ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

Genome scale Reconstruction of the saccharomyces Cerevisea Metabolic Network

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***Abstract:*-- Systems biology is the computational and mathematical modeling of complex biological systems. It is a biology-based interdisciplinary field of study that focuses on complex interactions within biological systems, using a holistic approach to biological research. Genome scale metabolic flux analysis is an emerging in the field of system biology. Metabolic flux analysis (FBA) is an experimental fluxomics technique used to examine production and consumption rates of metabolites in a biological system. FBA can give a large number of mathematically acceptable solutions to the steady-state. However solutions of biological interest are the ones which produce the desired metabolites in the correct proportion of these metabolites. For instance when modeling the growth of an organism the objective function is generally defined as biomass. Performing various experiments in an invivo system is a difficult task and an expensive and time consuming process. Through system biology it is possible to bring the difficult an easy process. By constructing the stoichiometric metabolic equation of an organism and simulation through software's make it easy to find the best possible solution for the higher production. This also helps in finding the better solution for knocking in and knocking out stratergies.**

***Keyword:*-Metabolic Flux, Stoichiometric Metabolites, knocking in, knocking out.**

1. INTRODUCTION

Genome scale represents the gene which is major role in a cells. Flux means flow in cell, cell is a living organism. In this project our aim is to develope and improve the product by feeding substrate to the cells. Substrate is a most important feed to the cells in fermentation technology. Substrate make the Sucrose ,Glucose, Fructose and various components to the cells. Substract will be converted as a product through fermentation process in living organism and different metabolites given as a final one.

Yeast is the organism commonly used for this process that is the organism we can say as saccharomyces cerevisiae. saccharomyces cerevisiae is a biological name of species or strain. In this we can analyse the pathway how the substrates are taken in to the cells through process and various metabolites are added in the cell to get the end product alcohol ,with the output it also gives the unwanted products.
This can be done in a practical approach but the main disadvantage is we have to do various experiments. It is a long time process. It takes too much of time to finish the work. To reduce the time and the process, the mathematical modeling is done through the matlab software. This software will provide the output as users requirement. It is an assumption of the product to improve the product through the software. If the user changes the substrate the pathway will be changed and also it will change the output.

S.cerevisiae is mostly used in biotechnological production organism as well as in eukaryotic model system. Eukaryotic microorganism is a cell that contain the nuclear surrounded by a membrane. It also contains DNA bounded together with protein. S.cerevisiae is used in the production of foods, alcoholic beverages and in different pharmaceutical industries. S.cerevisiae is a very attractive organism to work because it is nonpathogenic. The another reason, why S.cerevisiae is used in biotechnology is, it is susceptibility to genetic modifications by recombinant DNA technology.

The classical breeding and genetic crossing of two strain ie., combining two products that produces mutants(new generation). DNA technology has enabled us to change a given pathway of interest and to improve the cell by direct approach. It introduces a specific genetic perturbation for the modification the promoter strength of a given gene, to perform a gene deletion or to introduce a new gene or pathway to a cell.

Application of genetic engineering has been referred to as metabolic engineering. This consist of two important parts 1) analytical change of metabolic engineering which deals with

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the change of cells to identify the promising target for genetic manipulation. 2) genetic engineering of the cells, where the cell with genetic modification is constructed. Different target of metabolic engineering is as follows:

- ✓ Extension of substrate range.
- ✓ Improvement of productivity and yield.
- ✓ Elimination of by-products.
- ✓ Improvement of process performance.
- ✓ Improvements of cellular properties.
- ✓ Extension of product range including heterologous protein production.
- ✓ Careful analysis of the cellular system (analysis part).
- ✓ Construction of the recombinant strain. (synthesis part).

