Author's Accepted Manuscript

Catalase Enzyme: Application in Bioremediation and Food Industry

Jyoti Kaushal, Seema Gursharan Singh, Arun Raina, Shailendra Kumar Arya



PII:S1878-8181(18)30284-6DOI:https://doi.org/10.1016/j.bcab.2018.07.035Reference:BCAB833

To appear in: Biocatalysis and Agricultural Biotechnology

Received date: 11 April 2018 Revised date: 31 July 2018 Accepted date: 31 July 2018

Cite this article as: Jyoti Kaushal, Seema Gursharan Singh, Arun Raina and Shailendra Kumar Arya, Catalase Enzyme: Application in Bioremediation and Food Industry, *Biocatalysis and Agricultural Biotechnology*, https://doi.org/10.1016/j.bcab.2018.07.035

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Catalase Enzyme: Application in Bioremediation and Food Industry

Jyoti Kaushal, Seema, Gursharan Singh, Arun Raina, Shailendra Kumar Arya^{*}. University Institute of Engineering and Technology, Panjab University, Chandigarh, INDIA

*skarya_kr@yahoo.co.in

Abstract

The enzyme catalase is known to catalyse the breakdown of hydrogen peroxide into oxygen and water. Hydrogen peroxide metabolism is mainly regulated by this enzyme. Catalase is a common enzyme found in nearly all living organisms. It has one of the highest turnovers of all enzymes as it has the capacity to decompose more than one million molecules of hydrogen peroxide, per molecule of enzyme. Catalase has been used as an important enzyme in many biotechnological areas including bioremediation. This paper gives a review of its use and application in the field of bioremediation as an indicator of hydrocarbon degradation in soil (an important aspect in bioremediation of crude oil pollution), as a provider of oxygen in aerobic bioremediation process and in the removal of H_2O_2 from bleaching industry effluent and also its potential use in the food industry.

Keywords: Catalase; Hydrogen peroxide; Bioremediation; Food.

Introduction

Catalase (EC 1.11.1.6) enzyme is an oxidoreductase enzyme as it plays a crucial role in quenching the reactive oxygen species (ROS), i.e. hydrogen peroxide, often produced as a by-product of aerobic respiration (Beers and Sizer., 1952). Hence it acts as an antioxidant and protects the cell against oxidative stress (Abbott et al., 2009, Kirkman et al., 1987). The enzyme is found in a wide range of aerobic and anaerobic organisms. Catalase has one of the highest turnover number as one molecule of enzyme hydrolysing over a million molecules of the substrate i.e. hydrogen peroxide per second. New applications for catalases are constantly emerging thanks to their high turnover number (Lončar and Fraaije 2015, Zamocky et al., 1999), distinct evolutionary origin, reasonably simple (Alptekin et al. 2008) and well-defined reaction mechanisms (Baeza et al., 2013).

The basic mechanism of the working of this enzyme involves the breakdown and subsequent breakdown of the reactive oxygen specie i.e. hydrogen peroxide (H_2O_2) into oxygen and water thus relieving the oxidative stress caused by this substrate as depicted in the following reaction (Barynin et al., 2001).

$2 H_2O_2$	Catalase	2 H ₂ O +	O_2
(Substrate)	(Enzyme)	(Water)	(Oxygen)

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. Catalase has a fairly broad range of working from 4-11 optimum pH (Beers, 1961; Calera et al., 2000; Correia et al., 1994; Diaz et al., 2012). Catalase is usually located in a cellular, bipolar environment organelle called the peroxisome (Fita et al., 1985). As this enzyme is found in mainly all organisms (aerobic and anaerobic), it has been exploited in many applications including food processing, textile, paper, pharmaceutical industry and also in the field of bioremediation as one of the upcoming areas of its application (Gromada et al., 1997; Hussein 2012; . Beers et al., 1952; Youn et al 1995). This paper intends to give an account of how this enzyme has been exploited in the process of bioremediation in order to clean the environment of unwanted substances.

The quality of the life on earth has been linked intricately to the overall quality of the environment. Due to the advancement of technology, living standards and industrialisation many substances with toxic properties have been brought into the environment. The removal of these toxic substances from our environment has been a topic of concern over a long period of time so as to eliminate the hazardous effect of these substances (Vidali, 2001). A process of bioremediation invented by George M. Robinson (Sonawdekar, 2012) is a relatively low-cost, low-technology technique that uses natural biological processes/ activity to degrade, transform, and/or essentially remove contaminants or impairments of quality from soil and water (Dana et al., 2011). Many enzymes of microbial origin have been used as an indicator of hydrocarbon degradation in soil which includes lipases, catalases and dehydrogenase. In this paper the role of the enzyme catalase has been established as an indicator for testing hydrocarbon degradation in soil by monitoring the changes in its activity during the bioremediation of crude oil polluted soil.

Moreover as a result of expansion of the enzyme industry, the application of various enzymes in the food industry have been exploited recently including the use of catalase in various processes such as in determining the quality of milk, in packaging of food products, in production of cheese etc. A number of other applications of catalase enzyme in the industry are presented in the figure 1.



Fig.1: Applications of catalase enzyme

Bioremediation Technology

Ever since the advancement of the society and scientific technologies, one of the main concerns has been laid on the effect on the environment caused by the rapid industrialisation and human activities that pollute the environment by waste disposal into soil or water bodies and cause its degradation (Vidal, 2001). The conventional ways of waste disposal involved the dumping of the wastes into land pits or into water bodies causing environmental degradation and stress (Sulaimani et al., 2011). An alternative form of waste management technique i.e. bioremediation has been explored extensively lately due to its ease of employment and environment friendly technology (Dua et al., 2002).

Bioremediation is a waste management technique that involves the use of living organisms to remove or nullify pollutants from a contaminated site. It is a treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances (Dana et al., 2011). The employability of various organisms like bacteria, fungi, algae, and plants for efficient bioremediation of pollutants has been reported. The involvement of plants in the bioremediation of pollutants is called as phytoremediation (Hammel et al., 1997, Lehninger et al., 2004; Arora et al., 2010; Gianfreda et al., 1999; Ayotamuno et al., Williams, 2006; Park et al., 2006; Williams, 1997; Husain, 2006; Rubilar et al., 2008).

