International Journal of Scientific Research in Biological Sciences
Research Paper
Vol.7, Issue.5, pp.104-109, October (2020)
E-ISSN: 2347-7520

Cellulosic Textile Pretreatment Application with Alkali Active Catalase from *Serratia marcescens*

Govindaraj C.[1*], Ugamoorthi R.[2], Ramarethinam S.[3]

[1,2,3]R&D Division, Eastman Exports Global Clothing Pvt Ltd, Tirupur 641603, Tamil Nadu, India.

**Corresponding Author: research@eastmanexports.com, Tel.: + 91 7094484737*

Available online at: www.isroset.org

Received: 18/Sept/2020, Accepted: 08/Oct/2020, Online: 31/Oct/2020

Abstract— Preliminary treatment is a process for the extract of natural and added adulteration present in textile fibres to enhance the absorbency. Cotton pretreatment formula includes the Sodium hydroxide, and Hydrogen peroxide to eliminate the non-cellulosic polluting influences and characteristic shading matter. Hydrogen peroxide was profoundly utilized as a fading operator in the pretreatment of cellulosic and protein filaments. The lingering peroxide must be eliminated before the texture was exposed to coloring since it may meddle with the coloring cycle. Hence, compound peroxide executioners were utilized to get the texture before coloring eliminate the leftover peroxide. As another contagious catalase was utilized for killing the leftover peroxide, which acts just at acidic pH. This current investigation uncovers the utilization of catalase by Serratia marcescens, which has action on both acidic and antacid pH. The higher movement of catalase was screened by utilizing various mediums. Reactor preliminaries were performed to discover the specific boundaries for the creation of high catalase movement with low pigmentation and for medium advancement. Compound steadiness was concentrated by utilizing diverse pH and temperatures to plan the material application measure by utilizing catalase as a neutralizer to decrease steps and spare vitality.

Keywords— Serratia marcescens, Catalase, Pretreatment, Hydrogen peroxide, Utility savings

I. INTRODUCTION

Enzymes play a major role in the textile industry for pretreatment of fabric and in the textile process. Amylase, pectinase, protease, lipase, cellulase and xylanase are highly used as an alternate for chemicals used in the textile process [1]. Catalase is an enzyme that is used to remove the residual peroxide, after bleaching of cotton fabrics. The residual peroxide must be removed from the fabric before the fabric is subjected to dyeing, as residual peroxide can have a negative influence on the dyeing process, especially to reactive dyes [1,2]. Hence catalase is used to catalyze the decomposition of residual hydrogen peroxide and thus eliminate the peroxide residue on fabric originated from the bleaching process [3]. Peroxide residues on fabric can cause modification to reactive dyes by oxidation, which leads to colour loss. Thus, the dyed fabric will be rendered unacceptable in the quality test. In the conventional method, the peroxide residues were removed by draining the bleach bath and washing the fabric with water for numerous times [4]. This consumes a lot of water and energy. As an alternate mild reducing agent can be used to remove the peroxide residues on fabric, after draining the bleach bath. The neutralizing agent must be chosen with caution because it should not create any by-products in wastewater, any risks of harmful overdosing, or adverse effects on dyestuff [5]. By using the catalase enzyme, it only accepts hydrogen peroxide as substrate and doesn't require heating or extra washing step as required in chemical bleach cleanup [6]. Serratia marcescens is a catalase producing bacteria that also produces the red pigment called Prodigiosin. The catalase produced by this bacterium can be used for the textile treatment process. Due to the presence of the pigment Prodigiosin, it cannot be used in bleached cotton for removing the residual peroxide, because it might interfere with the cotton fabric's colour [7]. When the bacteria grown in warm temperatures such as 30 – 35oC the amount of pigment production is reduced. So, the growth of Serratia marcescens is controlled in a bioreactor, for enhanced enzyme activity and pigment suppression [8]. This present study concentrates on the influence of aeration and agitation in enzyme production and activity, by Serratia marcescens in a reactor.