This is well illustrated in attempts to extend the substrate range. Here the first step is clearly to introduce heterologous genes that enable metabolism of the substrate of interest, and for this purpose it is relevant to consider two different strategies: (i) introduction of a gene encoding a membrane-bound protein that transports the given substrate into the cell in addition to a gene encoding a protein responsible for cleavage of the substrate if necessary; and (ii) introduction of a gene encoding a protein that is secreted into the extracellular medium, whereby the substrate of interest is converted or cleaved into substrates that may be directly assimilated by the host organism. Independent of which strategy is chosen, it is important to ensure that the heterologous gene(s) is sufficiently expressed in the new host system. Once a recombinant organism that may use the substrate has been constructed, it often exhibits low uptake rates and low overall yields of product on the relevant substrate. To identify the underlying problem and direct the next synthesis step, it is necessary to perform a detailed analysis of the cell physiology. This is clearly illustrated in the attempt to convert xylose to ethanol by anaerobic fermentation of S. cerevisiae . Thus, metabolic engineering will almost always involve a close interaction between the synthesis and analysis parts, and often several rounds of strain construction are needed before an optimal recombinant strain is obtained. the synthesis part is relatively straightforward at least if the genes to be expressed are available and it is often the analysis part that is limiting. This is due to the complexity of the cellular metabolism, i.e., the metabolite levels may interact with gene expression and, conversely, gene expression might determine the metabolite levels via the enzyme concentrations. The analysis part has classically been referred to as physiology; in recent years a number of very powerful techniques have been developed that enable a far more in-depth analysis of the cellular physiology. These include DNA array technology for transcriptome analysis (simultaneous quantification of all gene transcripts in a cell), two-dimensional gel electrophoresis for proteome analysis (simultaneous quantification of a large number of proteins in a cell), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) methods for metabolome analysis (analysis of the intracellular metabolite levels), 13 C- labelling experiments for metabolic network analysis, advanced fermentation experiments with on-line monitoring of important cultivation variables, and bioinformatics (including mathematical models for analysis of pathway structures and control of pathway fluxes).

II. TRANSCRIPTOME AND PROTEOME ANALYSIS

The application of DNA arrays to transcriptome analysis is still a new technique, and there are presently no examples of how this technique has been used in the field of metabolic engineering. However, since many challenges in metabolic engineering involve multiple genetic changes, transcriptome analysis will be very important for metabolic engineering in the future, since this approach enables a study of the expression pattern of many genes. Furthermore, it is often found that a single mutation (disruption or overexpression of a certain gene) results in a completely different expression pattern, and DNA array technology will therefore be a very powerful technique for analysis of the consequences of the individual genetic changes. Furthermore, it is often found that a single mutation (disruption or overexpression of a certain gene) results in a completely different expression pattern, and DNA array technology will therefore be a very powerful technique for analysis of the consequences of the individual genetic changes.

As well as transcriptome analysis, proteome analysis is important in metabolic engineering. Often the pathway activity is directly correlated with the protein concentration, and when gene expression and/or protein-protein interactions are subjugated to metabolic regulation, it is important to quantify the protein levels in the different recombinant

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strains constructed. Clearly, a detailed proteome analysis may be valuable, but often it is sufficient to measure the levels of the proteins involved in the pathway studied and perhaps some of the regulatory proteins affecting the expression of the relevant genes.

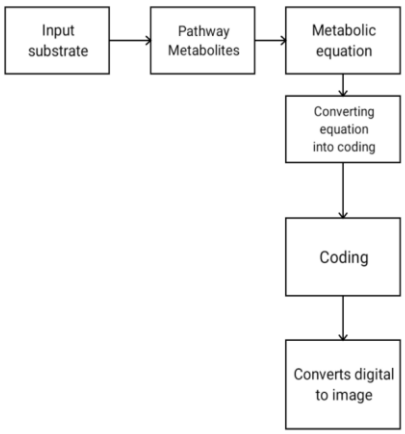

Fig.2.1 Overall Architecture Diagram

In this work the input substrate ie., glucose is taken as an input. For the next process it requires the pathway to create an equation. Then create a metabolic equation in mathematical representation. Covert this mathematical equation into coding .coding is done in matlab. The matlab equation will produce output in image format.

Pathway Analysis

Pathway analysis is often used to describe the application of metabolic flux analysis (MFA) and metabolic control analysis. Pathway analysis has proven successful as a guiding tool for the analytical part of metabolic engineering . MFA is a “global” cellular approach, where the complete network of intracellular reactions is considered and the fluxes, through the individual branches of the network, are quantified. The metabolic fluxes can be estimated from metabolite balances and measurements of a few fluxes, but introduction of 13 C-labelled substrates followed by measurement of the labelling distribution in intracellular metabolites are often used today, which serves as a far more powerful tool for quantification of the fluxes. In this case nuclear magnetic resonance spectroscopy or GC-MS can be used to measure the labelling pattern of the precursor metabolites.