Bioremediation is a cost effective and nature friendly biotechnology that is powered by microbial enzymes. The research activity in this area would contribute towards developing advanced bioprocess technology to reduce the toxicity of the pollutants and also to obtain novel useful substances (Duran et al., 2000). The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to innocuous products (Newman et al., 1998, Leisola et al., 2006). As bioremediation can be operative only where environmental conditions permit microbial activity and growth, its application often involves manipulation of the environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Chacon et al, 2009). The following figure 2, gives the basic idea of the bioremediation process.



Fig. 2: Simple bioremediation process

Therefore due to the simplicity and the environment friendly technique of bioremediation, this technology has gained enormous amount of appreciation and has been exploited for the waste management extensively (Allard et al., 1997). The enzyme catalase of microbial origin has been employed in various aspects of bioremediation where it has been known to act as an indicator of the hydrocarbon degradation of the crude oil polluted soil (Joergensen et al., 1995), in removal of the reactive oxygen specie hydrogen peroxide from the effluents of the textile industries, and also by providing oxygen in aerobic bioremediation process where it is used to give out oxygen by the breakdown of hydrogen peroxide (Kabana ,1982; Rila ,2008; Kabana, 1970; Margesin et al.,1997; Morgan et al.,1989; Achuba et al., 2014). All these applications of catalase enzyme are discussed further in this review.

Catalase as an indicator of Hydrocarbon degradation

Crude oil pollution has been an issue of concern as it pollutes the soil and water attenuating their quality. Crude oil is a naturally occurring mixture consisting predominantly of complex combination of hydrocarbons with other elements such as sulphur, nitrogen and oxygen appearing in the form of organic compounds (Achuba et al., 2014). It is a mixture of many different kinds of organic compounds, many of which are highly toxic and cancer causing (carcinogenic). They are formed by a chain of tens of thousands of different hydrocarbon molecules including the straight and branched – chain alkanes (paraffinic), cycloalkanes or cycloparaffins (naphthenic), aromatic hydrocarbons , and alkenes having a carbon number ranging from as small as four to as larger molecules having more than fifty carbons. Pollution arising from crude oil is a recurrent anomaly (Atlas et al., 1992). The area of oil-polluted soil was enlarged continuously due to development of petroleum industry (Frankenberger et al., 1982).

Crude oil contamination significantly increases the soil pH up to 8.0, and reduces available phosphorus concentrations in the soil. Therefore, crude oil contamination could potentially alkalinize marsh soils, adversely affect soil fertility and physical properties, and cause deterioration of its quality (Alinnor et al., 2013). The major causes of the crude oil pollution includes the oil spills, waste disposal, effluent disposal, gas flares etc. The soils polluted by the crude oil the hydrocarbons will absorb into the soil matrix which can be attacked by the microbial population residing in the soil. The oil-degrading microorganisms are widely distributed in the soils, which played an important role in remediating the polluted soils (Guwy et al., 1999). It is remarkably effective to treat the groundwater and soil polluted by tar and crude oil with the aid of aboriginal microorganisms. The crude oil permeated into soil by varying ways and interacted with soil enzymes. On one hand, the enzymes from oildegrading microorganisms could be as an indicator for assaying the amount and physiochemical property of crude oil. This technology is used mainly for the bioremediation of the soil either by the natural microbial species present in the soil or by artificially adding the desired microorganisms (bioaugmentation) that can degrade the hydrocarbons (Kimborough et al., 1997; Vainshtein et al., 198; Witteveen et al., 1992; Schellhorn, 1994; Alef et al., 1995, Pascual et al., 1998; Aebi 1974; Erdogan et al., 2011).

Many enzymes including microbial lipase, catalase and dehydrogenase have been used an indicator of the hydrocarbon degradation in the course of bioremediation (Karigar et al., 2011), where the activities of these enzymes before and after the bioremediation procedure is used as a parameter of the extent of bioremediation taking place in the particular area. Thus, the aim of that study is to monitor the changes in the activities of soil microbial catalase during the bioremediation of a crude oil polluted soil relative to bioremediation time. In one of the studies conducted on the crude oil polluted soil, the activity of the enzyme catalase present in the soil due to the catalase producing microorganisms was monitored. A control was established which contained the soil that was free of oil contamination and the catalase activity of this soil was used as a marker of normal enzyme activity. The soils polluted with 100, 200 and 400 ml crude oil were used as a test for the catalase activity. It was observed that there was a considerable decrease in the activity of the catalase enzyme in the soils that where polluted with crude oil and as the bioremediation procedure was carried out with the help of bacteria's such as arthrobactor species, there was a resurgence in the enzyme activity as the procedure progressed from 0 to 90 days (Ogbolosingha et al., 2015). This finding corroborates that of Achuba and Okoh (Achuba and Okoh, 2014) which create that there was

transformed of soil catalase activity when petroleum products were added to the soil, though, its activity amplified few days later which they predicated on the increased microbial activity towards biodegradation of available petroleum hydrocarbon (Seo et al., 2006). These activities place the enzyme catalase in a perfect stead as an indicator of the onset of the biodegradation process and therefore as an excellent marker for bioremediation (Ajao et al., 2011). Achuba and Peretiemo-Clarke also asserted that the initial reduction of catalase activity could be because being an enzyme its activity is altered by unfavourable conditions (Achuba et. al., 2008), such as hypoxia, unavailability of nutrient and changes in pH. This finding place catalase in a good stead as a useful biomarker for indicating the onset of the biodegradation process as their activities decline after the rate of biodegradation has decreased.

In yet an another study conducted with the *rhodococcus* strain, the impact of crude oil on the activity of the enzyme catalase from this strain was studied continuously for 120 days (Leilei et al., 2012). The study showed a decrease in the enzyme activity initially and as the bioremediation procedure progressed near the end days, there was restored catalase activity of the strain indicating the success of the bioremediation as now the enzyme was available from the Rhodococcus strain that was used for the bioremediation of the polluted soil (Leilei ; Azaizeh et al., 2011; Kariga 2011; Seo et al., 2006).