II. RELATED WORK

Enhanced alkaline catalase production by *serratia marcescens* its enzyme purification, characterization and recombinant expression [12]. Screening and potential of gram-negative bacterial isolates for their extracellular enzymatic activities isolated from aquatic environment. Susceptibility to hydrogen peroxide and catalase activity of root nodule bacteria [13]. *Serratia marcescens* catalase isolated from sludge containing hydrogen peroxide shows increased catalase production by regulation of carbon metabolism [14]. Optimization of catalase production and characterization of a novel cold adapted catalase from mesophilic bacterium *serratia marcescens* [15]. They have explored in catalase creating microbes' seclusion and

© 2020, IJSRBS All Rights Reserved

protein creation approach was distinguished. We have researched the catalase protein utilizing executing operator in leftover peroxide for material ventures. It's helpful to eliminate a peroxide content in material mechanical gushing in organic protein.

III. METHODOLOGY

Isolation and identification of *Serratia marcescens*

Soil sample was collected from the farm lands at Erode was used for isolation of *Serratia marcescens.* The soil sample was subjected to serial dilution and were pour plated on nutrient agar plates. The red colored colonies were isolated and subcultures to obtain purified colonies. Catalase positive colonies were chosen for further assessments. The pure isolate was further involved in biochemical characterization and 16S rDNA sequencing for species identification.

Catalase assay for enzyme activity estimation

The cell pellets were collected by centrifugation at 7,500 rpm for 5 minutes and the supernatant was stored at 4^oC. The cell pellets were suspended in phosphate buffer pH 7, in the ratio 1:100 (g: ml) and subjected to freeze-thawing for the lysis of the cells. Hydrogen peroxide with 415 ppm was used as a substrate for catalase assay. Iodometric titration for Hydrogen peroxide was performed to estimate the enzyme activity.

Medium optimization for Catalase Production

The different medium was screened for catalase enzyme production and activity. Complex general-purpose media such as nutrient and tryptic soy broth were used in the study. The defined basal medium was used to study the effect of glucose and phosphates. The medium selection was carried out as batch-wise in a conical flask, with an incubation period of 3 days at room temperature with pH 7. The catalase activity was tested for each trial to select the optimal medium for catalase production. The medium which showed high catalase activity was selected for further studies to assess the trial without bias.

Reactor studies – For enzyme activity enhancement

Culturing of *Serratia marcescens* under continuous agitation results in low or no pigment development, up to two days. The slight production of pigment starts, resulting in the change in colour of the medium. The pigment might interfere with the fabric and cause colour variation. Hence this pigment production must be suppressed while the enzyme production is being enhanced in the reactor. The agitation rates under test were in the range 150 – 200 rpm, and the aeration rates were in the range 0.5 – 1.6LPM, with a constant inoculum volume of 2%.

Nelder- mead simplex method for parameter optimization

The parameters under study for optimization were agitation (rpm) and aeration (LPM). The simplex method altered by Nelder and mead is used in this case of parameter optimization. This self-directing optimization technique is one of the widely used non-statistical technique, where the trials are done individually, in order to maximize or minimize the response. Here the enzyme activity response is highly influenced by the agitation and aeration parameters. Since the number of factors (n) under analysis is 2 (rpm& LPM), the simplex origin consists of 3 trials (n+1) i.e. the simplex consists of three vortexes. Based on the enzyme activity observed in the three origin trials, the new parameters were optimized. The modified new simplex was obtained by using the formula,

$$P = (B) + b\ (B-W)$$

Where, **P** - New simplex/ trial combination
B - Average of the best two trials out of the last three trials
W - Worst trial of the last three trials
b - coefficient of movement of the simplex,

The coefficient b might take up the values 1, 2, 0.5, and -0.5. (b = 1 reflection coefficient, b = 2 expansion coefficient, b = 1/2 contraction coefficient and b = -1/2 contraction coefficient with change of direction) [2]. This method hugely helps to reduce the number of trials for parameter optimization

Optimum pH and Temperature for enzyme activity

Every enzyme has its own pH and temperature range in which it is more stable and shows maximum activity. Beyond this range the enzyme loses its stable structure or denatures resulting in loss of activity [3]. The enzyme was taken at wide range with different pH and temperature for activity estimation and to find the optimum pH and temperature.