Starch Utilization

Starch is the storage carbohydrate in plants, and it serves as an important energy and carbon source in biotechnological processes. Starch is made up of long chains of glucose units joined by a-1,4 linkages and joined at branch points by a-1,6 linkages. Many microorganisms, including S. cerevisiae, are not able to degrade starch since they do not produce starch-decomposing enzymes such as a-amylase (which cleaves a-1,4-glycosidic bonds), b-amylase (which cleaves maltose units from the nonreducing end of starch), pullulanase or isoamylase (debranching enzymes that hydrolyze a-1,6-glycosidic bonds), and glucoamylase (which hydrolyzes glucose units from the nonreducing end of starch). Hence, it is necessary either to add starch-decomposing enzymes to the starch before fermentation or to use a recombinant strain that produces starch-decomposing enzymes in order to utilize this carbon source. In the baking industry, it is advantageous to use a certain recombinant strain that does not require a-amylase-enriched flour. For production of low-calorie beer in the brewing industry, it is of interest to use a recombinant strain of S. cerevisiae that secretes a glucoamylase whereby the larger oligomers (dextrins), which are formed from the partial hydrolysis of barley starch, are decomposed. Expression of a glucoamylase gene from As per gillus awamori and secretion of the heterologous protein from S. cerevisiae were successfully demonstrated, but the transformed strain grew on dextrins at a lower rate than observed when glucoamylase was added externally to the medium.

Lactose Utilization

Lactose is abundant in milk and is a major constituent of cheese whey, which is a by-product of cheese production. Only half of the cheese whey production is used for different purposes, including production of biogas, ethanol, and single-cell protein, whereas the other half is discarded as effluent . Thus, cheese whey causes a major environmental problem due to its high biological oxygen demand and the high chemical oxygen demand, primarily as a result of its high lactose content. S. cerevisiae does not have a lactose permease system, and hence lactose cannot be transported across the cell membrane;it also does not encode a b-galactosidase that cleaves theb-1,4-bond of lactose into a glucose residue.

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
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Melibiose Utilization

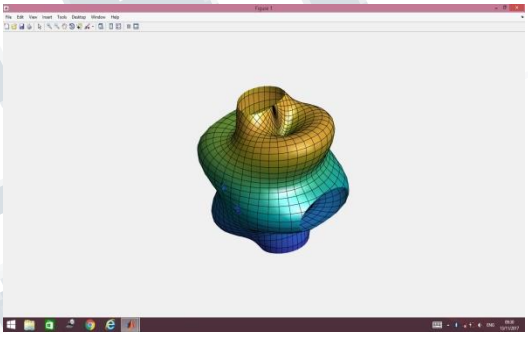
Molasses, which is used for ethanol and baker's yeast production, contains mainly glucose, fructose, and sucrose, but the trisaccharide raffinose is also present to some extent. Invertase, encoded by the SUC2 gene, decomposes raffinose into fructose and the disaccharide melibiose, which also contains a glucose residue and galactose residue similar to lactose, but the glucose and galactose moieties of melibiose are linked by an a-1,6-bond. Only a few strains of S. cerevisiae are able to assimilate melibiose, and many strains are not able to cleave the a-1,6-linkage of melibiose since they are missing one of the MEL1 to MEL11 genes, all encoding melibiase (a-galactosidase), which is secreted to the medium outside the cell. Baker's yeast strains able to utilize melibiose by expressing the MEL1 gene have been constructed by genetic engineering.

