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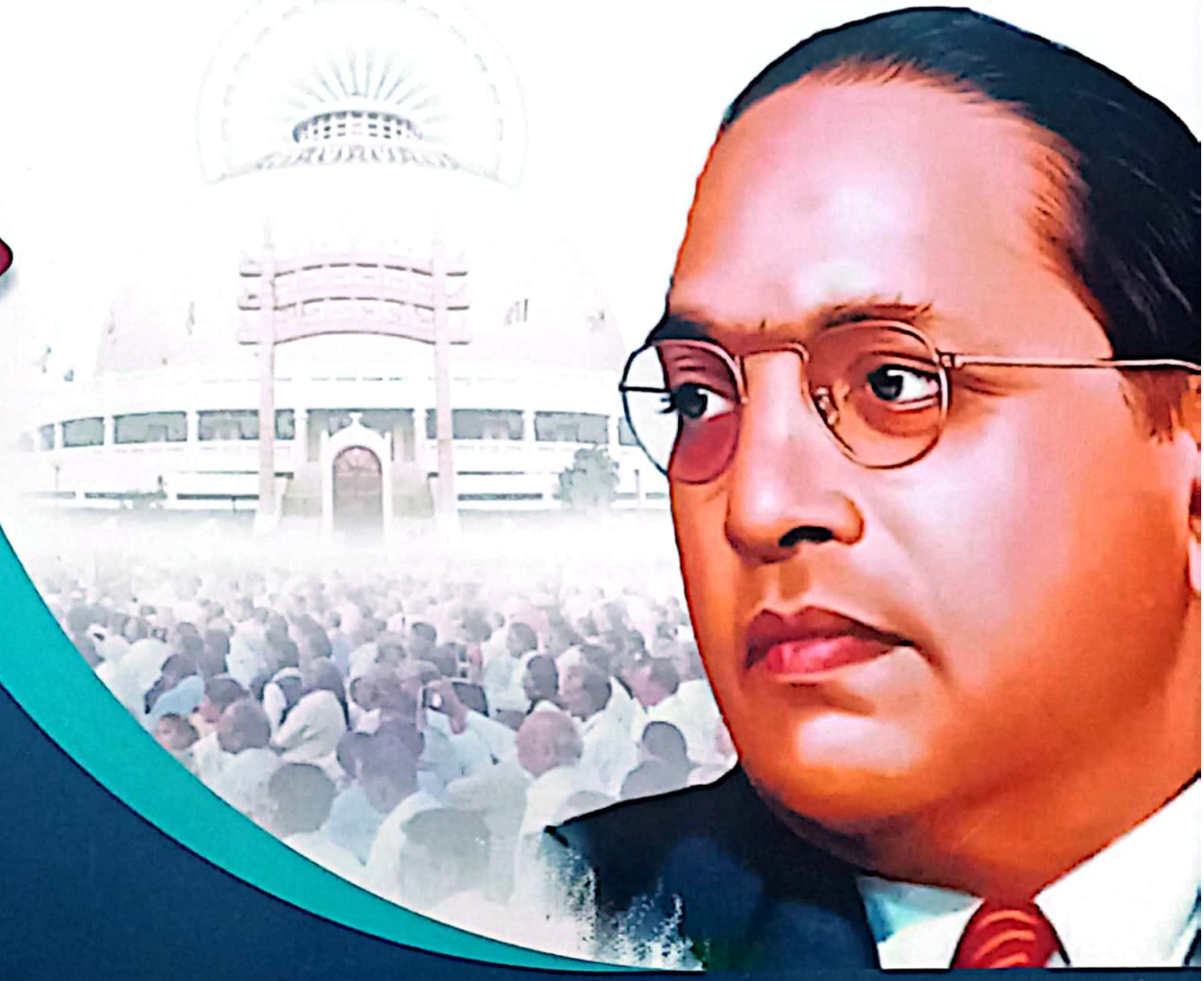

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Women Empowerment by Education

(An Analytical Study)

Dr. Manita Kaur Virdi, Assistant Professor (Political Science), Govt. College Bichhua, Distt. Chhindwara (MP)

Abstract

The Research Paper presented studies the contribution of education in women empowerment. The present world development landscape is changing very fast. Women are playing an important role in every field. Today, due to scientific and technical education, today women are acquiring knowledge of various disciplines of the engineer, knowledge of medicine, mathematics, and space, which is creating the background of their empowerment. Empowerment of women is being done through education. Women are very active in empowering themselves through education in the world, education has awakened the critical consciousness of women. It is full of new confidence in him. What better way than education can be for the uplifting and Empowerment of women.

- ***Key Words*: Empowerment, Education, Right, Upliftment**

Compilation of data: Compilation of data: Compilation of secondary data has been done through previously published topics, journals, newspapers, and research texts. facts are also collected through the internet.

Methodology: the research paper presented uses a dialectical, analytical study method along with a library study method.

Objective:

- Violence Towards women is a serious problem, for which scientific study and solution is very important.
- Understanding the actual scenarios by which women can be empowered.
- How women can be empowered through education.

Preface:

The history of women's education in India is linked to the ancient Vedic period. It is noteworthy that during the Vedic period, more than 3000 years ago women had considered an important part of the Society like men, Vedic scriptures say that "girls with boys are given proper care should be Nurtured and trained. Educating women can prove to be the key to overcome many social evils in India such as dowry, female feticide, and harassment at the workplace, etc.

We are making rapid progress to become the superpower of the world, but the challenge of gender inequality still stands before us as a harsh reality. Efforts are being made by the government for women's education such as "BETI BACHAO, BETI PADHAO" which was started in the year 2015 to address the issue of declining child sex ratio across the country. This was done to prevent female feticide, enforce the rules of the right to education, and arrange for primary level education for girls. The "MAHILA SAMAKHYA" Program was started in the year 1989 as per the goals of the national education policy1986 to improve and empower the education of women. UNICEF also working with the government of India to provide quality education to girls in the country, Vedas describes in detail the education, modesty, virtues, duties, and right of women, which is not observed in any scripture of the world. It is clear from the study of Vedic literature that women had a proud place in our country.

Women are the foundation of all progress. She is ready to build and is committed to her duties and rights and is entering in all field. During the Vedic period, the position of women was highly respected. He had the distinction of daughter wife and mother in all three forms. but gradually by the Middle ages, the situation of women had become very thoughtful. western culture and civilization to the British period women got a liberal Attitude Towards education among Indians. Gandhi Ji, Rajaram Mohan Roy Sati system, swami Dayanand Sarasvati, Vivekananda Ji all took action for the upliftment of women. as a result of this, after Attaining independence, the attitude of Indian society also changed Towards women and the issue of empowering women continued to get stronger.

The result of these efforts is that today women are waving their success in every field of development, and are occupying high positions Even that Indira Nooyi of PepsiCo is Kiran Majumdar Shaw, Kalpana Chawla and Sunita Williams of Indian origin. Or in posted of bank institutions NAINA Lal Kidwai, Chandra Kochhar, Meera Sanyal, Lalita Gupta or Sagarika and BARKHA DUTT, who lead the media. Sonia Gandhi, Smt. Pratibha Patil, who lead the country. She is performing her duties very efficiently in every post. Today women are maintaining their position in Parliament and gram panchayats. Along with all this corporate, administration, Management is doing amazing work like medical, education, police service, music singing, even flying airplanes, and running trains. today women are getting educated. and by education is reaching the culmination of the world. without education, Women can neither be aware nor have a strong. A question that how successful we have been in the matter of women empowerment.

The people of the village still shy away from sending girls to school, most of them are not educated. How women empowerment will be possible in the absence of education. Therefore, today it is necessary that we make the women so strong in the present time so that she

can enable herself in every sphere of life, and this work can be done through education. The darkness from the lamp of education will have to be eradicated.

Conclusion:

from pre-independence to the post-independence period, women have presented many examples of their abilities. Although many efforts were made by government institutions for their upliftment, many laws were also made, but their condition has not been completely improved so far. The efforts of the government of India, state government, voluntary organizations, and women's constitutional rights and modern education system and new thinking styles have resulted in the move towards women empowerment due to the increasing impact of the status of working women, struggle. but even today society in many parts of the world ignores their role, due to which women have to bear the brunt of largescale inequality, harassment, financial dependence, and other social evils. Women are legally empowered in society, but in practice, they are treated with inequality. women have progressed in a sustained manner against exploitation, oppression, and inequality and have taken meaningful steps. The tradition and culture of any nation are reflected by the women of that nation. to improve Tomorrow, we must improve the condition of women today. For this, we have to emerge from a conservative perspective and adopt a new evolutionary approach. Illiteracy is the mother of complete ignorance and exploitation, so if society has to uplift women and village, then we have to understand the importance of education.

Suggestion:

- Women should be given priority in the private sector also.
- Women should be made aware of their rights and duties so that they can collectively protest against issues related to gender discrimination female feticide domestic violence and women labor welfare provision etc.
- Equal opportunities should be provided to women at all educational levels.
- Training should be provided for vocational education in formal education and skill development.
- The Participation of women in public level policies should be increased so that problems related to women's safety, employment education can be recognized.
- Working women especially in rural areas related to agriculture work and Self Employment-based schemes should open Training centers in rural areas.
- In the new scenario towards women empowerment, the participation of women in socio-economic and political fields should be ensured.

