

Figure 3: Effect of sucrose on decolorization and biomass

*Effect of Different Nitrogen Sources on decolorization and biomass*

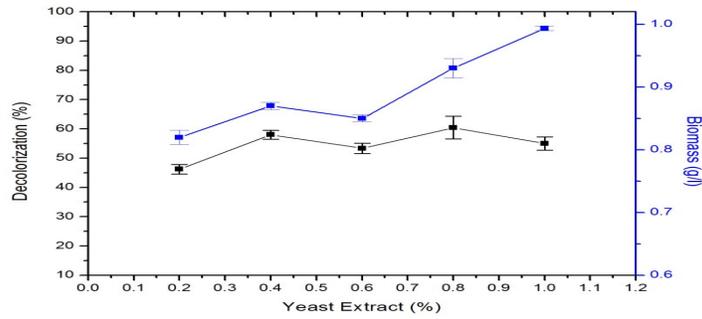


Figure 4: Effect of Yeast extract on decolorization and biomass

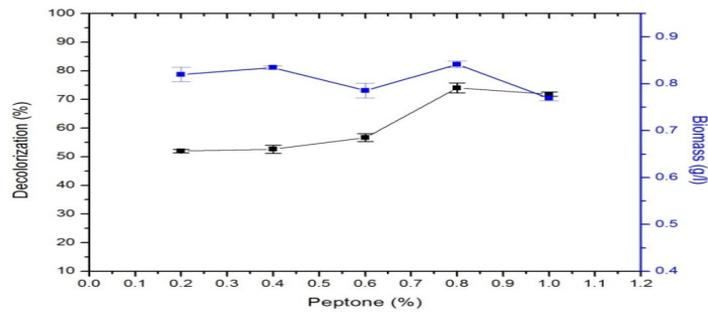


Figure 5: Effect of Peptone on decolorization and biomass

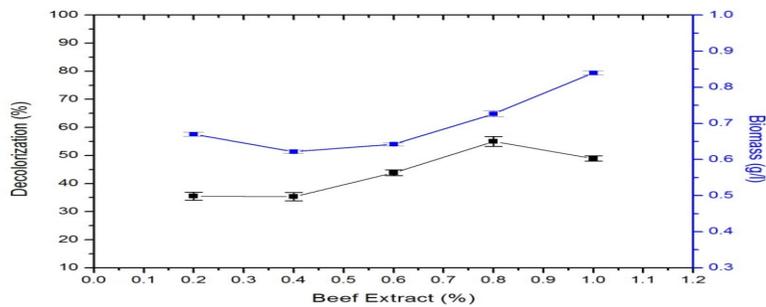


Figure 6: Effect of Beef extract on decolorization and biomass  
 Experimental run from Design expert

Table 1: Experimental runs

| Std | Run | Factor 1<br>A:Glucose | Factor 2<br>B:Peptone | Factor 3<br>C:KH <sub>2</sub> PO <sub>4</sub> | Response 1<br>Decolorization |
|-----|-----|-----------------------|-----------------------|---|------------------------------|
| 14  | 1   | 0.8                   | 0.8                   | 0.075   | 74                           |
| 15  | 2   | 0.8                   | 0.8                   | 0.075   | 74                           |
| 2   | 3   | 1                     | 0.6                   | 0.075   | 61                           |
| 3   | 4   | 0.6                   | 1                     | 0.075   | 70                           |
| 12  | 5   | 0.8                   | 1                     | 0.1   | 60                           |
| 13  | 6   | 0.8                   | 0.8                   | 0.075   | 74                           |
| 8   | 7   | 1                     | 0.8                   | 0.1   | 65                           |
| 6   | 8   | 1                     | 0.8                   | 0.05  | 69                           |
| 5   | 9   | 0.6                   | 0.8                   | 0.05  | 67                           |
| 16  | 10  | 0.8                   | 0.8                   | 0.075   | 74                           |
| 10  | 11  | 0.8                   | 1                     | 0.05  | 71                           |
| 17  | 12  | 0.8                   | 0.8                   | 0.075   | 74                           |
| 11  | 13  | 0.8                   | 0.6                   | 0.1   | 75                           |
| 4   | 14  | 1                     | 1                     | 0.075   | 68                           |
| 7   | 15  | 0.6                   | 0.8                   | 0.1   | 62                           |
| 1   | 16  | 0.6                   | 0.6                   | 0.075   | 66                           |
| 9   | 17  | 0.8                   | 0.6                   | 0.05  | 64                           |