Equations

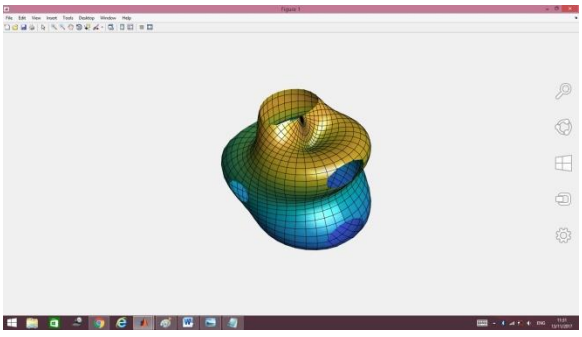
1. H2O[e] + sucrose[e] => fructose[e] + glucose[e]
2. glucose[e] => glucose[c]
3. fructose[e] => fructose[c]
4. ATP[c] + glucose[c] => ADP[c] + glucose 6
5. ATP[c] + fructose[c] => ADP[c] + fructose 6
6. glucose 6-phosphate[c] <=> fructose 6-phosphate[c]
7. ATP[c] + fructose 6-phosphate[c] => ADP[c]
8. fructose 1,6-bisphosphate[c] => dihydroxyacetone phosphate[c] + glyceraldehyde 3-phosphate[c]
9. dihydroxyacetone phosphate[c] <=>glucose
10. glyceraldehyde 3-phosphate[c] + NAD+[c]
11. 1,3-bisphosphoglycerate[c] + ADP[c] => ATP[c] + H2O[c] + PEP[c]
12. ADP[c] + PEP[c] => ATP[c] + pyruvate[c]
13. H2O[c] => H2O[e]
14. pyruvate[c] => pyruvate[e]
15. pyruvate[c] => acetaldehyde[c] + CO2[c]
16. acetaldehyde[c] + NADH[c] => NAD+[c]
17. ethanol[c] => ethanol[e]
18. CO2[c] => CO2[e]
19. sucrose[b] => sucrose[e]
20. pyruvate[e] => pyruvate[b]
21. H2O[e] <=> H2O[b]
22. CO2[e] => CO2[b]
23. ethanol[e] => ethanol[b]
24. ATP[c] + H2O[c] => ADP[c] + phosphate[c]

Performance analysis

Metabolic Flux Analysis (MFA) is a fundamental metabolic engineering tool to understand in vivo cell physiology by integrating system response for gene-protein- metabolites interaction, i.e. quantification of all steady state intracellular metabolite fluxes through the central metabolic reaction network . The final outcome of MFA is a metabolic flux map that comprises of catabolic and anabolic reaction fluxes for certain species under specific growth conditions.



a)The figure gives the required product, the top layer is the final product .Other two are unwanted products.



b)The product is obtained with the high throughput range.

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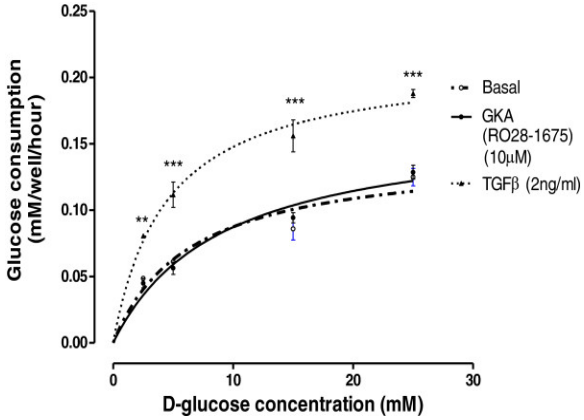


Fig: 3.1 Calculating the amount of glucose.

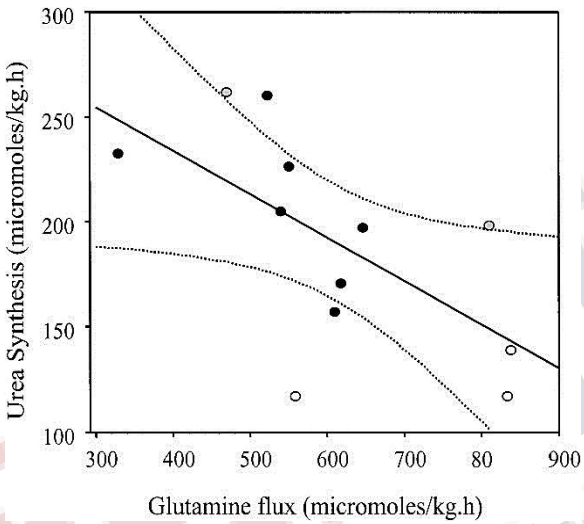


Fig: 3.2 Flux analysis

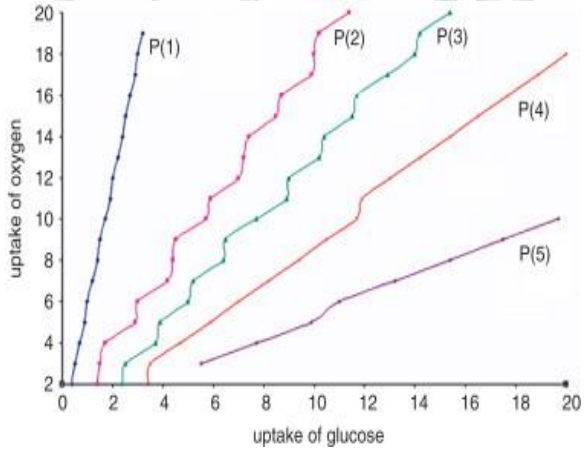


Fig:3.3 Uptake calculation.