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Rural Development Programs and Schemes

(*Analytical Study*)

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[1]Chhindwara University

Abstract:

Rural Economic activities have been the mainstay of the Indian economy. The economy of the entire country is adversely affected if the rural economy does not falter or develop. The concept of rural development was reinforced by Mahatma Gandhi's idea that the soul of India resides in the villages and until the villages are developed and they are not self-sufficient, the country cannot develop. Broadly rural development is meant to improve the standard of living of people living in rural areas. although there are ideological and theoretical differences in the concept of rural development, in practical terms the concept of rural development is clear, in which rural life is be improved. in the present context, the general meaning of rural development is derived from the economic, social, and political development of rural areas. Under the process of rural development, such a planning policy is adopted, through which the social and economic status of the weaker sections of the rural society can be raised by making optimal use of local resources and make them self-sufficient**.** There have been many studies un the past years to assess the state-run schemes of rural development, but the implementation of the schemes and studies related to public participation in the overall context of rural development has often been lacking. this study is an effort in this direction.

***Keywords*: Development, Economic, Rural, Planning, Implementation**

Compilation of data: The Collection of Secondary data has been compiled by Government Non-Government Publications, newspapers, magazines, and the internet.

Research Methodology: the research paper presented uses a dialectical, analytical study method along with a library study method.

Objective:

- To Test the durability and value effectiveness of the assets created by these programs.
- To evaluate the effects of programs in the context of poverty alleviation and productivity enhancement.
- To examine the role and functioning of Panchayati Raj Institutions in the implementation of these programs.

Preface: From the time of independence, the political leadership of India, created with the aim of public welfare, made its primary objective to formulate policies and programs for the eradication of rural poverty, it was felt that the strategy of poverty alleviation should be based on the growth of employment opportunities in the process of development. In making development policies, the emphasis was on poverty alleviation, ignorance, disease eradication, disparity, and availability of opportunities. Helps reduce extreme poverty with rapid growth. To provide opportunities for economic development to the villages, more participation of people in rural development programs, decentralization of schemes, better implementation of land reforms, and easy borrowing was included, the role of agriculture is most important in the development of the rural economy. therefore, it is possible to materialize rural development only by developing agriculture. because of this fact, several effective steps were taken by cooperative societies to improve the standard of living of farmers by developing agriculture the cooperative took special steps to implement crop loan schemes, prioritize loans to small farmers and develop the trend of saving among farmers. Despite all this, the cooperatives have been partially successful in fulfilling all the financial needs of the farmers.

Initially, the main thrust for the development was given to agriculture, industry, communication, education, health, and related fields, it was later realized that rapid development can only happen when government efforts have direct and indirect participation of lower-level people. because of this, the community project administration organization was established under the planning commission from 31 March 1952 to carry out community development programs. The community development program was launched on 2 October 1952. It was a milestone in the history of rural development. The Antyodaya Anna Yojana (AAY) scheme was launched by prime minister Atal Bihari Vajpayee on 25 December 2000. Under this scheme, about 2 crore families living below the poverty line (BPL) are provided food grains at a very subsidized rate, under the scheme total of 35 kg, food grain is provided the one family.

Many Poverty alleviation programs were restructured during the Navami plan period so that the efficiency of the programs could be increased to progressively benefit the poor people living in rural areas. The integrated rural development program (IRDP), development of Women and Children in Rural Areas (DWCRA), programs related to the supply of Improved toolkits of rural artisans (SITRA), Program

related to training of rural youth for self-employment (TRYSEM), Ganga Kalyani Yojana (GKY), and million wells Scheme (MWS), were merged into the overall self-employment schemes. which was named the Swarna Jayanti gram Swarozgar Yojana (SGSY). Keeping in mind the needs and aspirations of the local people, the cooperation of the Panchayati Raj Institution was taken in the implementation of this program. These institutions are a form of decentralized development of planning and its implementation. A new program called Hariyali was lanced by the prime minister on 27 January 2003 with the aims of seeking further cooperation in the development process of Panchayati Raj Institutions. The objective of starting a program called hariyali is to seek the cooperation of panchayat institutions in the implementation of wastelands development programs. Integrated watershed development program, drought Prone area program (DPAP) and, DDP. By the rural development department, the self-employment schemes and wage employment schemes, provision of houses for the rural poor and the Small Irrigation Means Scheme, social assistance schemes for the destitute, and the rural road construction sachems. Apart from this, the development provides support services and other quality resources for district rural development Agency (DRDA) administration, Panchayati Raj institutions, training, and research, human resource development, voluntary work development, etc. So that the programs can be properly implemented. The main programs run by the rural development department are Mahatma Gandhi National Rural Employment Guarantee Act (MNREGA), Pradhan Mantri gram Sadak Yojana (PMGSY), Indira Awash Yojana (IAY) and Swarna Jayanti gram Swarozgar Yojana (SGSY). Now known as national rural livelihoods mission (NRLM), national social assistance program (NSAP), and provision of urban facilities in rural areas (PURA). To eradicate poverty and unemployment from the rural areas of the country within the stipulated period, a multi-purpose and multi-pronged scheme maned "Swarna Jayanti Gram Swarozgar Yojana (SGSY) from 1st April 1999 to bringing prosperity setting up small enterprises there were started.

Conclusion:

Implementation of the programs run by the panchayat raj has increased the prospects of rural development and awakened the rural masses. They have become aware of their rights. However, the performance of gram panchayats is not as expected. No Matter how good a plan is its success depends on how it is implemented. There is a lack of trained and supported bureaucracy in India to determine the priorities of rural areas and effective solutions to the problems there. presently it becomes expedient to discuss those challenges in the Indian scenario and also it is necessary to identify the shortcomings in the implementation of rural development programs so that their remedial measures can be found.

Suggestion:

- The burden of Implementation of the program should be entrusted to such officers who are technically and mentally capable to bear it and who have a good knowledge of rural Psychology.
- Plans can be implemented realistically at the grassroots level by establishing public relations funds through local self-government.
- The social welfare schemes of the ministry of rural development can be obtained by the socially and educationally weaker sections only when their level of awareness is raised.
- In the Indian context, women do their utmost to do domestic work as well as in agricultural work, but being a patriarchal society, the rural areas also appear to be male-dominated. In such a situation it is felt necessary to have a certain percentage of women beneficiaries in rural development schemes.
- Road construction should be given to those agencies only. Those who are already employed in the respective districts and fully competent for this work. The Construction of rural roads should be done through selected contractors with technical knowledge.

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Production of biocatalyst α-amylase from agro-waste 'rice bran' by using *Bacillus tequilensis* TB5 and standardizing its production process

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ABSTRACT

Processing (milling, oil extraction) of agricultural products result in accumulation of massive amount of agro-waste residues. A sustainable alternative technique is required to utilize those agro-waste residues through biotechnology to convert them into useful products. α-Amylase is an enzyme of glycoside hydrolases family which hydrolyzes the α-D-(1,4) glycosidic bond. In the present research, *Bacillus tequilensis* TB5 was hired for the α-amylase production through SmF by utilizing agro-waste substrate rice bran. Also, the role of varying Physico-chemical parameters on α-amylase production was evaluated to determine the optimal conditions required for its maximum production. The findings of this research revealed that the optimal conditions for maximum yield (39.736 ± 0.296 U/ml) were found at pH 6.0, temperature 37 °C and incubation period of 72 h. On analyzing influence of various nutritional supplement on enzyme production, it was found that some of the nutrients like; peptone, beef extract, ammonium chloride, ammonium sulphate can enhance enzyme yield at a particular concentration. Purification of α-amylase was also done through ammonium sulphate precipitation method and then molecular weight of 54 kDa was determined by SDS-PAGE. The present research carried strongly supports, that rice bran is an efficient agro-waste substrate can possibilities of the commercial production of α-amylase.

1. Introduction

Enzymes are biocatalyst that are exceptionally fit and explicit under various environmental conditions and that's why they have numerous industrial applications (Pandey et al., 2017). Over the most recent decade many advancement can be seen in enzyme technology. Amylase hydrolyzes the α-1,4-glycosidic bond which occurs in starch, glycogen and various related polysaccharides to release simple sugar like glucose and maltose in an α-anomeric form (Xie et al., 2014; Elumalai et al., 2019; Herrera-Márquez et al., 2019). Amylases are the glycoside hydrolases which are ubiquitous in nature, produced by numerous animals, plants, bacteria, fungi and molds; but the majority applications of α-amylase in a number of modern biotechnological purposes are chiefly derived from bacteria and fungus (Kumar et al., 2013). Various species of Genus *Bacillus* are used for the industrial production of α-amylase (Paul et al., 2017). Commercial application of α-amylase enzyme is in textile, food, detergent, paper, sugar and pharmaceutical industry (Pandey et al., 2017; Asrat and Girma, 2018). Numerous strategies are developing day by day to produce large amount of amylase for industrial purposes and that too by utilizing various cost-effective substrate (Pascoal et al., 2011; Ahmed et al., 2019). Several characteristics of α-amylase enzyme including specificity, stability, optimum pH and temperature influence its performance, economics and feasibility (Finore et al., 2014). For its entire activity an enzyme requires a selected pH, temperature and incubation period (Paul et al., 2017). At present, amylase production covers up to 65% of the enzyme market globally and is constantly increasing (Simair et al., 2017).

Further, the selection of an appropriate substrate is moreover necessary for fermentation processes (Aullybux and Puchooa, 2013). Generally, at commercial scale starch is employed for the amylase production through bacteria. α-Amylase production by using synthetic media through SmF is extremely expensive and uneconomical. To minimize the production cost, utilization of agro-waste residue might be a po-

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tential alternative source for commercial-scale amylase production (Aullybux and Puchooa, 2013). Recently, agro-waste substrate like rice bran has attracted vast interest for amylase production underneath SmF because of their low cost, simplicity, easy availability, and lesser water output (Vijayaraghavan et al., 2015).