IV. RESULTS AND DISCUSSION

Isolation and identification of *Serratia marcescens*

The biochemical characterization and 16S rDNA sequencing were done to the isolate and identified to be *Serratia marcescens*. In 16S rDNA sequencing, a sequence of 1501 bp length was obtained. The obtained sequence was performed in the blast and it was again confirmed that the isolate was *Serratia marcescens.* The biochemical characterization results are shown in Table-1 and obtained sequence results are represented in Figure-1.

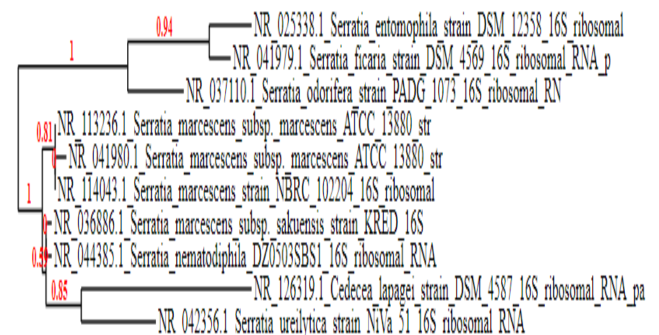

Figure-1 Phylogenetic tree construction of *serratia marcescens*

© 2020, IJSRBS All Rights Reserved

Table-1 Biochemical characterization results of the isolate

S.NO.	BIOCHEMICAL / MORPHOLOGY TEST	RESULT
1	Gram staining	Gram Negative
2	Capsule staining	Capsulated
3	Shape	Rod Shaped bacteria
4	Pigment production	Red pigment - Prodigiosin
5	Methyl red	Negative
6	Indole	Negative
7	Citrate test	Positive
8	Catalase test	Positive
9	H_2S	Negative
10	Urease test	Negative
11	Starch Hydrolysis	Positive
12	Gelatin Hydrolysis	Positive
13	Voges Proskauer	Positive
14	Oxidase	Negative

Media selection for catalase production

The Different medium was screened to find the optimal medium for catalase production with maximum activity. The cultures were incubated for 3 days at room temperature in medium with neutral pH. Nutrient broth, Peptone yeast broth, Soybean casein digest medium, glucose phosphate medium, and tryptic soy broth was under optimization for catalase production. It was found that *Serratia spp.* produced a high quantity of enzyme in nutrient broth. So nutrient broth was used as an optimized medium for further assessments.

Table-2 Activity of catalase in different media

S.NO.	MEDIA	ACTIVITY U/ML	WET CELL PELLET (G/L)
1	Nutrient broth	2166	11.12
2	Tryptic soy broth	1222	7.52
3	Glucose (5%) Basal media	638	1.72
4	Glucose phosphate media	872	3.64
5	Peptone broth	754	2.88

Reactor studies – For enzyme activity enhancement

The first three trials were designed such that the agitations and aeration parameter's high and low were covered, as to cover a broad range of the simplex [2]. Based on reactors capacity, the aeration can be varied from 0.5-2 LPM and agitation can be varied from 150 – 200 rpm. The parameters for the first three trials, aeration; 1, 2, 1.5 LPM and agitation; 150, 150, 200 rpm was taken for trial 1, 2 and 3 respectively. Table 3 shows all the trials conducted with their parameters, and the enzyme activity obtained. Based on several parameter assessments seven trials were designed. The best activity was observed in trial 1 and 3 out of seven trials. From the seven trials trial - 1 parameter showed the maximum activity. Thus aeration 1 LPM and agitation 150 RPM (Trial – 1/ Activity 5145 U/ml) was considered best for high catalase and cell production with pigment suppression. The same parameters were followed for further enzyme production.

Table-3 Trial parameters and activity of catalase obtained in each trial

TRIAL	AERATION (LPM)	AGITATION (RPM)	WET PACKED CELL WEIGHT (G/ L)	ACTIVITY (U/ ML)
1	1	150	10.33	5145
2	2	150	9.87	4603
3	1.5	200	7.91	4959
4	0.5	200	10.8	4651
5	0.8	180	6.28	3702
6	1.6	160	7.35	4682
7	1	100	7.13	4002

Optimum pH and Temperature for enzyme activity

To find the optimum pH, the enzyme was incubated in different pH for 5 minutes at 30°C and the activity was measured with hydrogen peroxide as substrate. The pH range was varied from 4 to 11 using buffers. The activity of the enzyme at different pH shows in fig-2. The highest activity of the enzyme was recorded at pH 7. The enzyme shows high stability from pH 6 – 11.