VI. ANOVA RESPONSE AND VALUES

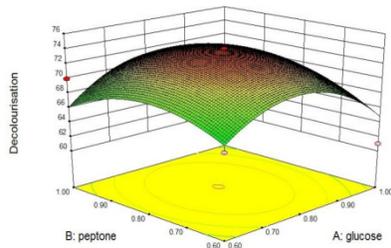
| Source      | Sum of Squares | df | Mean Square | F Value | p-value Prob > F | Significant     |
|-------------|----------------|----|-------------|---------|------------------|-----------------|
| Model       | 1.420E+007     | 9  | 1.577E+006  | 11.34   | 0.0004           | significant     |
| A-A         | 1.351E+005     | 1  | 1.351E+005  | 0.97    | 0.3476           |                 |
| B-B         | 6.683E+005     | 1  | 6.683E+005  | 4.80    | 0.0532           |                 |
| C-C         | 1.298E+006     | 1  | 1.298E+006  | 9.33    | 0.0122           |                 |
| AB          | 6728.00        | 1  | 6728.00     | 0.048   | 0.8304           |                 |
| AC          | 12482.00       | 1  | 12482.00    | 0.090   | 0.7706           |                 |
| BC          | 1.358E+006     | 1  | 1.358E+006  | 9.76    | 0.0108           |                 |
| A²          | 3.382E+006     | 1  | 3.382E+006  | 24.32   | 0.0006           |                 |
| B²          | 5.897E+006     | 1  | 5.897E+006  | 42.39   | < 0.0001         |                 |
| C²          | 3.494E+006     | 1  | 3.494E+006  | 25.12   | 0.0005           |                 |
| Residual    | 1.391E+006     | 10 | 1.391E+005  |         |                  |                 |
| Lack of Fit | 1.069E+006     | 5  | 2.139E+005  | 3.33    | 0.1066           | not significant |
| Pure Error  | 3.215E+005     | 5  | 64302.27    |         |                  |                 |
| Cor Total   | 1.559E+007     | 19 |             |         |                  |                 |

The Model F-value of 11.34 implies the model is significant. There is only a 0.04% chance that a "Model F-Value" this large could occur due to noise.

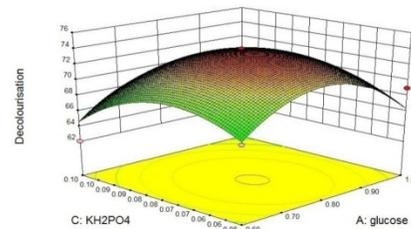
Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C, BC, A², B², C² are significant model terms.

| Std DEV | Mean    | C.V<br>% | PRESS      | R-<br>Squared | Adj R-<br>squared | Pred R<br>squared | Adeq<br>Recession |
|---------|---------|----------|------------|---------------|-------------------|-------------------|-------------------|
| 372.96  | 4925.65 | 7.57     | 8.775E+006 | 0.9108        | 0.8304            | 0.4370            | 10.037            |

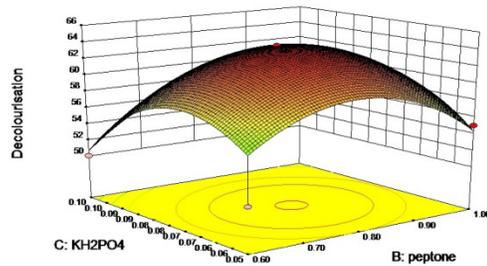
*Effect of Interaction between nutrients*



Glucose and Peptone

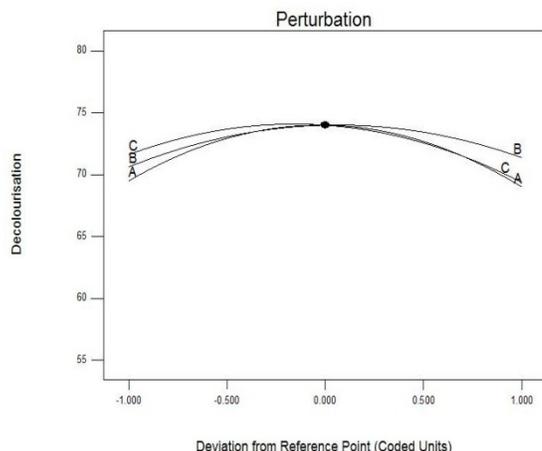


Glucose and KH<sub>2</sub>PO<sub>4</sub>



Interaction between Peptone and KH<sub>2</sub>PO<sub>4</sub>

The advantage of using RSM is that the prediction of limiting nutrient which is given by the perturbation graph. Here “A” i.e. glucose is the limiting nutrient