Rice bran is a solid agro-waste residue which is generated during milling process of paddy rice to yield polished rice (Moongngarm et al., 2012). Rice bran contains nutrients like carbohydrates 34–62%, lipids 15–20%, protein 11–15%, crude fibre 7–11% and ash 7–10% (Alauddina et al., 2017). Therefore, in the present study, agro-waste residue rice bran was used for high yield of α-amylase by *Bacillus tequilensis* TB5. Also, several production parameters were assessed and optimized. The efforts made may be of remarkable commercial significance because of effective waste usage and economic production.

2. Material and methods

2.1. Substrate

For this study, substrate rice bran (Fig. 1) was collected from the local market of Raipur Chhattisgarh, India and was stored in an airtight container at room temperature. Different chemicals used in this study were procured either from HiMedia Laboratories Private Limited or Sigma Aldrich Chemicals Private Limited.

2.2. Microorganism

The bacterial strain *Bacillus tequilensis* TB5 (Accession no. MK920157) prone to α-amylase production was isolated from vegetable waste and identified from Microbial Type Culture Collection (MTCC), Chandigarh, India. *B. tequilensis* was screened for amylase production via starch agar plate hydrolysis method. After exposure to iodine, the zone of clearance indicated amylase production activity.

2.3. α-Amylase assay

DNS (3, 5-Dinitrosalicylic acid) method was used for the quantification of produced α-amylase enzyme (Miller, 1959; Paul et al., 2017). Amylase was quantitatively assayed by estimating the amount of reducing sugar. For that, 1 ml of starch (1%) and 0.2 ml of the cell free crude enzyme extract were added in 0.8 ml of sodium acetate buffer (pH 6.0). After this 1 ml of DNS reagent was added to stop the reaction, then homogenized for 5 min in boiling water bath. After cooling this solution at room temperature, 1 ml of Potassium sodium tartrate (Rochelle salt) was added. Then making up the final volume to 12 ml, absorbance was measured at 540 nm. One unit (U) of amylase can be defined as the amount of amylase that is capable of releasing1 μg of reducing sugar per minute under the assay conditions (Deb et al., 2013; Gur et al., 2018; Paul et al., 2017).

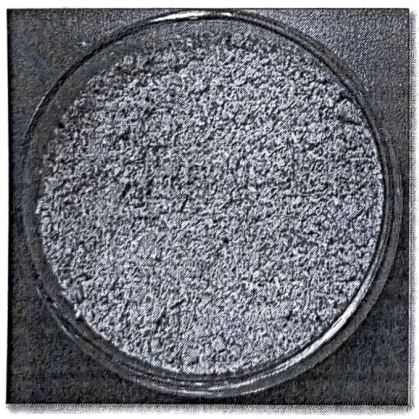

Fig. 1. Agro-waste substrate rice bran.

2.4. Enzyme production

Rice bran (15 g) was added in 150 ml of distilled water in the ratio 1:10 (w/v) in 250 ml of Erlenmeyer flask for the production of the enzyme. pH of this substrate hydrolysate was adjusted to 7.0. Substrate hydrolysate was autoclaved at 121 °C for 20 min and further used for the production of enzyme. For enzyme production, 1 ml of *Bacillus tequilensis* TB5 culture was inoculated in the substrate hydrolysate and was incubated at 37 °C.

2.5. Bioprocess parameter optimization for higher enzyme production

Several bioprocess parameters such as pH, temperature, incubation period and production medium supplements were assessed and optimized for maximum yield of α-amylase (Abdel-Fattah et al., 2012; Paul et al., 2017).

2.5.1. Optimization of different pH

The amount of α-amylase produced at varying pH (5, 6, 7, 8, 9, and 10) was measured to determine the optimum pH for maximum production. For this pH of the substrate hydrolysate was adjusted in the above mentioned range and then one ml of fresh bacterial culture was inoculated in it and incubated for 48 h at 37 °C. After production of enzyme, the cells were discarded and supernatant was assayed for amylase production by DNS method.

2.5.2. Optimization of different temperature

For determining the optimum temperature for enhanced enzyme yield, the amount of amylase produced at different temperatures such as 26, 30, 37, and 40 °C at previously optimized pH were measured. Substrate hydrolysate with inoculated bacterial culture was incubated at above different temperature individually for 48 h. After production of enzyme, the cells were discarded and supernatant was assayed for amylase production by DNS method.

2.5.3. Optimization of different incubation period

Optimization of incubation period for higher enzyme production was performed at formerly optimized pH and temperature. Substrate hydrolysate with inoculated bacterial culture was incubated at different incubation periods of 24, 48, 72, and 96 h for enzyme production. After production of enzyme, the cells were discarded and supernatant was assayed for amylase production by DNS method.

2.5.4. Effect of artificial medium supplement on enzyme production

At previously optimized process parameter (pH, temperature and incubation period) varied concentrations of 1, 2, 3, 4, and 5% (v/v) of modified production medium in (g/l), Yeast extract – 5 g, K_2HPO_4 – 0.5 g, $MgSO_4.7H_2O$ – 0.2 g, $CaCl_2$. $2H_2O$ – 0.1 g, Peptone – 5 g was supplemented in rice bran hydrolysate and analyzed its effect on production of α-amylase.

2.6. Effect of different nutrient supplements on enzyme production

In this study different type of nutrient supplements at various concentrations was added one at a time and analyzed for higher enzyme production. Nutrient supplements used were-peptone, beef extract, ammonium sulphate $[(NH_4)_2SO_4]$, ammonium chloride (NH_4Cl), ammonium nitrate (NH_4NO_3), magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$) and ferric chloride ($FeCl_3$).

2.6.1. Optimization of organic nitrogen sources

0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) concentrations of organic nitrogen source like peptone and beef extract were supplemented and analyzed for higher enzyme production by adding one nutrient at a time. Then the produced enzyme was estimated through α-amylase assay.

2.6.2. Optimization of inorganic nitrogen sources

Inorganic nitrogen sources were also used in the nutrient supplementation for higher enzyme production, for that different concentration 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) of ammonium sulphate ($(NH_4)_2SO_4$), ammonium chloride (NH_4Cl) and ammonium nitrate (NH_4NO_3) was added and analyzed individually for the higher production of enzyme.

2.6.3. Optimization of different ion salts for higher enzyme production

To analyze the influence of various ion salts on the level of enzyme production, varied concentrations like 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) of ion salts were supplemented in the substrate hydrolysate. Salts used were- Magnesium chloride ($MgCl_2$), Calcium chloride ($CaCl_2$), Ferric chloride ($FeCl_3$) and Magnesium sulphate ($MgSO_4$). Produced enzyme was quantified by the releasing of reducing sugar through DNS (3, 5-Dinitrosalicylic acid) method.

2.7. Optimization of different parameters for higher enzymatic activity

Enzymes can perform their maximum activity at optimized pH, temperature and incubation period. For assessing that specific enzymatic activity was calculated.

$$\text{Specific enzymatic activity(Unit/mg protein)} = \frac{\text{Unit /ml of enzyme}}{\text{mg protein /ml of enzyme}}$$

2.7.1. Effect of different pH on enzyme activity

For optimization of pH for complete and maximum enzymatic activity, buffer (sodium acetate) of different pH like 4, 5, 6, 7 and 8 was prepared. Then DNS method was used for the estimation of enzymatic activity.

2.7.2. Effect of different temperature on enzyme activity

To optimize temperature for proper enzymatic activity, at optimized pH different temperatures (26, 30, 37 and 40 °C) were assessed. The substrate hydrolysate was incubated at these temperatures individually one at a time for their optimum enzymatic activity. Then DNS method was used for the estimation of enzymatic activity.

2.7.3. Effect of various incubation periods on enzyme activity

Optimization of incubation period for maximum enzymatic activity was done by incubating substrate hydrolysate for various time intervals like 10, 15, 20, 25, 30, 35 and 40 min. Then DNS method was used for the estimation of enzymatic activity.

2.8. α-Amylase purification

After crude enzyme production, all the solid components i.e., bacterial cells and debris was removed by centrifugation of above solution at 10,000 rpm for 20 min. Then this cell free crude enzyme extract was precipitated by ammonium sulphate ($(NH_4)_2SO_4$) of 80% saturation for overnight at chilled condition. After 24 h precipitation, using the minimum volume of 0.1 M acetate buffer of pH 6, the sediment was dissolved and dialyzed (via dialysis membrane of 10-kDa cut-off) against 1 L of the same buffer at 4 °C for overnight by gentle shaking (Asoodeh et al., 2013; Paul et al., 2017). Process of dialysis was performed twice, similarly with the fresh buffer of 0.1 M acetate. After purification, specific enzymatic activity was also estimated to determine the enzyme purification fold.

2.9. Determination of protein concentration

For the estimation of specific enzymatic activity, protein concentration was determined by Lowry's method (Lowry et al., 1951) in which bovine serum albumin (BSA) was used as standard. Amylase total protein concentration was determined by comparing it with standard curve of BSA.

2.10. Molecular weight determination by SDS-PAGE

After purification of the enzyme, the molecular weight (MW) was determined through SDS polyacrylamide gel electrophoresis (PAGE) using 10% running gel of 1.5 M Tris–HCl, pH 8.8 of resolving buffer and 0.5 M Tris-HCl, pH 6.8 of staking buffer. For staining of protein bands coomassie brilliant blue stain (R-250) was used (Paul et al., 2017). MW of the purified amylase was determined with the help of standard protein marker. Commercial α-amylase enzyme (Himedia) was used for comparison.

2.11. Statistical analysis

All the data incorporated in this study were statistically analyzed by SPSS 16 software through the test significantly performed by analysis of variance (ANOVA). All experiment of this study was performed in three independent replicates and the results were followed by mean ± SE. Experimental mean values were determined by Duncan's Multiple Range Test which was compared at 5% level of significant differences.

