comprise culture media , temperature of incubation , values of pH , Periods of incubation , carbon and nitrogen sources .

3- Results and Discussion:

3-1 : greatest culture medium for peroxidase production :

The high creation of enzyme by *Bacillus subtilis* was remote from soil and growth in synthetic medium , it gave elevated average of peroxidase activity (9.261 U/ml) followed by tryptic soya broth with peptone (4.432 U/ml) while egg albumin gave small average of peroxidase activity (0.627 U/ml) . The choice of the appropriate fermentation medium is a vital feature for microbial development and enzyme creation , the growth of an organism in culture medium is inclined by the nutrient composition of the medium and the accessibility of these nutrients (15) . additional studies similar to (16) used the identical medium for production of peroxidase from *Bacillus* sp. , also (12) used the synthetic medium for production of peroxidase from *Bacillus* sp.

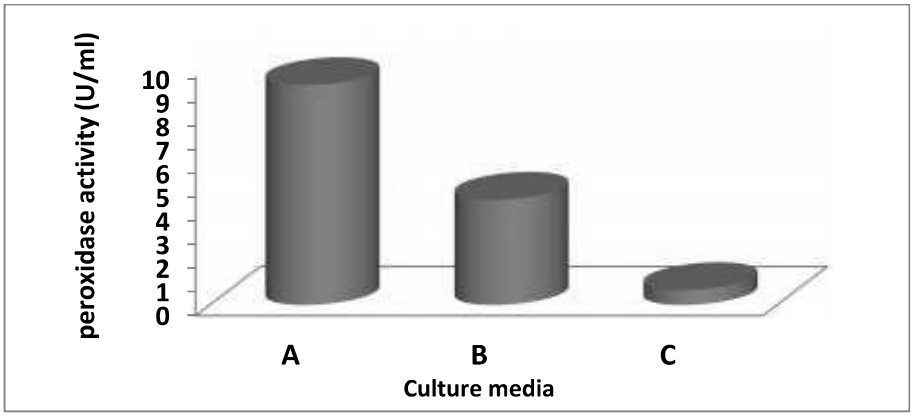

Figure (2) : Effect of culture media (A: synthetic medium, B: tryptic soya broth with peptone C: egg albumin) on the peroxidase production from *Bacillus subtilis* .

3-2 : Finest temperature for enzyme production:

The influence of heat on manufacture of the enzyme showed that peroxidase activity getting a highest in 30 °C (10.271 U/ml) higher than this temperature there was a lessening in the peroxidase activity (1.345 , 0.434 U/ml) in 50, 60 °C respectively (Figure 3) . Growth heat is a very significant factor which varies from organism to organism and minor changes in growth temperature may change enzyme creation (17) . Other results reported by (18) they publicized the main activity of peroxidase from for *Bacillus pumilus* and *Paenibacillus* sp. 25 ^{o}C and 35 ^{o}C respectively , also (19) shown the chief activity of peroxidase making by *Bacillus megaterium* 37 °C.

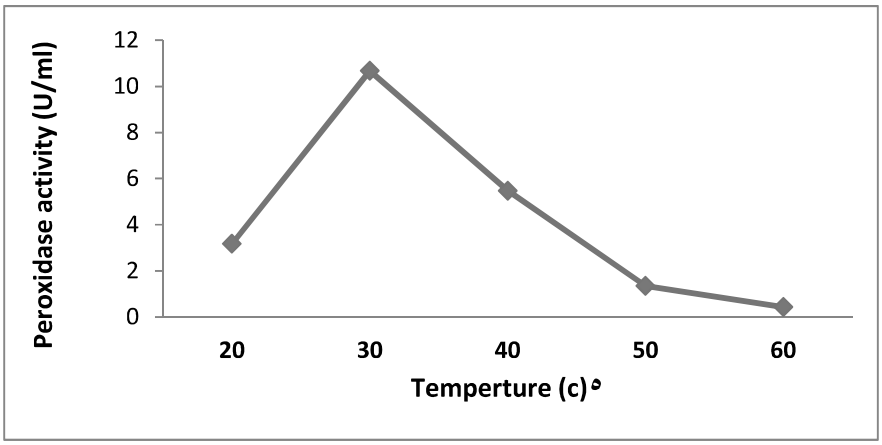

Figure (3) : Effect of incubation temperature on the production of peroxidase from *Bacillus subtilis*.