Fig. 3: Graph depicting catalase activity variation during bioremediation

The graph (figure 3) shows the variation in the catalase enzyme activity as the process of bioremediation progresses from day zero where a decrease in the enzyme activity can be observed in the polluted soil as compared to the remediated soil where the soil was remediated with the *rhodococcus* strain providing the catalase enzyme that was used for the degradation of the petroleum substrates due to crude oil pollution of the soil .The blue orange and grey colours in the graph represent the unpolluted, unremediated ,and remediated soil respectively. The catalase activity in soil could have modulated by many factors, such as microorganism, temperature, nutrients, oxygen, and moisture. In the present study was presence of petroleum hydrocarbon in the soil is acted both as a nutrient as well as oxygen limiting agent. Therefore the *rhodococcus* strain used in this study could have up regulated the catalase activity to provide more of free oxygen for utilization of petroleum hydrocarbon by the bacteria (Sharma et al., 2014).

Catalase as oxygen provider in aerobic bioremediation

Bioremediation involves the use of microorganisms for the breakdown of the wastes so as to provide an environmental friendly method of waste management. Microorganisms can be aerobic or anaerobic in nature. Aerobic microorganisms are those that require the provision of oxygen for their microbial activity (Margesin et al., 2000). The availability of oxygen is one of the main considerations in the aerobic bioremediation of the wastes. In the presence of aerobic conditions and appropriate nutrients, microorganisms can convert many organic contaminants to carbon dioxide, water, and microbial cell mass. Aerobic bioremediation uses oxygen as the electron acceptor. Aerobic metabolism is more commonly exploited and can be effective for hydrocarbons and other organic compounds, such as petroleum hydrocarbons and some fuel oxygenates (e.g., methyl tertiary-butyl ether [MTBE]) (Sutton et al., 2011; Brown et al., 1994).

Hydrogen peroxide can be used as one of the substrates that can provide the required oxygen demand of the microorganisms involved in the bioremediation (Zappi et al., 2000). The breakdown of H_2O_2 into oxygen and water gives out the oxygen required by aerobic microorganisms for the breakdown of organic wastes being treated. Hydrogen peroxide was used to supply oxygen for enhanced the bioremediation process and tolerance of the system to assessed (Brown et al., 1994; Zappi et al., 2000). Therefore catalase is one of the main enzymes that can cause the degradation of hydrogen peroxide into oxygen and water as

mentioned earlier. Since catalase is an enzyme that is most readily present in almost all the aerobic and anaerobic microorganisms, it can be used in the decomposition of the substrate hydrogen peroxide into oxygen and water thus giving the required oxygen supply in the process of bioremediation. According to the laboratory tests that were run, hydrogen peroxide was shown to rapidly decompose and produce pure gaseous oxygen (Margesin et al., 2000).

The decomposition of the hydrogen peroxide was shown to be caused by the enzymatic catalysts. As reported by Nagaprasad and Madhu when Hydrogen peroxide injection was provided into the wastes requiring the use oxygen for their decomposition by aerobic microorganisms, H_2O_2 decomposition took place by the action of the enzyme catalase present in the waste being bioremediated (Nagaprasad and Madhu, 2012).

.Catalase as discussed earlier has one of the highest turnover of all enzymes as it has the capacity to decompose more than one million molecules of hydrogen peroxide, per molecule of enzyme. It therefore rapidly decomposes the injected hydrogen peroxide into gaseous oxygen and water providing oxygen for the bioremediation by the aerobic microorganisms.

Catalase in treating bleaching effluents from textile industry

Catalase was also used for the removal of hydrogen peroxide from bleaching effluents of textile industries because of the stringent environmental impact of this reactive oxygen species. Catalase moreover has been used in the process of bioremediation where hydrogen peroxide (H₂O₂) is used as a source of oxygen in aerobic conditions as it leads to breakdown of H_2O_2 into water and oxygen. The effluents from the textile industries contain a high amount of hydrogen peroxide due to the bleaching process used in these industries. Particularly, textile bleaching is a water intensive process and thus, several methods have been suggested to degrade the bleaching agent hydrogen peroxide, which would allow recycling of the bleaching effluent in the dyeing process. Water consumption for processing of the textile raw material varies with different textiles like Lyocell fibre and cotton. Lyocell fibre fiber production stage is dependable for the lowest water consumption than cotton fibers. Lyocell fiber required on average 1384, 34.5, and 35.3 m³/t water as compared to 263, 2767, and 203 m³/t water for cotton (Chico et al., 2013, Amorim et al., 2002). Moreover it is now an important step to get rid of this reactive oxygen specie due to the problems of process costs and pollution of residual waters of the textile industry requiring increased attention due to the new ecological regulations and from an economic point of view (Dickinson et al., 1984).

The use of chemicals such as hydrosulphite or sodium bisulphite for the reduction of H_2O_2 can lead to unwanted raise in the salt concentration during the process. Alternatively Catalases can be used to degrade H_2O_2 (Vasudevan et al., 1994) so as to decrease water consumption or to recycle the washing liquor for dyeing. Catalase form the microorganisms can be used to bio-remediate the effluents from textile industry by decomposing the hydrogen peroxide into oxygen and water.

Catalases has been studied and its application has been exploited for a longer than any other type of enzyme with the first biochemical characterization reported almost 100 years ago (May D.W., 1901, Tzanov et al., 2001). Catalase was one of the first enzymes isolated in a high state of purity, and its crystallization from beef liver extracts ranked among the early triumphs of biochemistry. The use of catalase in treating the effluents of textile industry requires the exploitation of the alkalithermostable catalase from microorganisms so that the enzyme can withstand high PH values and temperature above 60^oc. There is very little known about catalases from alkalithermophilic micro-organisms, although enormous progress has been made over the last few years in the research area of extremophiles (Krulwich et al., 1989; Krulwich et al., 1997). Some thermoalkalophilic Bacilli are known, however, alkalithermophiles seem to be more abundant among anaerobic organisms (Wiegel, 1989).

Although catalase is one of the most effective biocatalysts in terms of turn-over number, cost of enzyme for the degradation of H_2O_2 of bleaching effluents could be reduced by immobilization of the enzyme. Many industrial processes use immobilized enzymes and catalases have been immobilized on numerous carrier materials such as alumina, gelatine, polyacrylamide and hen egg shell, artificial membranes, and carbon materials. Alumina pellets is one of the frequently used carrier material for catalases due to their stability at high pH and temperatures. In one of the studies, catalases from three newly isolated thermoalkophilic Bacillus sp. were immobilized on alumina pellets for the treatment of bleaching effluents. The reuse of the treated bleaching effluent for subsequent dyeing lead to savings in overall water consumption of up to 50%.