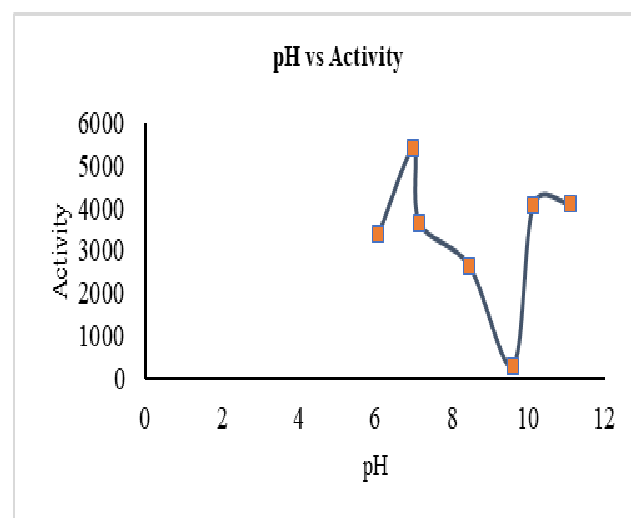

Figure – 2 pH Vs Activity of catalase

© 2020, IJSRBS All Rights Reserved

Similarly, the enzyme catalase with pH 7, was incubated at different temperatures for 5 minutes and the activity was checked. The activity at different temperatures shown in fig-3. Thus, it is evident that the catalase produced by *Serratia marcescens* is stable, in the pH range 6-11 with high activity exhibited at pH 7. The enzyme was also found to stable in the temperature range of 50-70°C, with high activity in 50 and 60°C.

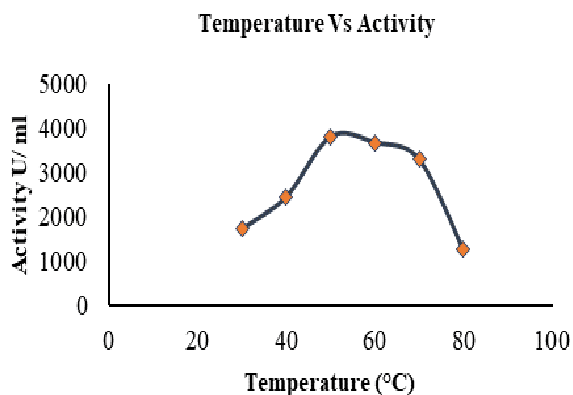

Figure – 3 Temperature Vs Catalase activity

Removal of Peroxide form Textile Substrate

Hydrogen peroxide residues present in form of nascent oxygen on cotton fabric causes the faulty dyeing. So, the removal of Hydrogen peroxide is much important to facilitate the fault-free dyeing of cotton and its blends. Various organic and inorganic chemicals are available to remove the residual nascent oxygen form bleached cotton fabric with its own limitations. Chemicals used to remove nascent oxygen residues cause environmental hazards. From the last decade, the application of biotechnology in the field of textile processing is becoming the most popular and significant technology to replace hazardous chemicals from the textile wet process. Catalase enzyme is used to remove the hydrogen peroxide residues from the cotton fabric before dyeing. Most of the industrial practice in the removal of nascent oxygen from fabric is followed by the neutralization process. But here we fermented both neutral and alkali active phosphate-buffered catalase enzyme which facilitate the removal in pH range from 6-11 and 50 to 60°C temperature. Industrial pretreatment on cotton fabric performed in 4 baths of water in 1:6 goods to liquor ratio. In order to save energy and water, we have a successful attempt in alkali treatment for removal of residual peroxide in a reduced bleach bath. Phosphate buffer reacts with enzyme and combination results in strong alkali pH 12±0.5 pH into pH 11 ±0.3.