3. Results and discussion

3.1. Optimization of pH

An alteration in hydrogen ion concentration influences various morphological and physiological attributes as well as the level of enzyme secretion in microorganisms. The present work shows that at pH 9.0, highest yield (25.976 ± 0.116 U/ml) of α-amylase was recorded (Fig. 2). The current finding indicates that bacteria can produce amylase even at slight alkaline pH.

The current finding was supported by Deljou and Arezi (2016) who also obtained the maximum amount of amylase at slightly alkaline pH (9.0) by inoculating 1% (v/v) *Bacillus licheniformis* AZ2 at 40 °C for 120 h. Optimum amylase production was achieved at pH (9.0) from *Chryseobacterium taeanense* in a medium containing mung

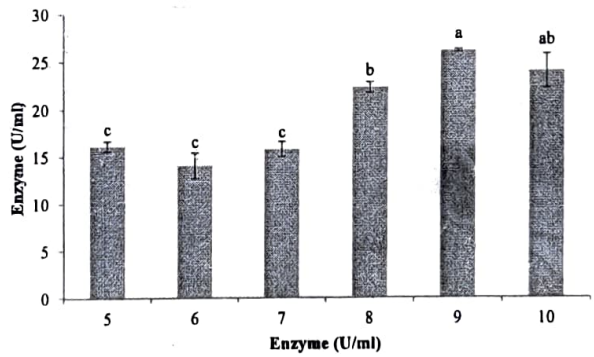

Fig. 2. Effect of different pH on enzyme production
ANOVA: $F = 23.506$, $p \leq 0.000$. Means bar followed by the same superscript letter did not differ significantly at 5% level of Duncan's Multiple Range Test.

bean as a sole carbon and nitrogen source as reported by Wang et al. (2011). Saxena et al. (2007) who worked on a highly thermostable and alkaline amylase from a *Bacillus* sp. PN5 found the maximum yield of amylase (45.9UmL^{-1}) at alkaline pH 10.0. Hasan et al. (2017) found maximum amylase production from *Chryseobacterium* and *Bacillus* isolates at pH 8 which makes a good agreement with our present finding. Elmansy et al. (2018) worked on the production and optimization of α-amylase from thermo-halophilic bacteria and obtained maximum yield of enzyme at pH 6 (12.00 ± 0.28 U/ml) which was quite lesser than the current finding. After going through several previous year findings it can be concluded that the enzyme produced in our current study is more tolerant to alkaline pH.

3.2. Optimization of temperature

Present work focuses on analyzing various temperatures (26, 30, 37 and 40 °C) for enhanced yield of α-amylase enzyme. Optimum temperature found for the maximum production of enzyme (34.288 ± 0.483 U/ml) was at 37 °C (Fig. 3).

Saha and Mazumdar (2019) who worked on production as well as optimization of α-amylase by *Bacillus cereus* amy3 also recorded the maximum yield of the amylase at 37 °C. Deljou et al. (2018) studied α-amylase production by *Bacillus licheniformis* strain AZ2 using rice husk as an elicitor also found maximum enzyme production at 37 °C. Paul et al. (2017) worked on production and temperature optimization of α-amylase by *Bacillus subtilis* and reported the maximum production of amylase at 37 °C. Serin et al. (2012) worked on production and optimization of α-amylase from *Bacillus circulans* ATCC 4516 and reported maximum yield of enzyme at 37 °C.

3.3. Optimization of incubation period

The amount of enzyme produced is influenced by incubation period also. Therefore, in this present work different time interval (24, 48, 72, and 96 h) was analyzed for maximized yield of enzyme. It was observed that after 72 h of incubation, higher enzyme production (39.736 ± 0.296 U/ml) was recorded (Fig. 4).

Asrat and Girma (2018) reported that incubation of 3 days is sufficient for production of 0.281 U/ml of enzyme using *Aspergillus niger* FAB-211. Elmansy et al. (2018) worked on the production of α-amylase from thermo-halophilic bacteria and obtained maximum production of enzyme after 3 days of incubation. Paul et al. (2017) worked on the α-amylase production and optimization of process parameters from *Bacillus subtilis* MB6 and found maximum yield of enzyme after 48 h of incubation. A further elevation in the incubation period resulted in a decrease in the level of amylase production. This result might be due to the accumulation of by products in the production medium or due to depletion of nutrients after 72 h.

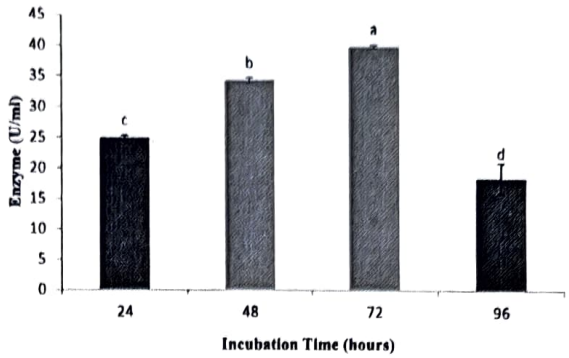

Fig. 4. Effect of different incubation period on enzyme production. ANOVA: F = 58.172, p ≤ 0.000. Means bar followed by the same superscript letter did not differ significantly at 5% level of Duncan's Multiple Range Test.

3.4. Optimization of medium supplement

After the optimization of process parameter like pH, temperature and incubation period, various concentration of modified production medium was supplemented in substrate hydrolysate and analyzed its effect on the level of enzyme production. It was recorded that the concentration of 3% (v/v) of production medium in substrate hydrolysate showed maximum enzyme production of 76.185 ± 5.591 U/ml and with further increase in the concentration of production medium, the level of enzyme produced was decreased (Fig. 5).

3.5. Effects of different nutritional supplement on enzyme production

It was observed that some of the nutrients like; ammonium chloride, peptone, beef extract, ammonium sulphate can enhance the production of enzyme at a particular concentration. After addition of peptone and beef extract individually one at a time, maximum amylase production (50.16 ± 6.72 U/ml) and (112.53 ± 4.84 U/ml) respectively was estimated at concentration of 0.4% (w/v). Ammonium nitrate at concentration of 0.2% (w/v) showed enhanced enzyme production (62.16 ± 1.69 U/ml). Ammonium chloride (NH_4Cl) and ammonium sulphate [$(NH_4)_2SO_4$] enhances the enzyme yield at concentration of 0.1% (w/v) (54.28 ± 1.34 and 68.20 ± 1.78U/ml) respectively (Table 1.).

Some previous findings support our current finding. Akcan (2011) reported that Ammonium nitrate (NH_4NO_3) shows higher enzyme pro-

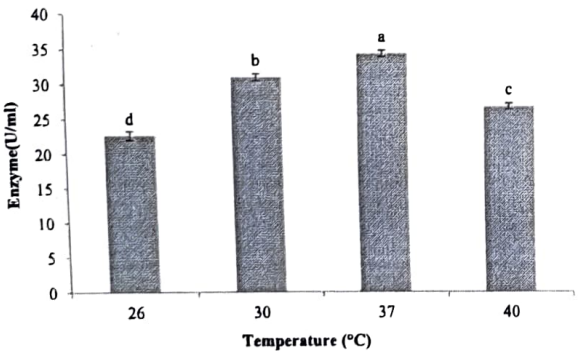

Fig. 3. Effect of different temperature on enzyme production. ANOVA: F = 90.120, p ≤ 0.000. Means bar followed by the same superscript letter did not differ significantly at 5% level of Duncan's Multiple Range Test.

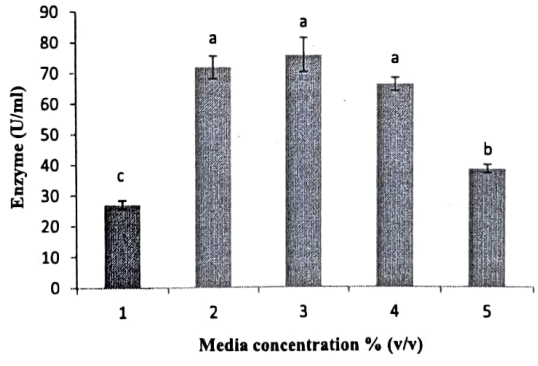

Fig. 5. Effect of media concentrations on enzyme production. ANOVA: F = 44.295, p ≤ 0.000. Means bar followed by the same superscript letter did not differ significantly at 5% level of Duncan's Multiple Range Test.

Table 1
Optimization of different nutrient supplements for amylase production.