A catalase peroxidase (CP) from the newly isolated Bacillus SF was also used to treat textilebleaching effluents (Gudelj et al., 2001, Zhenxiao Yu et al., 2016). The enzyme was high stable at high pH 6-10 and temperatures at 50 °C. The enzyme was immobilised on various alumina-based carrier materials with different shapes and the specific activity increased with the porosity of the carrier. The shape of the carrier had an important influence on the release

of oxygen formed during the catalase reaction from the packed-bed reactor and Novalox saddles were found to be the most suitable shape. Bleaching effluent was treated in a horizontal packed-bed reactor containing 10 kg of the immobilised CP at a textile-finishing company. The treated liquid (500 L) was reused within the company for dyeing fabrics with various dyes.

The enzyme Catalase Peroxidase has also been immobilised by glutaraldehyde-coupling to the silanised support for this purpose. A horizontal column reactor (3.6 cm diameter, 5.11 cm long) was loaded with 60 g of the biocatalyst (immobilised enzyme). After rinsing the reactor system with sodium phosphate buffer (50 mM, pH 7.0) until a constant A₂₄₀ was reached, this value was set to zero. The maximum Absorbance at 240 nm was determined via a bypass and then the measurement was started by pumping the hydrogen peroxide solution through the reactor system. Conversion was calculated from the difference in Absorbance at 240 nm and at the end of the reactor system. Hence catalase peroxidase is one of the main enzymes used in cleaning of textile effluents. Catalase has therefore provided an alternate way of bioremediating the effluents and making it possible to reuse the bleaching effluent in the subsequent dying steps that are involved in the textile industries (Paar et al., 2001; Allgood et al., 1986; Aebi ,1983; Fruhwirth et al., 2002; Chatterjee et al., 2002; Horst et al., 2006). The following table summarises various sources of catalase used in the process of bioremediation.

Bacterial strain	Catalase activity	Bioremediation process	References
Arthrobacter species	Catalase positive	Indicator of hydrocarbon degradation in soil bioremediation	Ogbolosingha et al., 2015
Rhodococcus strain	Catalase positive	remediation of the polluted crude oil	Leilei et al., 2012
Bacillus SF	Thermo alkali stable catalase	treatment and recycling of textile bleaching effluents	Paar et al., 2001
Bacillus SF	immobilised catalase peroxidase	treatment and recycling of textile bleaching effluents	Fruhwirth et al., 2002
<i>Escherichia coli</i> and <i>Saccharomyces</i> <i>cerevisiae</i> ,	Aerobic catalase producing microorganisms	aerobic bioremediation	Sutton et al., 2011

Table: So	urces of cata	lase for variou	s bioremediation	processes

Catalase in food industry

The enzyme industry as we all knows it today is the result of rapid development seen mainly over the past four decades thanks to the evolution of the modern biotechnology. Enzymes found in nature have been used since in the production of food products, such as cheese, sourdough, beer, wine and vinegar, and in many other food processing operations. All of these processes depend on either the enzymes produced by microorganisms or enzymes present in added preparations such as calves' rumen or papaya fruit. As a result the application of various enzymes in the food industry have been exploited recently including the use of catalase in various processes such as in determining the quality of milk, in packaging of food products, in production of cheese etc.

Catalase in production of cheese

The enzyme Catalase has been identified to be of use in one particular area of cheese production. Hydrogen peroxide (H_2O_2) is a potent oxidizer and toxic to cells. It is used instead of pasteurization, when making certain cheeses such as Swiss, in order to preserve the natural milk enzymes that are beneficial to the end product i.e. cheese and subsequent flavour development in the processed cheese. These enzymes would be destroyed by the high heat of pasteurization which may be as high as 140 $^{\circ}$ C. This process is referred to as cold pasteurization .The use of H_2O_2 for the purpose of cold pasteurization is legitimate and used for the production of cheese milk as permitted by the food and drug administration (FDA).

However, residues of hydrogen peroxide in the milk can hinder the bacterial cultures that are required for the actual cheese production process, so all traces of H_2O_2 must be removed. Catalase enzymes are typically obtained from bovine livers or microbial sources and are added to convert the hydrogen peroxide to water and molecular oxygen. In this way the enzyme catalase is used in order to get rid of the reactive oxygen species i.e. hydrogen peroxide from the milk after cold pasteurization (Geciova et al., 2002). The use of catalase has proven to be a very cost efficient and also a comparatively environmental friendly method of getting rid of excess hydrogen peroxide from milk samples for cheese production (Kilcawley et al., 1989). The process is depicted in the following figure 4.



Fig. 4: Catalase use in cheese production

Catalase in food wrappers

Active packaging is one of the most innovative food packaging perceptions that have been introduced in response to the continuous changes in the current consumer demands and market trends that are favourable. Active packaging or smart packaging has not only been employed in packaging of the food products but also in pharmaceutical and many other products requiring a larger shelf life.

Active packaging has been employed in protection of food and other products from moisture, metal chelation, temperature, oxidation, atmospheric degradation etc. For in package situation the enzyme may be added directly to the product to effect a reaction or may be incorporated into package structure. To function within a package material the enzyme must be immobilized. Immobilization of an enzyme or placing it in a static position where it may function for an indefinite period may be accomplished by making the enzyme an integral part of the packaging material. Recently, enzymes have been employed in the packaging materials for more cost effective and easier eco friendly purposes. Commercially available enzymes can be easily used for this purpose rendering the process of active packaging a promising way out for perishable products.

Major active packaging techniques are concerned with substances that absorb oxygen in order to prevent food products from being oxidized (Vermeiren et al., 1999). In many cases, food deterioration is caused by oxidation of food constituents or spoilage by moulds in the presence of O_2 . Glucose oxidase – catalase based food wrappers or packaging materials can be employed in the oxygen scavenging technique. Glucose oxidase is a highly specific enzyme, from the fungi *Aspergillus niger* and *Penicillium*, which catalyses the oxidation of glucose by transferring two hydrogen's from the CHOH group of glucose to O_2 with the formation of to glucono-1, 5 - lactone (which spontaneously hydrolyses non-enzymically to gluconic acid using molecular oxygen) and releasing hydrogen peroxide. Thus the oxygen is used in this reaction and is removed from subsequent reaction. Since hydrogen peroxide being one of the most dangerous by product of this reaction, catalase enzyme is used along with glucose oxidase in food wrappers for the protection of food products from reactive oxygen specie i.e. hydrogen peroxide (Grigoras A. G., 2017, Nikola L., 2015, Floros et al., 1997; Day 1998).