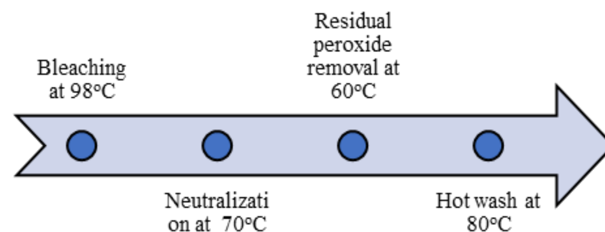

Figure – 4 Industrial cotton pretreatment processes

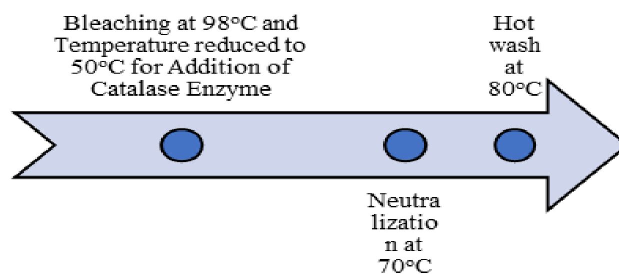

Figure – 5 Optimized process routes

Optimized cotton pretreatment process

The optimized process has greater environmental benefits and it saves more Time, Water and energy. The Benefits of the optimized process with phosphate-buffered catalase enzyme is shown in table-6. Each utility consumption graph is shown in table 6 as Water consumption, Time consumption, Energy consumption, and Chemical oxygen demand.

Table -6 Comparison between conventional and optimized process

S.N.	PARTICULATE	CONVENTIONAL PROCESS	OPTIMIZED PROCESS	CONSUMPTION SAVINGS (%)
1	Water	24 liters	18 liters	25
2	Time	314 Minutes	270 Minutes	14
3	Energy for complete Pre-treatment cycle	1.29 kWh	1.09 kWh	15
4	Chemical Oxygen Demand	940 mg/l	756 mg/l	19.5

V. CONCLUSION

The bacterial isolate, obtained from the farmland soil at Erode was found to be Serratia marcescens by biochemical characterization and 16S rDNA sequencing. The isolated Serratia marcescens was observed to grow well in nutrient broth with a catalase activity of 2166 U/ ml and approximate biomass of 11.12 g/ L (Centrifuged wet cell pellet). On the observation of reactor trials, 1 LPM and 150 rpm are the optimal culture conditions in the reactor for which the catalase activity was recorded to be 5145 U/ ml. The produced enzyme has stability at the pH range 6.1 – 11.3, with the high activity observed in pH 7 and 11 ± 0.3. The enzyme is stable up to 80°C and it exhibits high

© 2020, IJSRBS All Rights Reserved

activity at 50 and 60°C. The Optimum process designed for Serratia marcescens phosphate-buffered catalase was proved to be efficient over conventional process. Industrial pretreatment on cotton fabric performed in 4 baths of water in 1:6 goods to liquor ratio. In order to save energy and water, we have a successful attempt in alkali treatment for removal of residual peroxide in a reduced bleach bath. it saves 25% water, 14% time and 15% energy of complete pretreatment cycle and 19.5% COD reduction. The eventual outcome didn't present any noteworthy adjustments in its physical and substance properties. Contrasted and the synthetic reducer and progressive washes, the utilization of the catalase demonstrated to be a reasonable option for textile processing.

REFERENCES

[1] Alexandra M. Amorim, Marcelo D. G. Gasques, Jürgen Andreaus and Mauro Scharf. The application of catalase for the elimination of hydrogen peroxide residues after bleaching of cotton fabrics. Anais da Academia Brasileira de Ciências vol. **3**, pp. **433-436**, **2001**.

[2] Edith Ducros, Miriam P. Ferrari, Matias Santiago Pellegrino, and Claudia Raspanti. Effect of aeration and agitation on the protease production by Staphylococcus aureus mutant RC128 in a stirred tank bioreactor. Bioprocess BiosystEng vol **32,** pp. **143**–**148**, **2009**.

[3] Hans Bisswanger. Reporting Enzymology Data – STRENDA Recommendations and Beyond. Perspectives in Science vol. **1**, pp. **41**–**55**, **2001**.

