Conclusion
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The versatility of GO, has stimulated research on biochemical and molecular aspects of this important enzyme belonging to the Oxidoreductase family. It is very essential to study characteristic of GO with respect to regulation of biosynthesis and mechanical action. An increasing number of publications in recent years have reported that GO is possible to extract from new sources. Their sequencing and strain improvement is done by immobilization and mutagenesis. Many reports are available on applications in Food and Pharmaceutical industry in the form of biosensor, which explains the unique specificity and safe use of biocatalyst. Since GO is industrially important enzyme, many reviews have been published covering various aspects of enzyme. At present, most of the available commercial glucose oxidase is obtained from fungal sources which are not thermo stable and active in pH range up to 6.0.

To optimise the bio-processing parameters for enhanced production; increase the stability and efficiency of enzyme and to expand the areas of applications it is the need of industry to search for new strains producing glucose oxidase. Keeping in mind these objectives we have carried out research on screening of such novel strain and studied them in detail for production and applications of novel glucose oxidase. Sampling plays an important role in isolating new microbes which are habituate of different environment. Likewise we have isolated various thermophilic/alkalophilic and thermoduric microorganisms and by applying various strategies we have selected a strain of Aspergillus which was identified at molecular level by 28S rDNA technology as Aspergillus niger F-C405-2. The fermentation medium was modified and newly designed by using native components and other supplementary carbon and nitrogen sources which were found to increase the production. Similarly other physiological and environmental parameters were optimised and used for large scale production in the form of stirred batch fermentation. Similarly strain improvement was carried out and random mutagenesis with UV radiations and treatment with ethidium bromide gave satisfied results. By considering associated risk we have selected UV mutagenesis further for programme mutagenesis. From the point of biotechnology it is every important to introduce novel methods. The production of glucose oxidase was carried out using different immobilising matrices. Immobilisation has lot of advantages as to keep the product intact and pure; one can reuse the intact cells for many times for production, protecting the cells as well as enzyme against various corrosive agents in fermentation medium etc. In present study we were successful in getting near about all these advantages.
Immobilised cells with cellulose acetate produced GO in short period of time (48 hours) as compared to intact cells, enhanced thermal (50°C for 180 min) and pH stability and many other properties. The physico-chemical characters of our glucose oxidase were found to be matching with what is recorded by various scientists as per their reviews. Overall our enzyme is homodimeric protein confirmed by zymogram analysis with molecular weight of 90,000 D of each subunit. Its pI was found to be 4.2. Though amino acid sequencing was not possible for us we have tried to determine approximately the presence of amino acids in polypeptide of glucose oxidase by simple mechanism of acid hydrolysis. The located amino acids matched with that reported by others. Kinetic parameters revealed that there is positive impact of immobilization on enzyme activity. Significant decrease in $K_m$ in case of immobilised glucose oxidase indicated matrix of cellulose acetate provided intact environment to increase affinity of enzyme towards substrate. For scale up process it is important to study about how the gene of glucose oxidase is regulated at molecular level. It was observed that metabolism of glucose is carried out by different enzymes using various pathways. Expression of glucose oxidase is carried out due to shift in metabolic pathway from Glycolysis into Pentose Phosphate Pathway. In our study we observed that glucose oxidase is an inducible enzyme and its synthesis is induced by glucose and calcium carbonate. In presence of both of these inducers it was observed that in *Aspergillus niger* F-C405-2 the metabolic pathway was shifted from glycolytic to different pathways mostly PPP pathway due to which intracellular concentration of glucose oxidase and catalase was found to be increased. While in presence of copper ions the gene expression was found to be suppressed. These regulation studies will be helpful while designing expression vectors for over expression of glucose oxidase.

In vitro utility of our enzyme was studied in various fields. It was found to be a novel preservative and stabiliser as glucose oxidase results into synthesis of gluconic acid. It not only reduces alcohol content (by 2%) in wine but also increases shelf life of wine. Similarly we have also used this enzyme in baking, production of gluconic acid etc. In our last phase of research work more emphasis is given on designing biosensor. The biosensor we have designed was used to determine blood sugar of different patients. As per our observations we have proved the efficiency of our biosensor by comparing its efficiency with that commercially available glucometer. Statistical evidences have proved its efficacy.

Overall our research findings have presented new strain of *Aspergillus niger* F-C405-2 which is moderately thermophilic. Generally this phenomenon of thermophily was very well studied in bacteria than in fungi. Hence in depth study for thermophilic fungi is demand
of research. Thus such novel strain which produces glucose oxidase at elevated temperature like 50°C and can be produced using cheap carbon sources like molasses, beat sugar etc which is always economical. Another important aspect of enzyme is thermo stability which will help for broad spectrum applications of said enzyme. Our findings will definitely add values in upcoming research.

**Future Prospect:**

- Optimization of Glucose oxidase production by recombinant DNA technology in *E.coli* and *Saccharomyces cerevisiae*.

- Application of glucose oxidase in textile industry as a bleaching agent along with hydrogen peroxide.

- Other application of GO-CAT enzyme system in various food products as a preservative.

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