Catalase-glucose oxidase system in food industry

Catalase has been used along with glucose oxidase in many applications of food industry. The enzyme system has been used to demonstrate the antioxidant effect of the combined enzyme mixture in food products like mayonnaises and in the removal of glucose from the egg whites before drying for use in the baking industry. A mixture of the enzymes is used (165 U kg⁻¹) together with additional hydrogen peroxide (about 0.1 % (w/w)) to ensure that sufficient molecular oxygen is available, by catalase action, to oxidise the glucose. It therefore aids in the removal of glucose from egg whites by providing oxygen for oxidation of glucose by breaking down its substrate i.e. hydrogen peroxide into oxygen and water (Scott et al.,1953).

It was observed according to the literature, that the antioxidative effect of the GOW/CAT enzyme system depends on the concentration of the fish oil in the mayonnaises i.e on the vulnerability to oxygen. It was observed that the mayonnaises with soybean oil and enzyme system did not show any oxidation when stored for 12 weeks. Moreover it was observed that the increase in the concentration of enzymes from 400 to 800 U/kg had no substantial effect on the antioxidant effect of the enzymes made with the soybean oil, but a light decrease in the mayonnaises containing 200 g/kg fish oil and a more significant effect on fish oil mayonnaises having 400 g/kg fish oil (Isaksen et al., 1997).

These results suggested that oxygen competition between two parallel reactions that is enzymatic removal of oxygen and lipid oxidation influences the antioxidative efficiency of the enzyme system GOX/CAT. Furthermore the formation of the volatile oxidation products also further indicate the potential antioxidative effect of the enzyme system .It was demonstrated in the literature that this enzyme system was able to retard the lipid oxidation in mayonnaises when stored between the temperatures of 5 °C and 25 °C (Loliger 1989; Frankel 1993).

According to the literature, the GOX/CAT enzyme system has also been used to control the browning of the fruit purees, which has always remained a challenge for the fruit processing industry. The commonly used techniques used involve the use of chemical antioxidants for example ascorbic acid and sulphites, and also the use of high temperature (Parpinello et al., 2002). These additives have however shown to cause an adverse effect on the health of the consumers making the use of these chemical antioxidants questionable. The enzyme system GOX-CAT, however provides an alternative, acting as natural antioxidant option for protection of purees from browning. It was observed that the treatment of fruit purees with GOX/CAT enzyme system removed 99% of dissolved oxygen responsible for browning within 120s (Amiot et al., 1992; Ashie et al., 1996).

Catalase test for mastitis to determine milk quality

Infectious Bovine Mastitis is one of the major diseases plaguing the livestock industry today. It renders the milk obtained from the cow altered in its constituency. The catalase test has been used as an indicator of the milk quality to separate the infected milk from the cow from the normal healthy milk. The Lind apparatus for catalase determination was developed by Orla (Orla-Jensen. S., 1931). It consists of a 20 ml. test tube with a one-holed rubber stopper with a piece of S-shaped glass tubing inserted in it (Fig. 5). It has been reported to be used for catalase Mastitis test. The test consists of placing 15 ml of the milk to be tested in the tube and adding 5 ml H₂O along with the milk. Catalase in the milk breaks down the substrate i.e. hydrogen peroxide into oxygen and water. The amount of oxygen collected acts as an indicator of the quality of the milk as amount above 1.5 to 2.5 is considered to be abnormal. The catalase test rarely misses a case of infectious mastitis unless the infection is almost completely walled off. (Martin, 1979; Maddy, 1954).



Fig. 5: The Lind apparatus

Conclusion

The role of catalase enzyme in various aspects of bioremediation has been demonstrated by this review paper. Catalase has proven to be one of the most abundant and easily available enzymes of the microbial origin that can effectively act as an indicator of bioremediation step involved in the remediation of the crude oil polluted soil as its activity is highly affected by hydrocarbon pollution. It has also shown to provide oxygen by breaking down of hydrogen peroxide into water and oxygen during the aerobic bioremediation of wastes thus acting as a source of oxygen for the aerobic microorganisms. Its application in the removal of hydrogen peroxide from the bleaching effluents and making it possible for the water to be reused again for subsequent dying steps has been one of the main use this enzyme in bioremediation of waste effluents from textile industries. There is very little known about catalases from alkalithermophilic micro-organisms, although enormous progress has been made over the last few years in the research area of extremophiles. This class of catalase can solve many problems involved in the use of catalase by increasing its tolerance towards high temperature (above 60 0 C) and high PH.

Its use in the food industry has been established in various field that include production of cheese, removal of glucose from egg whites for bakery purposes, as an antioxidant enzyme system along with glucose oxidase, in food wrappers and in checking milk quality. It therefore proves to be of use in many food processing areas. This enzyme has been studied

since almost 100 yrs. however, research on catalases is still going on and this class of enzymes continues to surprise us.

Conflict of interest

Authors have no conflict of interest.

References

- Abbott D.A., Suir E, Duong G.H., Hulster E.D., Pronk, J,T, Maris. A.J., "Catalase overexpression reduces lactic acid induced oxidative stress in Saccharomyces cerevisiae", Appl Environ Microbiol 2009;75(8):2320–25.
- Achuba F.I and Okoh P.N., "Effect of Petroleum Products on Soil Catalase and Dehydrogenase Activities", Open Journal of Soil Science 2014, volume 4, 399-406.
- Achuba F.I and Peretiemo-Clarke B.O., "Effect of Spent Engine Oil on Soil Catalase and Dehydrogenase Activities", International Agrophysics 2008, volume 22, 1-4.
- Aebi , H.E. , "Catalase. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis" , Weinheim: Verlag Chemie (1983), Vol. 3. 273 286.
- Aebi H., "Methods of enzymatic analysis", London: Academic Press, 1974. p. 673– 84.
- Ajao, A.T., Oluwajobi, A.O., Olatayo and V.S., "Bioremediation of Soil Microcosms from Auto-Mechanic Workshops" J. Appl. Sci. Environ. Manage 2011, volume 15 (3), 473 -477.
- Alef K, Nannipieri P., "Methods in applied soil microbiology and biochemistry" London: Academic Press, 1995. p. 362–3.
- AlinnorI.J and Nwachukwu M.A., "Determination of total petroleum hydrocarbon in soil and groundwater samples in some communities in Rivers State, Nigeria" Journal of environmental chemistry and ecotoxicology 2013, volume 5(11), 292-297.
- Allard Ann-Sofie, Alasdair H Neilson, "Bioremediation of organic waste sites: a critical review of microbiological aspects", Int. Biodeterioration Biodegradation 1997, volume 39, 253-285.