Sr. No.	Concentration % (w/v)	Amount of enzyme (U/ml) in different nutrient supplements								
		Peptone	Beef extract	NH_4NO_3	$MgSO_4$	NH_4Cl	$(NH_4)_2SO_4$	$MgCl_2$	$CaCl_2$	$FeCl_3$
1	0.1	35.25 ± 8.82^a	33.32 ± 0.56^{cd}	56.73 ± 2.57^a	58.22 ± 1.17^a	54.28 ± 1.34^a	68.20 ± 1.78^a	47.07 ± 15.70^a	73.68 ± 1.01^e	49.54 ± 2.86^a
2	0.2	39.02 ± 1.55^a	63.87 ± 10.24^b	62.16 ± 1.69^a	38.50 ± 3.49^b	27.72 ± 1.80^b	47.73 ± 2.26^b	34.42 ± 3.92^{ab}	97.34 ± 0.96^d	35.67 ± 2.36^b
3	0.3	40.05 ± 7.78^a	49.64 ± 9.10^{bc}	43.86 ± 1.03^b	33.85 ± 3.54^{bc}	17.80 ± 1.22^c	26.30 ± 1.87^c	24.56 ± 1.16^{ab}	114.4 ± 2.48^c	33.36 ± 3.41^{bc}
4	0.4	50.16 ± 6.72^a	112.53 ± 4.84^a	42.76 ± 2.89^b	27.27 ± 1.64^{cd}	15.67 ± 3.26^c	25.14 ± 1.90^c	21.97 ± 0.59^{ab}	141.3 ± 3.68^b	24.39 ± 4.23^c
5	0.5	46.97 ± 11.18^a	25.73 ± 1.67^d	37.02 ± 2.20^b	21.81 ± 0.93^d	14.50 ± 2.60^c	24.74 ± 2.44^c	20.01 ± 3.68^b	154.1 ± 2.36^a	25.30 ± 0.45^c

ANOVA of different nutrient supplements: Peptone- $F = 0.600$, $p < 0.671$; Beef extract- $F = 27.533$, $p \leq 0.000$; Ammonium nitrate- $F = 23.291$, $p \leq 0.000$; Magnesium sulphate- $F = 32.872$, $p \leq 0.000$; Ammonium chloride- $F = 57.315$, $p \leq 0.000$; Ammonium sulphate- $F = 88.033$, $p \leq 0.000$; Magnesium chloride- $F = 20,277$, $p < 0.133$; Calcium chloride- $F = 195.199$, $p \leq 0.000$; Ferric chloride- $F = 11.847$, $p < 0.001$.Means of each nutrient sources within a column followed by the same superscript letter did not differ significantly at 5% level of Duncan's Multiple Range Test.

duction of ~800 U/ml using *Bacillus licheniformis* ATCC 12759. Hasan et al. (2017) worked on production and optimization of extracellular amylases by using *Chryseobacterium* and *Bacillus* isolates also found that organic nitrogen sources, like peptone and beef extract was the best for enhancing the level of amylase production. Deb et al. (2013) found maximum enzyme production of ~60 U/ml at 0.2% concentration of ammonium nitrate. Kaur et al. (2015) worked on amylase production using agro-waste by *Bacillus licheniformis* also reported that after the addition of nutrient like ammonium nitrate, there is an increase in the level of amylase production. Sivakumar et al. (2012) also found maximum enzyme production (70 ± 3.0U/ml) at 0.5% concentration of ammonium sulphate. Tiwari et al. (2014) observed that amylase production using *Bacillus tequilensis* RG-01 was highest at 0.3% concentration of peptone.

3.6. Effect of various ions on enzyme production

Influence of various ions at different concentration on enzyme production revealed that $MgSO_4$, $MgCl_2$, and $FeCl_3$ show higher production at concentration of 0.1% (w/v) i.e. 58.22 ± 1.17 U/ml, 47.07 ± 15.70 U/ml and 49.54 ± 2.86 U/ml of enzyme respectively. Maximum yield of amylase was obtained at 0.5% (w/v) concentration of $CaCl_2$ i.e., (154.1 ± 2.36 U/ml) as compared with the control in which production was 39.736 ± 0.296 U/ml (Fig. 6).

Deljou and Arezi (2016) reported highest production of the enzyme at 0.3 g/L concentration of $CaCl_2$ by thermophilic *Bacillus licheniformis*-AZ2. Gupta et al. (2003) reported maximum enzyme production in the presence of Mg^{2+} and also found that the enzyme production gets reduced to half when Mg^{2+} was removed. Akcan (2011) also reported that the production of enzyme increases due to the addition of $CaCl_2$ (0.1%) by *Bacillus licheniformis* ATCC 12759 in submerged fermentation. Serin et al. (2012) studied production and optimization of α-amylase from *Bacillus circulans* ATCC 4516 and observed that $CaCl_2$ increases the production of enzyme.

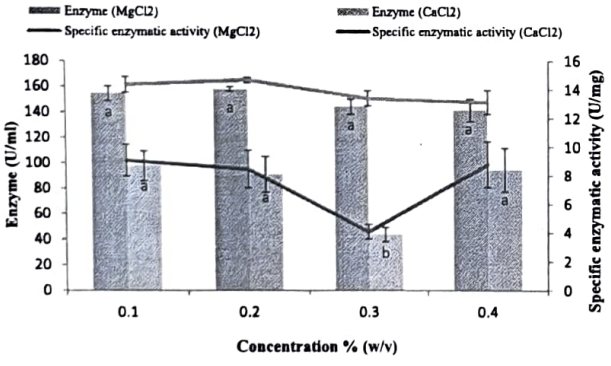

Fig. 6. Effect of different ions on enzyme production and on enzyme activity. ANOVA: $MgCl_2$- Enzyme (U/ml) and Specific activity (U/mg); $F = 1.67$, $p < 0.249$; $CaCl_2$- Enzyme (U/ml) and Specificactivity (U/mg); $F = 3.85$, $p < 0.056$. Each data point followed by similar superscript letters in each ion does not differ significantly at 5% level of Duncan's Multiple Range Test.

3.7. Effect of pH on enzyme activity

An enzyme can perform its complete activity at optimum pH, so in this study different pH (4, 5, 6, 7 and 8) was analyzed. It was observed that at pH 6, maximum enzymatic activity of 148.73 ± 6.75 U/ml was recorded with a specific activity of 13.80 ± 0.62 U/mg of protein. Also, it was observed that enzymatic activity gradually decreases with the further elevation in pH (Fig. 7).

Elmansy et al. (2018) also reported that pH 6 was suitable for higher enzymatic activity. Deb et al. (2013) reported that the enzyme shows its highest activity of 28 U/ml at pH 6.5. Abdulaal (2018) found that amylase shows its complete activity at a pH of 7. Tiwari et al. (2014) also support the present investigation; they got nearly similar optimum pH of 7 for the maximum activity of the enzyme.

3.8. Effect of temperature on enzyme activity

In this study different temperature of 26, 30, 35 and 40 °C was analyzed at optimized pH. It was observed that a temperature of 37 °C showed maximum activity of 177.09 ± 0.61 U/ml with a specific activity of 16.44 ± 0.58 U/mg of protein. Enzyme activity decreased when the temperature was further increased (Fig. 8).

Temperature influences performance of enzyme in two different ways. Firstly, by influencing the reaction rate constant and the other one is by thermal denaturation of the enzyme at higher temperatures Elmansy et al. (2018). Divakaran et al. (2011) worked on α-amylase

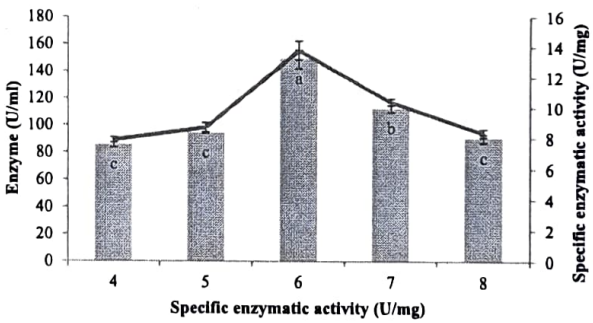

Fig. 7. Effect of different pH on activity of enzyme. ANOVA Enzyme (U/ml) and Specific activity (U/mg); $F = 52.907$, $p \leq 0.000$. Each data point of Enzyme (U/ml) and Specific activity (U/mg) followed by similar superscript letters do not differ significantly at 5% level of Duncan's Multiple Range Test.

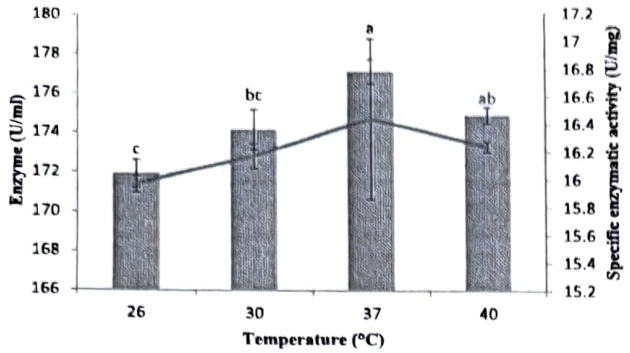

Fig. 8. Effect of different temperature on activity of enzyme. ANOVA Enzyme (U/ml) and Specific activity (U/mg); F = 8.778, p < 0.007. Each data point of Enzyme (U/ml) and Specific activity (U/mg) followed by similar superscript letters do not differ significantly at 5% level of Duncan's Multiple Range Test.

production by two different strains of *Bacillus licheniformis* and obtained maximum enzymatic activity at 37 °C. Gupta et al. (2003) found optimum temperature for the enhanced activity of α-amylase to be in the range of 25–30 °C. Kaur et al. (2015) found 30 °C as the optimum temperature for the highest enzymatic activity of 2.07 ± 0.02 IU/ml. Paul et al. (2017) reported enzyme activity to be high at 55 °C. Pinjari and Kotari (2018) studied that the optimal temperature for maximum amylase activity was 45 °C.

3.9. Effect of incubation period on enzyme activity

Proper incubation is required for the completion of any chemical reactions. So, it is essential to provide suitable time for the complete and maximum activity of enzyme. It was found that complete and maximum enzymatic activity was recorded after 35 min of incubation and obtained 174.27 ± 0.53 U/ml of enzyme with a specific activity of 16.19 ± 0.05 U/mg of protein. It was also observed that activity of enzyme decreased as the incubation period was further increased (Fig. 9).