[4] Schmidt MG. Bleach Clean up with Catalase. In: International conference and exhibition AATCC, Philadelphia, pp. **248**-**255**, **1995**.

[5] Tzanov T, Costa S, Guebitz GM and Cavaco-Paulo A. Effect of temperature and bath composition on the dyeing of cotton with catalase-treated bleaching effluents. Coloration Technology. pp. **166-170**, **2001**

[6] Buschle-Diller G, El Mohgahzy Y, Inglesby MK and Zeronian SH. Effects of Scouring with Enzymes, Organic Solvents, and Caustic Soda on the Properties of Hydrogen Peroxide Bleached Cotton Yarn. Textile Res J. vol. **68**, pp. **920**-**929**, **1998**.

[7] Cavaco-Paulo A. Processing Textile Fibres with Enzymes. K. E. Eriksson and A. Cavaco-Paulo, Eds. ACS Symp Ser vol. **687**, pp. **180**, **1998**.

[8] Uygur A. An overview of oxidative and photooxidative decolorisation treatments of textile waste waters. JSDC. vol. **113**, pp. **211**-**217**, **1997**.

[9] Xianbo Jia, Xinjian Lin, Chenqiang Lin, Lirong Lin, Jichen Chen, Enhanced alkaline catalase production by Serratia marcescens FZSF01: Enzyme purification, characterization, and recombinant expression. Electronic journal of biotechnology. vol.**30**, pp. **110**-**117**, **2012**.

[10] Hua-Wei Zeng, Yu-Jie Cai, Xiang-Ru Liao, Feng Zhang, Da-Bing Zhang Production, characterization, cloning and sequence analysis of a monofunctional catalase from Serratia marcescens SYBC08. Journal of Basic Microbiology. Vol. **2**, pp. **205**–**214**, **2011**.

[11] Ondarza J. Ozone Sensitivity and Catalase Activity in Pigmented and Non-Pigmented Strains of *Serratia Marcescens*. *Open Microbiol J*. vol. **11**, pp. **12**–**22**, **2017**.

[12] Anuradha S. Pendse, K. Aruna, "Physicochemical and analytical characterization of biosurfactant produced by Serratia rubidaea KAP," *International Journal of Scientific Research in Biological Sciences*, Vol.**7**, Issue.**2**, pp.**25**-**40**, **2020**

[13] V. Calandrelli, A. Gambacorta, I. Romano, *et al.*A novel thermo-alkali stable catalase–peroxidase from *Oceanobacillus oncorhynchi*subsp. *incaldaniensis*: Purification and characterization. World J Microbial Biotechnology, vol. **24**, pp. **2269**-**2275**, **2008**.

[14] B.S. Sooch, B.S. Kauldhar, M. PuriRecent insights into microbial catalases: Isolation, production and purification. Biotechnol Adv, vol. **32**, pp. **1429**-**1447**, **2014**.

[15] Deisseroth, A.L. DounceCatalase: Physical and chemical properties, mechanism of catalysis, and physiological role. Physiol Rev, vol. **50**, pp. **319**-**375**, **1970**.

[16] H.W. Zeng, Y.J. Cai, X.R. Liao, *et al.*Production, characterization, cloning and sequence analysis of a monofunctional catalase from *Serratia marcescens* SYBC08 J Basic Microbiol, vol. **51**, pp. **205**-**214**, **2011**.

[17] J.M. Nagy, A.E. Cass, K.A. BrownPurification and characterization of recombinant catalase-peroxidase, which confers isoniazid sensitivity in *Mycobacterium tuberculosis. J* Biol Chem, vol. **272**, pp. **31265**-**31271**, **1997**.

[18] X. Shi, M. Feng, Y. Zhao, *et al.* Overexpression, purification and characterization of a recombinant secretary catalase from *Bacillus subtilis.* Biotechnol Lett, vol. **30**, pp. **181**-**186, 2008**.

[19] H.W. Zeng, Y.J. Cai, X.R. Liao, *et al.Serratia marcescens* SYBC08 catalase isolated from sludge containing hydrogen peroxide shows increased catalase production by regulation of carbon metabolism Eng Life Sci, vol. **11**, pp. **37**-**43**, **2011**.