- Allgood G.S., Perry J.J, Characterization of a manganese-containing catalase from the obligate thermophile Thermoleophilum album", J. Bacteriol 1986, volume 168 (2), 563–567.
- Alptekin O., Tukel S.S., Yildirim D. "Immobilization and characterization of bovine liver catalase on eggshell", J Serb Chem Soc 2008; volume 73(6):609–18.
- Amiot M.J., Tacchini, S M. Aubert And Nicolas J., "Phenolic composition and browning susceptibility of various apple cultivars at maturity". Journal of Food Science 1992, 57, 958–962.
- Amorim A.M., Gasques M.D. G., Andreaus J. and Scharf M., "The application of catalase for the elimination of hydrogen peroxide residues after bleaching of cotton fabrics", Anais da Academia Brasileira de Ciências (2002), 74(3): 433-436.
- Arora P. K., Srivastava A., and . Singh V. P, "Application of Monooxygenases in dehalogenation, desulphurization, denitrification and hydroxylation of aromatic compounds," Journal of Bioremediation & Biodegradation 2010, vol. 1, pp. 1–8.
- Ashie I.N.,. Simpson B.K And Smith J.P., "Mechanism for controlling enzymatic reactions in foods", Critical Review of Food Science and Nutrition 1996, 36, 1–30.
- Atlas, R.M., Bartha, R., "Hydrocarbon biodegradation and oil spill bioremediation", Adv. Microbial Ecol., 1992, 12, 287-338.
- Ayotamuno M.J., Kogbara R.B., Ogaji S.O.T., Probert S.D., "Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt. Niger", Appl. Energ 2006, 83: 1249–1257.
- Azaizeh H, Castro PML, Kidd P, "Biodegradation of Organic Xenobiotic Pollutants in the Rhizosphere", Org. Xenobiotics and Plants 2011, volume 8: 191-215.
- Baeza S., Vejar N., Gulppi M., Azocar M., Melo F., Monsalve A., "New evidence on the role of catalase in Escherichia coli mediated biocorrosion", Corros Sci 2013;volume 67:32–41.
- Barynin V.V, Whittaker M.M., Antonyuk S.V., Lamzin V.S., Harrison P.M., Artymiuk P.J., "Crystal structure of manganese catalase from Lactobacillus plantarum. Structure, 2001; 9:725–38.
- Beers R.F. (1961), "Method of recovering catalase from bacterial sources thereof", Indian Patent No. 76635.
- Beers R. F. Jr., and Sizer I. W, A Spectrophotometric method for measuring the breakdown of Hydrogen Peroxide by Catalase*, J. Biol. Chem. (1952), 195:133-140.

- Brown, R. A., & Norris, R. D., "The evolution of a technology: hydrogen peroxide in in situ bioremediation", Hydrocarbon Bioremediation 1994, 148-162.
- Calera J.A., Weatherby J.S, Medrano R.L , Leal F. , "Distinctive properties of the catalase B of Aspergillus nidulans", FEBS Lett 2000;475:117–20.
- Chacon N., Herrera I., Flores S., González J.A., Nassar J.M., "Chemical, physical, and biochemical soil properties and plant roots as affected by native and exotic plants in Neotropical arid zones", Biol. Fertil. Soils 2009, 45: 321-328.
- Chatterjee U., Kumar A, Sanwal G.G, "Goat liver catalase immobilized on various solid supports", J Ferment Bioeng 1990, volume 70:429–430.
- Chico. D., Aldaya, M.M., Garrido, A., 2013. A water footprint assessment of a pair of jeans: the influence of agricultural policies on the sustainability of consumer products. J. Clean. Prod. 57, 238-248. https://doi.org/10.1016/j.jclepro.2013.06. 001
- Correia V.M., Stephenson T., Judd S.J., "Characterization of textile wastewaters: a review", Environ Technol 1994;15:917–29.
- Dana L. D. and Bauder J. W., "A General Essay on Bioremediation of Contaminated Soil", Montana State University, Bozeman, Mont, USA, 2011.
- Day, B.P.F., "Active packaging of foods", CCFRA New Technologies Bulletin 1998, volume 17, 23pp.
- Diaz A., Loewen P.C., Fita I., Carpena X., "Thirty years of heme catalases structural biology", Arch Biochem Biophys 2012;525:102–10.
- Dickinson K., "Preparation and bleaching", Rev. Prog. Coloration 1984, volume 14: 1–7.
- Dua M., Singh A., Sethunathan N., and Johri A., "Biotechnology and bioremediation: successes and limitations," Applied Microbiology and Biotechnology 2002, vol. 59, no. 2-3, pp. 143–152.
- Duran N. and Esposito E., "Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review", Applied Catalysis B 2000, vol. 28, no. 2, pp. 83–99.
- Erdogan, E.E. and Karaca, A. "Bioremediation of crude oil polluted soil", Asian Journal of biotechnology, 2011, 3(3), 206-213.
 Fita I., Rossmann M.G., "The active center of catalase", J Mol Biol 1985; 185:21–27.
- Floros, J.D., Dock, L.L. and Han, J.H. , "Active packaging technologies and applications' in Food Cosmetics and Drug Packaging" 1997, 20, 10 -17.