3-3 : Best incubation period for peroxidase creation:

The enzyme activity was improved with increasing the incubation period awaiting arrive at highest activity (11.533 U/ml) after 18 hrs of incubation , then it began to decreased (4.865 , 0.734U/ml) after 24,30 hrs of incubation respectively figure (4) . additional study similar to (20) when they shown the greatest activity of peroxidase from *Bacillus subtilis* LPTK achieved after 36 hrs of incubation .

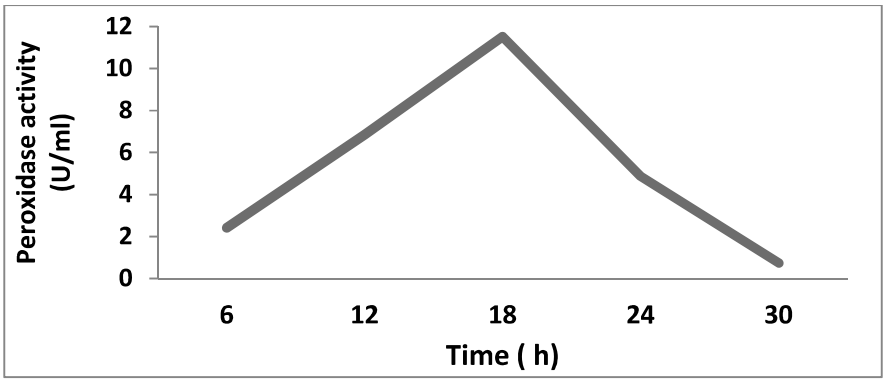

Figure (4) : Effect of incubation period on the peroxidase production from *Bacillus subtilis*.

3-4 : Best pH for enzyme creation: The rising of peroxidase activity with rising the pH until arrive at to greatest activity (12.225 U/ml) in pH = 7 , then it began to decreased in upper pH values (1.471, 0.373 U/ml) in pH= 8, 9 respectively figure (5) . best pH is very vital thing , the composition of plasma membrane of microorganism is famous to be artificial by the culture pH , the alteration in the first pH of the medium may

guide to alteration in the character of the cell membrane and thus disturbing the peroxidase creation and growth of organisms (21) . This work was decided with (19) when he shown the main activity of peroxidase from *Bacillus megaterium* occurred at the equal pH , whereas (11) establish the main activity of peroxidase from *Bacillus subtilis* occurred at the pH= 6 .

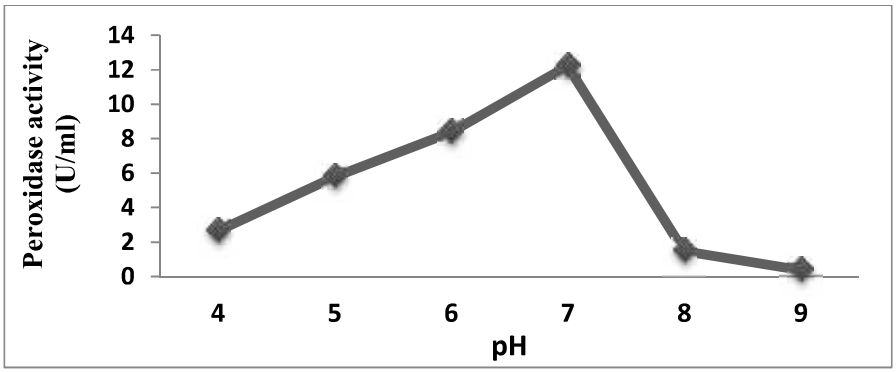

Figure (5) :Effect of pH on the creation of peroxidase from *Bacillus subtilis*.

3-5 : Best nitrogen source for peroxidase creation :

The over making of enzyme by *Bacillus subtilis* was occurred via the yeast extract as nitrogen source , it gave elevated titer of peroxidase activity (13.403 U/ml) followed by peptone nitrate (8.372 U/ml) , whereas the other sources (ammonium nitrate, ammonium chloride, and ammonium tartarate) gave small titer of peroxidase activity (3.824 , 1.419, 0.752 U/ml) respectively figure (6) . like results were also reported by (22) showed the highest activity of peroxidase from *Streptomyces albus* ATCC 3005 using the similar source , also (12) when they establish the main activity of peroxidase from *Bacillus* sp. occurred using the yeast extract as nitrogen source.