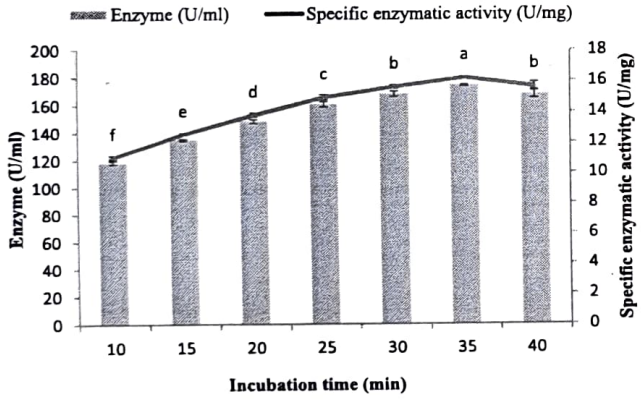

Fig. 9. Effect of different incubation period on activity of enzyme. ANOVA Enzyme (U/ml) and Specific activity (U/mg); F = 165.618, p ≤ 0.000. Each data point of Enzyme (U/ml) and Specific activity (U/mg) followed by similar superscript letters do not differ significantly at 5% level of Duncan's Multiple Range Test.

Similar result was obtained in previous study of Amoozegar et al. (2003) who worked on amylase production by strain MA-2 of *Halobacillus* sp. and found complete enzymatic activity after 30 min of incubation period. Najafi and Kembhavi (2005) reported that 30 min of incubation was suitable for the complete enzymatic activity which strongly supports our finding. Paul et al. (2017) also analyzed enzymatic activity of produced enzyme and found maximum activity of enzyme after 30 min of incubation. Deb et al. (2013) found maximum enzymatic activity after 40 min of incubation.

3.10. SDS-PAGE

The purified α-amylase was subjected to SDS-PAGE for MW determination. The relative MW of the amylase produced by *B. tequilensis* TB5, on comparing it with the standard marker found was ~54 kDa (Fig. 10) which was similar to the MW of commercial α-amylase. The purification of α-amylase was estimated to be about 1.84-fold with a specific enzymatic activity of 29.81 U/mg of protein which is greater than that of the crude enzyme extract i.e., 1.0 fold and specific enzymatic activity of 16.19 U/mg of protein (Table 2.)

Similar finding of 53 kDa α-amylase by *Bacillus* sp. MB6 was reported by Paul et al. (2017). Xian et al. (2015) also found MW of the purified enzyme to be 58 kDa which also supports our current finding. Hmidet et al. (2008) also stated MW of α-amylases to be 58 kDa from *Bacillus licheniformis* NH1. Kannan and Kanagaraj (2019) reported amylase of ~60.5 kDa MW from *Bacillus lichenformis*. A similar study

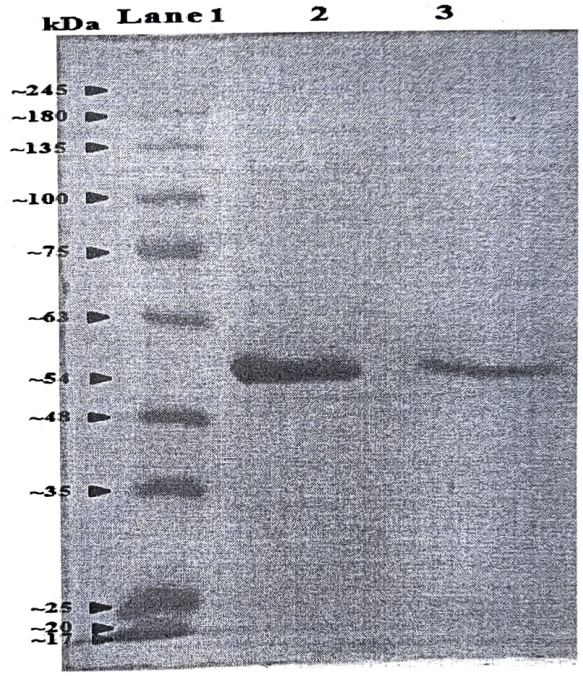

Fig. 10. SDS-PAGE Analysis of α-amylase enzyme. (Lane 1- Protein ladder, 2- Commercial α-amylase, 3- Purified α-amylase).

Table 2
Purification fold and specific enzymatic activity of *Bacillus tequilensis* TB5 purified enzyme.

S. No.	Enzyme sample	Enzyme (U/ml)	Total Protein (mg/ml)	Specific enzymatic activity (U/mg Protein)	Activity yield (%)	Purification (fold)
1.	Crude enzyme extract	174.27 ± 0.53	10.76 ± 0.13	16.19 ± 0.05	100	1
2.	Purified enzyme	138.35 ± 0.74	4.64 ± 0.20	29.81 ± 0.21	79.38	1.84

by Tiwari et al. (2014) revealed MW to be 67 kDa produced from synthetic media using *B. tequilensis* which is slight higher than the current finding of MW. Enzyme purification fold increased from 1 to 1.63 as reported by Paul et al. (2017) who isolated amylase from *Bacillus subtilis* subspecies MB6. Aguilar et al. (2000) obtained 1.36 fold of purified enzyme after ultrafilteration and dialysis of amylase obtained from *Lactobacillus manihotivorans* which is quite lower than our current finding of 1.84 fold.

4. Conclusion

α-Amylase is one of the most frequently used glycoside hydrolase in industries. Therefore, it requires large-scale production and that too by utilization of cost-effective substrate. The findings of this investigation uncovered that rice bran is a cheaper as well as eco-friendly agro-waste substrate for industrial-scale amylase production by *Bacillus tequilensis* TB5 which can replace high-cost synthetic media. Further, amylase production can be enhanced by optimizing various process parameters like pH, temperature, incubation period, nutritional supplement etc. A combination of optimized bioprocess parameter like pH 9.0, the temperature of 37 °C and 72 h incubation yielded a maximum amount of amylase by *B. tequilensis*. Purification via ammonium precipitation followed by dialysis can efficiently purify the enzyme and increases its purification fold. These output can aid to the development of potential strategies in enzyme technology for manufacturing high yield as well as cheaper α-amylase by utilizing agro-waste substrate i.e., Rice bran which might be of great industrial significance.

CRediT authorship contribution statement

Jai Shankar Paul: Conceptualization, Writing - original draft, Formal analysis. **Esmil Beliya:** Data curation. **Shubhra Tiwari:** Formal analysis. **Karishma Patel:** Investigation. **Nisha Gupta:** Methodology. **S.K. Jadhav:** Conceptualization, Writing - review & editing.

Declaration of competing interest

There is no any conflict of interest in any part of this article to author's and other.

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Production of biocatalyst α-amylase from agro-waste 'rice bran' by using *Bacillus tequilensis* TB5 and standardizing its production process

Jai Shankar Paul [a], Esmil Beliya [b], Shubhra Tiwari [a], Karishma Patel [a], Nisha Gupta [a], S.K. Jadhav [a, *]

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ABSTRACT

Processing (milling, oil extraction) of agricultural products result in accumulation of massive amount of agro-waste residues. A sustainable alternative technique is required to utilize those agro-waste residues through biotechnology to convert them into useful products. α-Amylase is an enzyme of glycoside hydrolases family which hydrolyzes the α-D-(1,4) glycosidic bond. In the present research, *Bacillus tequilensis* TB5 was hired for the α-amylase production through SmF by utilizing agro-waste substrate rice bran. Also, the role of varying Physico-chemical parameters on α-amylase production was evaluated to determine the optimal conditions required for its maximum production. The findings of this research revealed that the optimal conditions for maximum yield (39.736 ± 0.296 U/ml) were found at pH 6.0, temperature 37 °C and incubation period of 72 h. On analyzing influence of various nutritional supplement on enzyme production, it was found that some of the nutrients like; peptone, beef extract, ammonium chloride, ammonium sulphate can enhance enzyme yield at a particular concentration. Purification of α-amylase was also done through ammonium sulphate precipitation method and then molecular weight of 54 kDa was determined by SDS-PAGE. The present research carried strongly supports, that rice bran is an efficient agro-waste substrate can possibilities of the commercial production of α-amylase.

1. Introduction

Enzymes are biocatalyst that are exceptionally fit and explicit under various environmental conditions and that's why they have numerous industrial applications (Pandey et al., 2017). Over the most recent decade many advancement can be seen in enzyme technology. Amylase hydrolyzes the α-1,4-glycosidic bond which occurs in starch, glycogen and various related polysaccharides to release simple sugar like glucose and maltose in an α-anomeric form (Xie et al., 2014; Elumalai et al., 2019; Herrera-Márquez et al., 2019). Amylases are the glycoside hydrolases which are ubiquitous in nature, produced by numerous animals, plants, bacteria, fungi and molds; but the majority applications of α-amylase in a number of modern biotechnological purposes are chiefly derived from bacteria and fungus (Kumar et al., 2013). Various species of Genus *Bacillus* are used for the industrial production of α-amylase (Paul et al., 2017). Commercial application of α-amylase enzyme is in textile, food, detergent, paper, sugar and pharmaceutical industry (Pandey et al., 2017; Asrat and Girma, 2018). Numerous strategies are developing day by day to produce large amount of amylase for industrial purposes and that too by utilizing various cost-effective substrate (Pascoal et al., 2011; Ahmed et al., 2019). Several characteristics of α-amylase enzyme including specificity, stability, optimum pH and temperature influence its performance, economics and feasibility (Finore et al., 2014). For its entire activity an enzyme requires a selected pH, temperature and incubation period (Paul et al., 2017). At present, amylase production covers up to 65% of the enzyme market globally and is constantly increasing (Simair et al., 2017).