- Frankel, E. N. "In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids", Trends in Food Science and Technology 1993, volume 4, 220–225.
- Frankenberger, W.T., Johanson, J.B., "Influence of crude oil and refined petroleum products on soil dehydrogenase activity", J. Environ. Qual, 1982, 11, 602-607.
- Fruhwirth G. O,. Paar A, Gudelj M.,. Cavaco-Paulo A,. Robra K.H, Gbitz G. M., "An immobilised catalase peroxidase from the alkalothermophilic Bacillus SF for the treatment of textile-bleaching effluents", Appl Microbiol Biotechnol (2002) 60:313–319.
- Geciova J.,.Bury D,Jelen P, "Methods for disruption of microbial cells for potential use in the dairy industry-a review" International Dairy Journal, 2002, Volume 12, Issue 6, Pages 541-553.
- Gianfreda L., Xu F., and Bollag J. M., "Laccases: a useful group of oxidoreductive enzymes," Bioremediation Journal 1999, vol. 3, no. 1, pp. 1–25.
- Grigoras A. G., Catalase immobilization A review, Biochemical Engineering Journal, 117, (2017), 1–20
- Gromada A., Fiedurek J., "Optimization of catalase biosynthesis in submerged cultures of Aspergillus niger mutant", J Basic Microbiol 1997;37(2):85–91.
- Gudelj M, Fruhwirth G.O., Paar A., Lottspeich F., Robra K.H, Cavaco-Paulo A.,. Gübitz G. M, "A catalase-peroxidase from a newly isolated thermoalkaliphilic Bacillus sp. with potential for the treatment of textile bleaching effluents",) Extremophiles (2001) 5:423–429.
- Guwy A.J, Martin S. R., F.R. Hawkes D.L. Hawkes, "Catalase activity measurements in suspended aerobic biomass and soil samples", Enzyme and Microbial Technology 25 (1999) ,669–676.
- Hammel K. E., "Fungal degradation of lignin, Driven by Nature: Plant Litter Quality and Decomposition", G. Cadisch and K. E. Giller Editors ,CAB International, Wallingford, UK 1997, pp. 33–45.
- Horst, F.,. Rueda E. H, and Ferreira M. L., "Activity of Magnetite Immobilized Catalase in Hydrogen Peroxide Decomposition", Enzyme and Microbial Technology 2006, volume 38(7): 1005–1012.
- Husain Q, "Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water: a review", Critical Reviews in Biotechnology 2006, vol. 26, no. 4, pp. 201–221.
- Hussein A.A, "Purification and characterization of thermo-alkali stable catalase from Bacillus sp", Int Res J Biotechnol 2012; 3(10):207–14.

- Isaksen A. and Adler-Nissen J., "Antioxidative Effect of Glucose Oxidase and Catalase in Mayonnaises of Different Oxidative Susceptibility". I. Product Trials, Lebensm.-Wiss. U.Technol. 1997, volume 30, 841–846.
- Joergensen R.G., Schmaedeke F., Windhorst K., Meyer B., "Biomass and activity of microorganisms in a fuel-oil contaminated soil", Soil Biol. Biochem 1995, volume 27, 1137-1143.
- Kabana R. R., Truelove B., "Effects of crop rotation and fertilization on the catalase activity in a soil of the south-eastern United States", Plant Soil 1982, volume 69, 97–104.
- Kabana R.R and Truelove B. , "The determination of soil catalase activity", Enzymologia 1970, volume 31, 217–236.
- Karigar C.S. and Rao S.S. " Role of microbial enzymes in the bioremediation of pollutants: A Review", Enzyme Research Volume 2011 (2011), Article ID 805187, 11 pages.
- Kilcawley K.N., Wilkinson M.G. and Fox P.F., "Enzyme-modified cheese", International Dairy Journal 1998, volume 8: 1-10.
- Kirkman HN, Galiano S, Gaetani GF. The function of catalase-bound NADPH. J Biol Chem. (1987), 262, 660–666.
- Kimborough D.R., Magoun M.A., Langfur M., "A laboratory experiment investigating different aspects of catalase activity in an inquiry-based approach", J Chem Ed 1997; volume 74,210–12.
- Lehninger A. L., Nelson D. L., and Cox M. M., "Lehninger's Principles of Biochemistry", W.H. Freeman, New York, NY, USA, 4th edition, 2004.
- Leilei Z., Mingxin H. and Suiyi Z., "Enzymatic remediation of the polluted crude oil by Rhodococcus", African Journal of Microbiology Research, 2012 Vol. 6(6), pp. 1213-1220.
- Leisola M, Jokela J., . Pastinen O, and Turunen O., "Industrial use of Enzymes— Essay, Laboratory of Bioprocess Engineering", Helsinki University of Technology, Helsinki, Finland, 2006.
- Loliger J., "Natural antioxidants for the stabilization of foods", In: Min, D. B. And Smouse, T. H. (Eds), Flavor Chemistry of Lipid Foods. Campaign, IL: The American Oil Chemists' Society 1989, pp. 302–325.
- Lončar N. and Fraaije M. W., Catalases as biocatalysts in technical applications: current state and perspectives, Appl Microbiol Biotechnol (2015) 99:3351–3357

- Maddy K.T., "Studies and Observations on Bovine Mastitis. II. Catalase Test for Mastitis", Iowa State University Veterinarian 1954, Vol. 16: Issue.1, Article1.
- Margesin R,. Zimmerbauer A,. Schinner F, "Monitoring of bioremediation by soil biological activities", Chemosphere, February 2000, Volume 40, Issue 4, pages 339-34.
- Margesin R., Schinner F, "Bioremediation of diesel-oilcontaminated alpine soils at low temperatures", Appl. Microbiol. Biotechnol 1997, volume 47, 462 468.
- Martin P. G., Food and Agriculture Organization of the United Nations, "Manuals of Food Quality Control: Commodities", Food & Agriculture Org., 1979 Food 409 pages.
- May D.W., Science, Catalase, a new enzym of general occurrence, 1901 Nov 22; 14(360):815-6. DOI:10.1126/science.14.360.815
- Morgan, P., Watkinson, R.J., "Hydrocarbon degradation in soils and methods for soil biotreatment", CRC Crit. Rev. Biotechnol, 1989, 8, 305-333.
- Nagaprasad K. S., Madhu D., Effect of Injecting Hydrogen Peroxide into Diesel Engine, International Journal of Engineering Sciences & Emerging Technologies, (2012), Volume 2, Issue 1, pp: 24-28
- Newman L. A., Doty S. L., Gery K. L., "Phytoremediation of organic contaminants: a review of phytoremediation research at the University of Washington", Soil and Sediment Contamination 1998, vol. 7, no. 4, pp. 531–542.
- Nikola L. and Marco W. F., Catalases as biocatalysts in technical applications: current state and perspectives, Appl Microbiol Biotechnol (2015) 99:3351 3357
- Ogbolosingha A.J, Essien E.B., Ohiri R.C.' and "Variation of Lipase, Catalase and Dehydrogenase Activities during Bioremediation of Crude Oil Polluted Soil" Journal of Environment and Earth Science 2015, Vol 5, No.13, No.14.
- Orla-Jensen, S. Dairy Bacteriology, 2nd ed. Blakiston Co. Philadelphia, Penn. 1931.
- Paar, S. Costa, T. Tzanov, M. Gudelj, K.H. Robra, A. Cavaco-Paulo, G.M. Gu^{*}bitz, "Thermo-alkali-stable catalases from newly isolated *Bacillus* sp. for the treatment and recycling of textile bleaching effluents", Journal of Biotechnology (2001), volume 89 147–153.
- Park J.-W., Park B.-K., and Kim J.-E, "Remediation of soil contaminated with 2,4dichlorophenol by treatment of minced shepherd's purse roots", Archives of Environmental Contamination and Toxicology 2006, vol. 50, no. 2, pp. 191–195.