Perturbation graph for prediction of limiting nutrient

## REFERENCES

- [1] Aoshima, I., Tozawa, Y., Ohmomo, S. and Ueda, K. (1985) 'Production of decolourizing activity for molasses pigment by *Coriolor versicolor Ps4a*', Journal of Agric. Biol. Chem Vol. 49, pp. 2041–2045.
- [2] D'souza, D. T., Tiwari, R., Sah, A. K. and Raghukumar, C. (2006)'Enhanced production of Laccase by a marine fungus during treatment of coloured effluents and synthetic dyes', journal of Enz. Micro.Technol., Vol.38, pp. 504-511.
- [3] Ikan, R., Dorsey, T. and Kaplan, I. R. (1990) 'Characterization of natural and synthetic humic substances (melanoidins) by stable carbon and nitrogen isotope measurements and elemental compositions', journal of Anal.Chim Acta Vol. 232, pp. 11–18.
- [4] Jiranuntipon, S., Chareonpornwattana, S., Damronglerd, S., Albasi, C. and Delia, M. L. (2008) 'Decolorization of synthetic Melanoidins-containing wastewater by a bacterial consortium', journal of Ind.Microbiol. Biotechnol., Vol. 35, pp. 1313-1321.
- [5] Kalavathi, D. F., Uma, L. and Subramanian, G. (2001) 'Degradation and metabolization of the pigment-melanoidin in distillery effluent by the marine *Cyanobacterium Oscillatoria boryana* BDU 92181', journal of Enzyme Microb.Technol., Vol. 29, pp. 246-251.
- [6] Kirk, T. K., Schultz, E., Connors, W. J., Lorenz, L. F. and Zeikus, J. G. (1978) 'Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*', journal of Arch. Microbiol., Vol. 117, pp.177-185.
- [7] Kumar, V., Wati, L., FitzGibbon, F., Nigam, P. and M., B. e. a. I. (1997)'Bioremediation and decolorization of anaerobically digested distillery spent wash', journal of Biotechnolog., Vol. 19, pp. 311-313.
- [8] Kumar, V., Wati, L., FitzGibbon, F., Nigam, P. et al (1997)'Microbial decolorization and bioremediation of anaerobically digested molasses spent wash effluent by aerobic bacterial culture, Microbios., Vol. 89, pp. 81-90.
- [9] Miyata, N., Mori, T., Iwahori, K. and Fujita, M. (2000) 'Microbial decolorization of melanoidin-containing wastewaters: Combined use of activated sludge and the fungus *Coriolor hirsutus*', journal of Biosc. Bioeng., Vol. 89, pp. 145-150.
- [10] Ohmomo, S., Aoshima, I., Tozawa, Y., Sakurada, N. and Ueda, K. (1985a)'Purification and some properties of melanoidin decolourizing enzymes,P-3 and P-4, from mycelia of *Coriolor vericolor Ps4a*', Journal of Agric. Biol. Chem Vol. 49, pp. 2047–2053.
- [11] Ohmomo, S., Daengsabha, W., Yoshikawa, H., Yui, M., Nozaki, K., Nakajima, T. and Nakamura, I. (1987a) 'Screening of anaerobic bacteria with the ability to decolorize molasses melanoidin', journal of Agric. Biol. Chem Vol. 57, pp. 2429-2435
- [12] Painter, T. J. (1998) 'Carbohydrate polymers in food preservation: an integrated view of the Maillard reaction with special reference to discoveries of preserved foods in Sphagnum-dominated peat bogs.', journal of Carbohyd. Polym Vol. 36, pp. 335–347.
- [13] Pant, D., Singh, A., Satyawali, Y. and Gupta, R. K. (2008) 'Effect of carbon and nitrogen source amendment on synthetic dyes decolourizing efficiency of white-rot fungus, *Phanerochaete chrysosporium*', journal of Environ. Biol., Vol. 29, pp. 79-84.
- [14] Raghukumar, C. and Rivonkar, G. (2001) 'Decolorization of molasses spent wash by the white-rot fungus *Flavodon flavus*, isolated from a marine habitat.Applied Microbiol', journal of biotechnology Vol. 55, pp. 510-514.
- [15] Ravikumar, R., Vasanthi, N. S. and Saravanan, K. (2011) 'Single factorial experimental design for decolorizing anaerobically treated distillery spent wash using *cladosporium cladosporioides*', journal of Environ. Sci.Technol., Vol. 8, pp. 97-106.
- [16] Sirianuntapiboon, S. and Phothilangka, P. O., S. (2004) 'Decolourization of molasses wastewater by a strain no. BP103 of acetogenic bacteria', journal of Biores. Technol., Vol. 92, pp. 31-39
- [17] Watanabe, Y., Sugi, R., Tanaka, Y. and Hayashida, S. (1982) 'Enzymatic decolourisation of melanoidin by *Coriolor sp.* no. 20', journal of Agric. Biol. Chem Vol. 46, pp. 1623–1630.
- [18] Zhao, Y. C., Yi, X. Y., Zhang, M., Liu, L. and Ma, W. J. (2010)'Fundamental study of degradation of dichlorodiphenyl trichloroethane in soil by laccase from white rot fungi', journal of Int. Environ. Sci. Technol., Vol. 7, pp. 359-366.
- [19] Chavan,M.N.,Kulkarni,M.V.,Zope.V.P.,Mahulikar,P.P.,(2006)'Microbial degradation of melanoidins in distillery spent wash by an indigenous isolate',Indian J. biotechnol.,Vol.5,pp.416-421.
- [20] Chavan,M.N.,Kulkarni,M.V.,Zope.V.P.,Mahulikar,P.P.,(2006)'Microbial degradation of melanoidins in distillery spent wash by an indigenous isolate',Indian J. biotechnol.,Vol.5,pp.416-421.
- [21] Harley,J.H., and Prescott,L.M.,(eds)(1996)'Laboratory exercise in microbiology'Vol.3,pp 46-116.
- [22] Shah,V., Joshi,J.P., Kulkarni,P.R., (1989)'Aerobic biological treatment of alcohol distillery waste:Kinetics and microbiological analysis',Indian Chem. Eng.,Vol.1,pp.61-66.