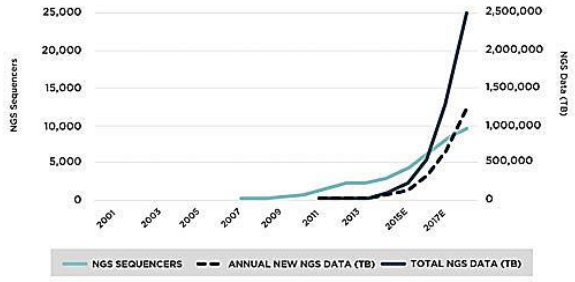


Fig: 3.4 NGS sequences

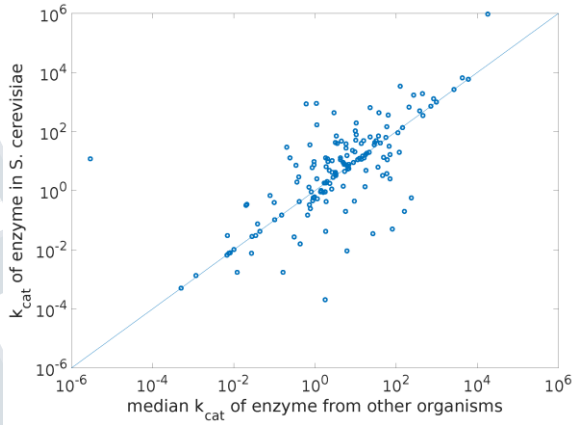
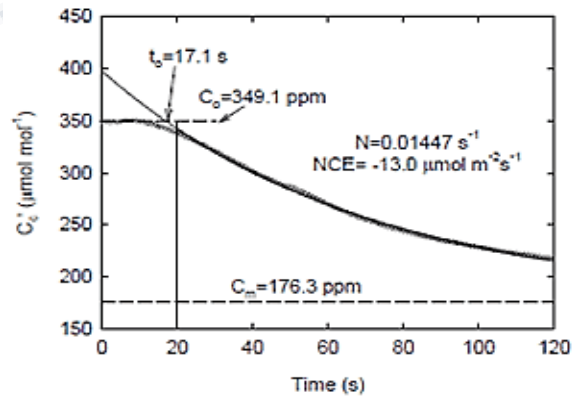


Fig: 3.5 Collection of enzymes



Fig; 3.6 Metabolite analysis

CONCLUSION

Taking the above considerations together, it is obvious that successful performance of metabolic engineering is a multidisciplinary field that requires inputs from several

specialists. Clearly, geneticists and molecular biologists are the drivers in implementing the appropriate genetic modifications, but analytical chemists, biochemists, and biochemical engineers also play important roles in the analysis part of metabolic engineering. Thus, to support the analytical side of metabolic engineering, which includes the theoretical tools mentioned above, analytical chemistry contributes methods necessary for quantifying the extra- and intracellular metabolite levels and biochemistry provides valuable information about pathway regulation and enzyme kinetics. Additionally, biochemical engineering is needed to integrate the information obtained by the different analytical techniques and, based on this, to define appropriate strategies for manipulation of the cell. This can be achieved through the use of advanced bioreactor systems, where the important cultivation variables are monitored on-line. With these bioreactor systems, it is possible to study the influence of a single medium component on cellular function while keeping all other factors constant. By changing the feed rate of medium to a continuous bioreactor system, it is possible to change the dilution rate, which equals the specific growth rate under steady-state conditions.

ACKNOWLEDGEMENT

We would like to thank all the authors of different research papers, to be referred during writing this paper. It was very knowledge gaining and helpful for the further research to be done in future. In account of we are much indebted to thank anna university Chennai, Department of biotechnology for their support.

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