Further, the selection of an appropriate substrate is moreover necessary for fermentation processes (Aullybux and Puchooa, 2013). Generally, at commercial scale starch is employed for the amylase production through bacteria. α-Amylase production by using synthetic media through SmF is extremely expensive and uneconomical. To minimize the production cost, utilization of agro-waste residue might be a po-

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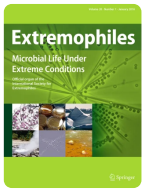

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Abstract

α-Amylase is the most significant glycoside hydrolase having applications in various industries. It cleaves the α,1–4 glucosidic linkages of polysaccharides like starch, glycogen to yield a small polymer of glucose in α-anomeric configuration. α-Amylase is produced by all the three domains of life but microorganisms are preferred sources for industrial-scale production due to several advantages. Enormous studies and research have been done in this field in the past few decades. Still, it is requisite to work on enzyme stability and catalysis, as it loses its functionality in extreme. As the enzyme loses its structural and catalytic property under extreme environmental conditions, it is mandatory to confer some potential strategies for enhancing enzyme behaviour in such conditions. This limitation of an enzyme can be overcome up to some extent by extremophiles. They serve as an excellent source of α-amylase with outstanding features. This review is an attempt to encapsulate some structure-based strategies for improving enzyme behaviour thereby enabling researchers to selectively amend any of the strategies as per requirement during upstream and downstream processing for higher enzyme yield and stability. Thus, it will provide some cutting-edge strategies for tailoring α-amylase producing organism and enzyme with the help of several computational biology tools.

Extremophiles
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REVIEW

Molecular strategies to enhance stability and catalysis of extremophile-derived α-amylase using computational biology

Nisha Gupta[1] · Esmil Beliya[1,2] · Jai Shankar Paul[1] · Shubhra Tiwari[1] · Shriram Kunjam[3] · Shailesh Kumar Jadhav[1]

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Keywords α-Amylase · Computational biology · Extremophiles · Glycoside hydrolase · Structural insights

Introduction

Enzymes are the most vital bio-product needed for sustaining life on earth. In recent years, α-amylase has significantly replaced the chemical hydrolysis of starch in industries. α-Amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) is an endo-acting hydrolyzing enzyme responsible for the breakdown of α,1–4 glucosidic linkages of starch and other related polysaccharides to yield maltooligosaccharides, glucose, and limit dextrin in an α-anomeric form (Machius et al. 1995; Yadav 2012; Al-Dhabi et al. 2020; Abd-Elaziz et al 2020; Janeček and Zámocká 2020). The total contribution of α-amylases in the enzyme market is about 30% and hence occupies the second position after proteases (Wu et al. 2018; Allala et al. 2019; Wang et al. 2019a; Abd-Elaziz et al. 2020). It is synthesized by microorganisms, plants, and animals. But for large-scale production, microorganisms are generally selected. Microorganisms are preferred because they offer cheaper large-scale production, ease of genetic engineering approaches, enormous strain availability etc. (Abdel-Fattah et al. 2013; Abd-Elhalem et al. 2015; Afrisham et al. 2016). It is extensively used in several industries and plays a substantial role in them (Table 1).

Despite having lots of industrial applications there are certain shortcomings related to the use of α-amylase. They tend to drop their structural conformations, stability, and catalysis when allowed to work in extreme conditions (Ahmed et al. 2020). To overcome this sensitivity of α-amylase towards harsh conditions, researchers are seeking sources living in extreme environmental conditions. Extremophiles are the organism inhabiting such harsh environment

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Nisha Gupta, Esmil Beliya have contributed equally as first author.

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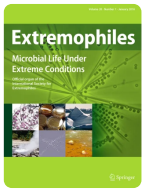

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Keywords α-Amylase · Computational biology · Extremophiles · Glycoside hydrolase · Structural insights

Introduction

Enzymes are the most vital bio-product needed for sustaining life on earth. In recent years, α-amylase has significantly replaced the chemical hydrolysis of starch in industries. α-Amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) is an endo-acting hydrolyzing enzyme responsible for the breakdown of α,1–4 glucosidic linkages of starch and other related polysaccharides to yield maltooligosaccharides, glucose, and limit dextrin in an α-anomeric form (Machius et al. 1995; Yadav 2012; Al-Dhabi et al. 2020; Abd-Elaziz et al 2020; Janeček and Zámocká 2020). The total contribution of α-amylases in the enzyme market is about 30% and hence occupies the second position after proteases (Wu et al. 2018; Allala et al. 2019; Wang et al. 2019a; Abd-Elaziz et al. 2020). It is synthesized by microorganisms, plants, and animals. But for large-scale production, microorganisms are generally selected. Microorganisms are preferred because they offer cheaper large-scale production, ease of genetic engineering approaches, enormous strain availability etc. (Abdel-Fattah et al. 2013; Abd-Elhalem et al. 2015; Afrisham et al. 2016). It is extensively used in several industries and plays a substantial role in them (Table 1).

Despite having lots of industrial applications there are certain shortcomings related to the use of α-amylase. They tend to drop their structural conformations, stability, and catalysis when allowed to work in extreme conditions (Ahmed et al. 2020). To overcome this sensitivity of α-amylase towards harsh conditions, researchers are seeking sources living in extreme environmental conditions. Extremophiles are the organism inhabiting such harsh environment

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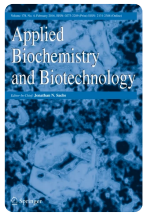

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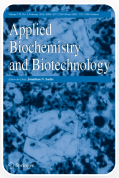

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Abstract

α-Amylases are the oldest and versatile starch hydrolysing enzymes which can replace chemical hydrolysis of starch in industries. It cleaves the α-(1,4)-D-glucosidic linkage of starch and other related polysaccharides to yield simple sugars like glucose, maltose and limit dextrin. α-Amylase covers about 30% shares of the total enzyme market. On account of their superior features, α-amylase is the most widely used among all the existing amylases for hydrolysis of polysaccharides. Endo-acting α-amylase of glycoside hydrolase family 13 is an extensively used biocatalyst and has various biotechnological applications like in starch processing, detergent, textile, paper and pharmaceutical industries. Apart from these, it has some novel applications including polymeric material for drug delivery, bioremediating agent, biodemulsifier and biofilm inhibitor. The present review will accomplish the research gap by providing the unexplored aspects of microbial α-amylase. It will allow the readers to know about the works that have already been done and the latest trends in this field. The manuscript has covered the latest immobilization techniques and the site-directed mutagenesis approaches which are readily being performed to confer the desirable property in wild-type α-amylases. Furthermore, it will state the inadequacies and the numerous obstacles coming in the way of its production during upstream and downstream steps and will also suggest some measures to obtain stable and industrial-grade α-amylase.

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Contamination of Heavy Metal in Water of Bhimgadh Dam at Seoni District Mp: Transporting, Toxicity and Treatment

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Contamination of Heavy Metal in Water of Bhimgadh Dam at Seoni District Mp: Transporting, Toxicity and Treatment

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Abstract
The term ‘‘heavy metal'' refers to any metal and metalloid element that has a relatively high density ranging from 2.1 to 7 g cm^3 in any water body. Generally heavy metals can a water body supply by industrial consumer waste or even from acidic rain breaking down soil and releasing heavy metals into streams, lakes, rivers and ground water. Toxic or poisonous at low concentrations, and includes mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), zinc (Zn), nickel (Ni), Copper (Cu) and lead (Pb); Although ‘‘heavy metals'' is a general term defined in the literature, it is widely documented and frequently applied to the widespread pollutants of soils and water bodies. This study was carried out to the concentration of heavy metals in water of Bhimgarh dam near Seoni city. The detection of heavy metals by different analytical process and confirmation by Atomic Absorption spectrophotometer as required.

***Keywords:** Heavy metals; Concentration; Spectrophotometer; Toxic metal; Water pollution*

Introduction

Among Water pollution is contamination of water by foreign matter that decrease the quality of the water. Water pollution occurs in the oceans, lakes, streams, rivers, underground water and bays, in short liquid containing areas. It involves the release of toxic substances, radioactivity, that becomes deposited upon the bottom and their accumulations will interfere pathogenic germs, substances that require much oxygen to decompose, easy soluble substances, with the condition of aquatic ecosystems e.g. Lack of oxygen in a water body caused by excessive algae growths because of enrichment of pollutants. (According to the water cycle, naturally water around us will be absorbed to the land (soil) and rivers will stream from the upstream to the downstream and released to the sea). In normal situation organic pollutants are biodegraded by microbes and converted to a form that brings benefits to the aquatic life. And for the inorganic pollutants, in the same situation, don't bring too much hazards because they are widely dispersed and have almost no effect to the environment which they are released. Some of the pollutants like lead (Pb), arsenic (As), mercury (Hg), chromium (Cr) specially hexa valent chromium, nickel (Ni), barium (Ba), cadmium (Cd), cobalt (Co), selenium (Se), vanadium (V), oils and grease, pesticides, etc are very harmful, toxic and poisonous according WHO [1,2]. There are some minerals which are useful for human and animal health in small doses, which these are e.g. Zinc (Zn), copper (Cu), iron (Fe) [3,4] etc of all into this category. In agriculture field some elements like zinc, copper, manganese (Mn), sulphur (S), iron, boron (B), together with phosphates, nitrates, urea, potassium, etc are useful in insecticides and pesticides as prescribed quantities.

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Materials and Methods

Sources of heavy metal

Environmental pollution from hazardous metals and minerals can arise from natural well as anthropogenic sources. Natural sources like: seepage from rocks into water, volcanic activity, forest fires, partitioning of polluting elements (which are concentrated in clay minerals with high absorption capacities) between sedimentary rocks and their precursor sediments and water, with rapid industrialization and consumerist life style, sources of environmental pollution have increase. The pollution occur both level of industrial production as well as end use of the products. These toxic elements enter the human body mostly through food and water and to a lesser extent through inhalation of polluted air, use of cosmetics, drugs, poor quality herbal formulations and `Unani' formulations, even items like toys which have paints containing lead. Sources of heavy metals Chromium Mining, industrial coolants, chromium salts manufacturing and leather tanning (Table 1).