- Parpinello G. P., Chinnici F., Versari A., Riponi C., "Preliminary Study on Glucose Oxidase Catalase Enzyme System to Control the Browning of Apple and Pear Purees", LWT - Food Science and Technology, 2002, Volume 35, Issue 3, Pages 239-243.
- Pascual J.A,. Hernandez T, Garcia C., Ayuso M., "Enzymic activities in an arid soil amended with urban organic wastes: laboratory experiment", Biores Tech 1998;64:131–8.
- Rila J.P. , "Development and application of bioassays for a site-specific risk assessment of contaminated soil", Thesis, Utrecht University 2008.
- Rubilar O., . Diez M. C, and Gianfreda L., "Transformation of chlorinated phenolic compounds by white rot fungi", Critical Reviews in Environmental Science and Technology 2008, vol. 38, no. 4, pp. 227–268.
- Schellhorn H.E, "Regulation of hydroperoxidase (catalase) expression in Escherichia coli". FEMS Microbiol Lett 1994; 131:113–9.
- Scott D., "Food Stabilization, Glucose Conversion in Preparation of Albumen Solids by Glucose Oxidase-Catalase System", J. Agric. Food Chem., 1953, 1 (11), pp 727– 730.
- Seo J.S, Keum Y.S., Hu Y, Lee S.E., and Li Q.X. "Phenanthrene degradation in *Arthrobacter* sp. P11: initial 1,2-, 3,4- and 9,10-dioxygenation, and meta- and orthocleavages of naphthalene-1,2-diol after its formation from naphthalene-1,2-dicarboxylic acid and hydroxyl naphthoic acids.", Chemosphere 2006, 65(11), 2388-94.
- Sharma, A., Kumar, P. and Rehman, M.B. "Biodegradation of Diesel Hydrocarbon in Soil by Bioaugmentation of Pseudomonas aeruginosa: A Laboratory Scale Study ", International Journal of Environmental Bioremediation & Biodegradation, 2014, 2 (4), 202-212.
- Sonawdekar S., Bioremediation: A boon to hydrocarbon degradation, International Journal of Environmental Sciences, (2012), 2, 2408- 2424; doi: 10.6088/ijes.00202030122
- Sulaimani H.A, Joshi S., Wahaibi Y. Al, S. Bahry N. A, Elshafie A., and Bemani, A. (2011) "Microbial biotechnology for enhancing oil recovery: current developments and future prospects," *Biotechnology, Bioinformatics and Bioengineering Journal*, vol. 1, no. 2, pp. 147-158.
- Sutton, N. B., Grotenhuis, J. T. C., Langenhoff, A. A., & Rijnaarts, H. H. "Efforts to improve coupled in situ chemical oxidation with bioremediation: a review of optimization strategies", Journal of Soils and Sediments 2011, 11(1), 129-140.

- Tzanov T., Costa S., G. M. Guebitz and A.Cavaco-Paulo, "Dyeing in catalase-treated bleaching baths", Color Technol 2001, 117: 1-5.
- Vainshtein K., Melik–Adamyan W.R., Barynin V.V., Vagin A.A., Grebenko A.I, "Three-dimensional structure of the enzyme catalase" Nature 1981; 293:411–2.
- Vasudevan P.T.,. Thakur D.S (1994), "Soluble and immobilized catalase effect of pressure and immobilization on kinetics and deactivation", Appl. Biochem. Biotechnol 1994, volume 49: 173–189.
- Vermeiren L.,. Devlieghere F, van Beest M., Kruijf N. De Debevere J, "Developments in the active packaging of foods", Trends in Food Science &Technology1999, volume10, 77-86.
- Vidali M., "Bioremediation. An overview," Pure and Applied Chemistry 2001, vol. 73, no. 7, pp. 1163–1172.
- Williams P. P., "Metabolism of synthetic organic pesticides by anaerobic microorganisms," Residue Reviews 1977, vol. 66, pp. 63–135.
- Witteveen C.F., Veenhuis M., Visser J., "Localization of glucose oxidase and catalase activities in Aspergillus niger", Appl Environ Microbiol 1992; 58:1190–4.
- Youn H.D, Yim Y.I., Kim K, Hah Y.C., Kang S.O, "Spectral characterization and chemical modification of catalase-peroxidase from Streptomyces sp", J Biol Chem 1995; 270(23):13740–7.
- Zamocky M., Koller F., "Understanding the structure and function of catalases:clues from molecular evolution and in vitro mutagenesis, Progress in Biophysics & Molecular Biology 1999, volume 72 :19-66.
- Zappi M., White K., Hwang H.M, Bajpai R., Qasim M. "The fate of hydrogen peroxide as an oxygen source for bioremediation activities within saturated aquifer systems", Journal of the Air & Waste Management Association 2000, 50(10), 1818-1830.
- Zhenxiao Y., Hongchen Z., Xingya Z., Shufang L., Jianyong X., Hui S., High level extracellular production of a recombinant alkaline catalase in E. coli BL21 under ethanol stress and its application in hydrogen peroxide removal after cotton fabrics bleaching, Bioresource Technology 214 (2016) 303–310