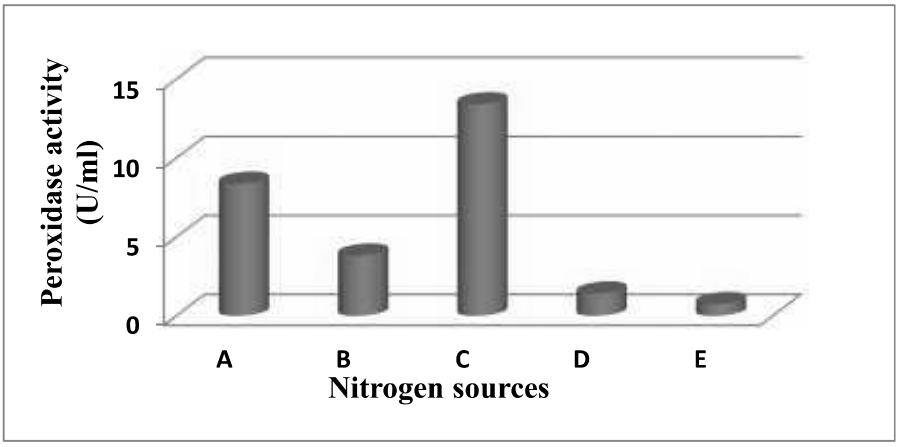

Figure (6) : Effect of nitrogen sources (A: peptone, B: ammonium nitrate, C: yeast extract, D: ammonium chloride, E: ammonium tartarate on the production of peroxidase from *Bacillus subtilis*.

3-6 : Best carbon source for enzyme creation: The best peroxidase manufacture by *Bacillus subtilis* was occurred using the lactose as the carbon source , it gave elevated titer of peroxidase activity (11.624U/ml) , followed by fructose (6.174 U/ml) , whereas the additional (glucose ,sucrose , mannitol) gave little titer of peroxidase activity (3.241, 2.109, 0.487U/ml) respectively figure (7). The peroxidase making by microbes is powerfully effected by the environment and concentration of carbon source payable the carbon source used in the synthesis of polysaccharide and as energy source (23) . This work was fixed with (14) when they shown the main activity of peroxidase from *Lentinus kauffmanii* achieved via the fructose as the carbon source, whereas (24) reported the main activity of peroxidase from *Grammothele fuligo* achieved via the xylose as the carbon source .

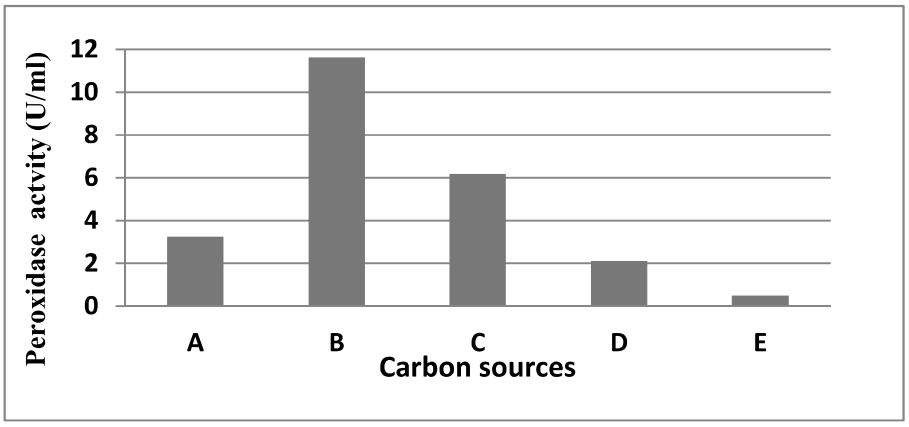

Figure (7) : Effect carbon sources (A: glucose , B: lactose , C: fructose , D: sucrose, E: mannitol) on the production of peroxidase from *Bacillus sutilis.*

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ظروف النمو المثالية لاقصى انتاج لانزيم البيروكسيديز من بكتريا *Bacillus subtilis*

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الخلاصة : تضمنت الدراسة التي اجريت في مختبرات قسم علوم الحياة – كلية العلوم- جامعة الكوفة عزل انزيم البيروكسيديز وتحديد المعايير المثالية للانتاج من بكتريا *Bacillus subtilis* المعزولة من التربة بطريقة التخمر وباستخدام بيروكسيد الهيدروجين كمادة اساس ، اعلى انتاج للانزيم كان باستخدام الوسط التركيبي واللاكتوز كمصدر كاربوني وخلاصة الخميرة كمصدر نتروجيني وعند درجة حرارة 30 م والاس الهيدروجيني 7 وبعد فترة حضانة 18 ساعة .

الكلمات المفتاحية : البيرو كسيدز , بكتريا *Bacillus subtilis*