[20] P. Nigam, "Microbial enzymes with special characteristics for biotechnological applications," *Biomolecules*, vol. **3**, no. **3**, pp. **597**–**611**, **2013**.

[21] S. C. B. Gopinath, P. Anbu, and A. Hilda, "Extracellular enzymatic activity profiles in fungi isolated from oil-rich environments," *Mycoscience*, vol. **46**, no. **2**, pp. **119**–**126**, **2005**.

[22] D. Sharma, P. K. Gill, S. S. Bhullar, and P. Singh, "Improvement in inulinase production by simultaneous action of physical and chemical mutagenesis in Penicillium purpurogenum," World Journal of Microbiology and Biotechnology, vol. **21**, no. **6**-**7**, pp. **929**–**932**, **2005.**

[23] Blanco CA, Caballero I, Barrios R, Rojas A. Innovations in the brewing industry: Light beer. Int J Food Sci Nutr. Vol. **65**, pp. **655**–**60**, **2014**.

[24] Choi, J.M., Han, S.S. and Kim, H.S. 2015. Industrial applications of enzyme biocatalysis: Current status and future aspects. Biotechnology Advances, vol. **7** pp. **1443**–**1454**, **2015**.

[25] Lee, D.H., Oh, D.C., Oh, Y.S., Malinverni, J.C., Kukor, J.J. and Kahang, H.Y. 2007. Cloning and characterization of monofunctional catalase from photosynthetic bacterium Rhodospirillum rubrum S1. Journal of Microbiology and Biotechnology, vol. **9** pp. **1460**–**1468, 2007**.

[26] Oluoch, K.R., Welander, U., Andersson, M.M., Mulaa, F.J., Mattiasson, B. and Hatti-Kaul, R. 2006. Hydrogenperoxide degradation by immobilized cells of alkaliphilic Bacillus halodurans. Biocatalysis and Biotransformation, vol. **3**, pp. 215–222.

[27] Orozco, J., García-Gradilla, V., D'Agostino, M., Gao, W., Cortes, A. and Wang, J. ArtiFICIALcial enzyme-powered microfish for water-quality testing. ACS Nano, vol. **1,** pp. **818**–**824**, **2012**.

[28] Paar, A., Costa, S., Tzanov, T., Gudelj, M., Robra, K.H. and Cavaco-Paulo, A. Thermoalkalistable catalases from newly isolated Bacillus sp. for the treatment and recycling of textile bleaching effluents. Journal of Biotechnology, vol **8**, pp. **147**–**153**, **2001**.

[29] Vetrano, A.M., Heck, D.E., Mariano, T.M., Mishin, V., Laskin, D.L. and Laskin, J.D. 2005. Characterization of the oxidase activity in mammalian catalase. Journal of Biological Chemistry, vol. **42,** pp. **35372**–**35381**.

[30] Welinder, K.G. Bacterial catalase–peroxidases are gene duplicated members of the plant peroxidase superfamily. Biochimica et Biophysica Acta, vol.**3**, pp. **215**–**22, 1991.**

AUTHORS PROFILE

Mr. C Govindaraj pursued M. Tech Biotechnology in K S Rangasamy college of technology. He is currently working in Eastman Exports Global Clothing Pvt Ltd, R&D Division, Tirupur, Tamilnadu, India. His main research work focuses on biosurfactant production, microbial pigment synthesis, enzyme fermentation and plant tissue culture.

Mr. R Ugamoorthi M.Sc., MPhil pursued Environmental science in Bharathiar university. He is currently working in Leading team of Textile testing Laboratory, Sustainability, Research and Development at Eastman Exports Global Clothing Pvt Ltd, Tamilnadu, India. His main work focuses on organic chemistry, quality control, Auditing and method development.

Dr. S Ramarethinam M.Sc., PhD. He is currently working as Principal scientist, R&D Division at Eastman Exports Global Clothing Pvt Ltd, Tirupur, Tamilnadu, India. He has 20 years of research experience and has published more than 20 papers in numerous journals. His main research work focuses on biofertilizer, pesticide, crop improvement and plant sciences.

© 2020, IJSRBS All Rights Reserved