- **Mercury (Hg)**: Chlor alkali plants, thermal power plants, fluorescent lamps, hospital waste, electrical appliances etc [5].
- **Lead (Pb)**: lead acid batteries, paints, E waste, Smelting operations, coal based thermal power plants, ceramics, bangle industry
- **Arsenic (As)**: Geogenic/Natural processes, smelting operations, thermal power plants and fuel
- **Copper (Cu)**: Mining, electroplating, smelting operations
- **Vanadium (V)**: Spent catalyst, sulphuric acid plant
- **Nickel (Ni)**: Smelting operations, thermal power plants, battery industry.
- **Cadmium (Cd)**: Zinc smelting, waste batteries, e waste, paint sludge, incinerations and fuel combustion
- **Molybdenum (Mo)**: Spent catalyst.
- **Zinc (Zn)**: Smelting, electroplating.

TABLE 1. **Standard level of heavy metal concentration in drinking water and their effects on human health.**

SN	Metal	Effects	Drinking water standards
1	Lead	• Phytotoxic [6,7]. • Tiredness, irritability anemia and behavioral changes of children. • Toxic to humans, aquatic fauna and livestock High doses cause metabolic poison [8]. • Hypertension and brain damage.	• maximum concentration: 0.1 mg L^{-1}. • By European Community: 0.5 mg L^{-1}. • According to water quality (India) 0.1 mg L^{-1}.
2	Nickel	• High phytotoxicity Damaging fauna • High conc. can cause DNA damage [9]. • Eczema of hands..	• By the Environmental Protection Agency. • maximum concentration: 0.1 mg L^{-1} By (India): 0.1 mg. • According to water quality (India) 0.1 mg L^{-1}.
3	Chromium	• Necrosis nephritis and death in man (10 mg kg^{-1} of body weight as hexavalent chromium). • Irritation of gastrointestinal mucosa.	• By the Environmental Protection Agency. • maximum concentration: (hexavalent and trivalent) total 0.1 mg L^{-1}. • By European Community: 0.5 mg L^{-1}. • Regulation of water quality (India) 0.1 mg L^{-1} [10].
4	Copper	• irritation followed by depression • Causes damage in a variety in aquatic fauna. • Mucosal irritation and corrosion Central nervous system .	• By the Environmental Protection Agency maximum concentration: 1.3 mg L^{-1}. • By European Community: 3 mg L^{-1} • According to water quality (India)

			0.01 mg L^{-1}.
5	Zinc	• Lack of muscular coordination abdominal pain etc [11]. • Phytotoxic Anemia.	• By European Community: 5 mg L^{-1} • According to water quality (India), 0.1 mg L^{-1}. • By the Environmental Protection Agency maximum concentration: 0.4 mg L^{-1}.
6	Cadmium	• Emphysema. • Anemia Acute effects in children. • Cause serious damage to kidneys and bones in humans Bronchitis.	• By the Environmental Protection Agency maximum concentration: 0.004 mg L^{-1}. • By European Community: 0.02 mg L^{-1}. • Regulation of water quality (India) 0.005 mg L^{-1}.
7	Mercury	• Poisonous. • Disturbs the cholesterol. • Causes mutagenic effects.	• By the Environmental Protection Agency maximum concentration of Hg: 0.002 mg L^{-1}. • By European Community: 0.001 mg L^{-1}. • According to water quality (India) 0.003 mg L^{-1}.
8	Arsenic	• Causes toxicological and carcinogenic. • Effects Causes melanosis. • Keratosis and hyperpigmentation in humans Genotoxicity through generation of reactive oxygen species and lipid. • Peroxidation. • Immunotoxic Modulation of co-receptor expression.	• World Health Organization guideline of 0.01 mg L^{-1}. • By European Community: 0.01 mg L^{-1}. • According to water quality (India): 0.05 mg L^{-1}.

Experiment

Water samples were collected from different sample sources of the different region of the Bhimgadh Dam, Seoni city in the period of October 2018 to Decemebr 2018. The water samples volume 500 ml in polythene bottles which acidify with nitric acid to bring down the pH up 2.0. The samples for heavy metals analysis were collected separately and acidify immediately. Metals like Pb, Ni, Hg, As, Cd, Cr, Cu, Fe, Mn and Zn were analyzed by different analytical estimation method and after analyzed by Perkin Calmer Flame AAS (Model 2380) using standard methods. All water samples were analyzed in January 2019.

Purification techniques

Bioaccumulation of heavy metals in food chains and their toxicity to biological systems due to increased concentration over time have led to tremendous pressure for their separation and purification. Heavy metals can enter into water bodies through agricultural runoff, industrial effluents, household uses and from commercial applications. We can remove heavy metals from drinking water very easily with reliable technology. Several technologies available in the market remove a huge range of metals commonly found in drinking water and wastewater effluents. There are various remediation technologies that have been used for the removal of heavy metals from waste water. These remediation technologies are summarized as:

1. Precipitation and coagulation
2. Ion exchange
3. Membrane filtration
4. Bioremediation
5. Heterogeneous photo catalysts
6. Adsorption

Electro-coagulation

Electro-coagulation consists of electrodes that act as the anode and cathode, where oxidation and reduction takes place. Many physicochemical processes such as oxidation reduction, coagulation and adsorption govern the electro-coagulation. Similarly to other treatment techniques, the electro-coagulation of heavy metals offers a cost effective and easy handling technique on an industrial scale.

a. Clays/Layered Double Hydroxides (LDHs)
b. Biomass and Bio-sorption of Metal Ions
c. Magnetic Nano particles as Nano sorbents
d. Removal of Iron and Manganese from Water

Ion exchange

Ion exchange resins provide many advantages and are one of the most widely techniques used for treatment of wastewater effluents. Lee and Nicol have used the Diphonix resin to remove ferric iron from a cobalt sulfate solution with various pH ranges. A lower pH and higher dose of resin gives a higher removal of iron from solution. Elution of iron was observed with an increase of Ti (III) in the sulfuric acid eluent. These workers found that the iron elution enhancement with Ti (III) was due to the combined effects of a reduction of Fe (III) and competitive adsorption of Ti (III) and Ti (IV) ions. A mathematical mode was used to predict the equilibrium, which gave a good fit for the experimental data in various solutions.

Membrane filtration

Membranes are complex structures that contain active elements on the nanometer scale. Modern day reverse osmosis membranes are typically homogeneous polymer thin films supported by a porous support structure.

Phytoremediation

Bioremediation is the technological process whereby biological systems, plants and animals, including microorganisms, are harnessed to affect the cleanup of pollutants from environmental matrices.

Heterogeneous catalysts and catalysis

Remarkable discovery much research has been carried out on the efficiency of Cr (VI) and TiO_2 as a photo catalyst. During the past few years, the applications of TiO_2 for environmental cleanups have been performed by several laboratories for the treatment of industrial effluents.

Activated carbons

Activated Carbon is used in water filter purifiers because activated carbon removes from the water most toxic organic compounds in water like pesticides and heavy metal organic compounds. It makes water safe to drink by removing most toxic organic compounds in water like pesticides and heavy metal organic compounds. Activated carbon water filter works is because activated carbon is an extremely porous material that attracts and holds on its surface harmful chemicals by a process known as 'Adsorption'. Adsorption happens due to electrostatic forces of attraction known as 'Van-der Waals forces' or 'chemisorption'. Activated carbon is very effective in removing bad odour from air or water. Activated carbon can also remove bad taste from water.

Result and Discussion

In the above study (Table 2) heavy metal Fe (iron) and copper (Cu) are found nearest level of standard value according to WHO. On the other side Ni (0 mg/l), and Hg (0 mg/l) are absent in the water sample, Whereas Pd, As, Cd, Cr, Mn and Zn are found less quantity respect to standard value of WHO [10].

TABLE 2. **Analytical results of heavy metal in various sample of different water sample of Bhimgadh Dam at Seoni City.**

S.N	Heavy Metal	standard value in (mg/L) According (WHO)	observed values in (mg/L)
1	Pb (Lead)	0.05 mg/l	0.001 mg/l
2	Ni (Nickel)	0.02 mg/l	0 mg/l
3	Hg (Mercury)	0.001 mg/l	0 mg/l
4	As (Arsenik)	0.05 mg/l	0.001 mg/l

5	Cd (Cadmium)	0.005 mg/l	0.003 mg/l
6	Cr (Chromium)	0.1 mg/l	0.04 mg/l
7	Cu (Copper)	1 mg/l	0.8 to 0.2 mg/l.
8	Fe (Iron)	0.1 mg/l	0.1 mg/L
9	Mn (Mangnige)	0.5 mg/l	0.01 to 0.03 mg/l
10	Zn (Zink)	5.0 mg/l	2.0 mg/l

Conclusion

The presence of heavy metals and their toxicity to the water and to human beings is posing a serious challenge to environmental engineers [12,13] with respect to the treatment of Waste water effluents prior to discharge into the nearby water bodies. Several removal techniques have been developed and applied for the treatment of water to remove the toxic metal ions. Techniques such as microbe assisted: phyto-remediation [14,15], ion exchange, membrane filtration, photo-catalytic oxidation and reduction and adsorption [16,17] have their own advantages and disadvantages over metal ion sequestrations from environmental matrices. Adsorbents such as clays, LDHs [18], zeolites, carbon nano-tubes and their composites, activated carbons, biomass derived bio-sorbents, inorganic nano-materials, inorganic organic hybrid nano-composites and magnetic nano-materials have been synthesized and investigated for their ability to sequester metal ions from water [19]. Magnetic nano-particles are very promising for applications in catalysis, bio-labeling and bio-separation [20,21]. In liquid-phase extraction of heavy metals and dyes in particular, such small and magnetically separable particles may be useful as they combine the advantages of high dispersion, high reactivity [22], high stability under acidic conditions and easy separation [23].

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