Chapter-V
Summary
And
Conclusion
5.1. Summary:

Fermentation is one of the oldest traditions of the human beings and it is most comprehensive method which has been used for a wide variety of useful products such as bread, acids, antibiotics, enzymes, cheese, yogurt, proteins, and biofuel using biocatalysts. Most important application of this method is the production of biofuel such as bioethanol and biodiesel using cheap carbohydrate source, because bioethanol is ecofriendly fuel and an alternative for Non-renewable energy sources like petroleum fuels, diesel. It can be blended with gasoline, diesel, and petrol. As the fossil fuel crisis increases, the demand of biofuel also increases. To meet the required fuel quantity, bioethanol production through fermentation process in industrial scale is the only alternative method to avoided fuel crisis.

In the present study, the impact of the physical-chemical and nutritional parameters were studied individually using batch fermentation process in 5l bioreactor for the optimization of factors that effects bioethanol productions. After the optimization of standard fermentation factors was completed, central composite design (CCD) was applied to design cost effective fermentation method to produce highest yield of bioethanol with a potential substrate mahua flower extract (MFE) as substrate. Initially, medium-I was formulated with standard optimum fermentation factors and also the medium-II was designed with optimum fermentation factors to enhance the production of bioethanol through response surface methodology in 5l bioreactor.

Bioethanol production process uses a potential microorganism, which converts sugars to fuel alcohol. During this process, the selection and availability of carbohydrate rich feedstock occupies primary stage. A number of feedstocks have been studied by many scientists for bioethanol production. Amongst, mahua flower was proved as alternative
feedstocks for the production of bioethanol at economical cost, because it has good keeping quality.

The present scientific studies evaluated the potential usage of mahua flower extract (MFE) because it is renewable and cost effective substrate. The biochemical analysis of the mahua flower: moisture content of 17 %, total fermentable sugars of 73.13 %, reducing sugars of 18%, protein of 4.6 mg and fat of 0.5 mg. The biochemical analysis of constituents of mahua flower extract indicated that it could stimulate the yeast cells to produce bioethanol in large scale industrial process.

In order to evaluate the fermentative ability of microorganisms, different strains of microorganisms such as bacteria strains and yeast strains of *S. cerevisiae* were subjected to alcoholic fermentation. Amongst, eight strains had a profound effect on bioethanol production, out of which the yeast strain *Saccharomyces cerevisiae*-3190 (NCIM) exhibited significant effect on the conversion of fermentable sugars to bioethanol.

*Saccharomyces cerevisiae*-3190 was grown in medium containing glucose of 1 %, yeast extract of 0.3 %, malt extract of 0.3 % and peptone of 5 % and agar of 2% (GYMP) were mixed in Erlenmeyer flasks and the pH 5 was adjusted by the addition of 0.1 N NaOH and 0.1 M HCL. The growth medium was autoclave at 1210°C temperature for about 30 minutes. After autoclave was completed, the volume 10 ml of sterilized medium was aseptically transferred to petri plates and 20 ml tubes and then one loop full of the original culture of *S.cerevisiae*-3190 was aseptically inoculated on agar slopes under sterile conditions. Using the medium composition, *S. cerevisiae*-3190 culture was maintained in agar slants under physical conditions of pH 5 and incubation temperature of 300°C after 48 hours of incubation. For every 30 days, yeast culture medium was freshly prepared for maintaining cell stability and for entire experimental studies.
Initially, the medium-I was designed and its impact was studied on bioethanol production was carried out using Mahua flower juice as source of carbon with varying substrate concentrations ranging from 40 g.l\(^{-1}\) to 480 g.l\(^{-1}\) with an increment of 40 g.l\(^{-1}\) in 5L bioreactor in the presence of \(S.cerevisiae-3190\). It was revealed that the substrate concentration 400 g.l\(^{-1}\) was optimum and the bioethanol yield obtained was 98.251 g.l\(^{-1}\) at the rate of productivity of 2.044 g.l\(^{-1}\).h\(^{-1}\) and the bioethanol percentage was 48.013 g.l\(^{-1}\). The substrate concentration was found to be optimum at 409.916 g.l\(^{-1}\) with response surface methodology. At this concentration, a bioethanol yield obtained was 113.570 g.l\(^{-1}\) and the percentage of bioethanol yield was 55.563\% after 48 hours of fermentation time with central composite design. Form the data, it can be concluded that the statistical optimization of fermentation parameters for bioethanol production would be better method than the bioethanol production under initial optimized conditions.

Temperature of the fermentation medium is most crucial factor for successful bioethanol production. The bioethanol yield increased gradually from 10 \(^0\)C to 30 \(^0\)C and above 30 \(^0\)C temperature, the bioethanol yield, and growth of yeast was decreased. The bioethanol observed at 30 \(^0\)C was 103.15g.l\(^{-1}\) and the rate of productivity was 2.148g.l\(^{-1}\).h\(^{-1}\) and the percentage of bioethanol was 50.464 g.l\(^{-1}\) after 48 hours of fermentation time. The statistical optimization using central composite design (CCD) of temperature was 31.4302 \(^0\)C. The bioethanol yield obtained was 113.57077g.l\(^{-1}\) at the rate of productivity of 2.366077 g.l\(^{-1}\).h\(^{-1}\) and the percentage of bioethanol yield was 55.563\% with the pH 4.9753 after 48 hours of fermentation time.

The pH of the fermentation medium influences the production of bioethanol during large scale alcoholic fermentations. The optimization of pH was studied on bioethanol production using mahua flower extract. The results shown that the yield of bioethanol obtained was 108.69 g.l\(^{-1}\) at 5 pH and rate of productivity of 2.25 g.l\(^{-1}\).h\(^{-1}\) without using central
composite design. The pH was found to be optimum at 4.97 in 5l bioreactor for bioethanol production with statistical optimization. The pH 4.97 revealed that the bacterial contamination risk was reduced below 5 pH.

The addition of inoculum volume to the fermentation medium is crucial in industrial scale up of bioethanol production. The maximum yield of bioethanol observed was 111.19g.l^{-1} at 8v/v of inoculum volume and the percentage of bioethanol was 54.398% at the rate of productivity of 2.316g.l^{-1}.h^{-1}. The statistical optimization of inoculum volume using central composite design indicated that the high yield of bioethanol was 121.8783g.l^{-1} at 9.0003 v/v of seed culture. The percentage of bioethanol obtained was 58.185% at the rate of productivity 2.8159g.l^{-1}.h^{-1}. It was noticed that the bioethanol yield (3.787%) was improved after statistical optimization.

The uniform mixing of fermentation medium in bioreactor along with the yeast strain is the main important factor affecting the fermentation efficiency in the batch process during the industrial production of bioethanol. The effect of agitation was studied using mahua fermentation medium and it was noticed that maximum bioethanol concentration obtained was 110.63g.l^{-1} at 120 RPM and the rate of productivity of 2.179g.l^{-1}.h^{-1}. The percentage of bioethanol obtained was 54.124g.l^{-1}. The statistical optimization of fermentation process for high metabolic production of bioethanol obtained was 121. 878g.l^{-1} and the rate of productivity of 2.5391g.l^{-1}.h^{-1} at the agitation rate was 117.2861 RPM and the percentage of bioethanol was 58.185% at 48 hours of fermentation time. The improved bioethanol yield observed was 4.041% with central composite design.

The age of yeast strain and their physical nature during the submerged fermentation is significant in bioethanol production. The effect of inoculum age on bioethanol yield was examined with yeast cultures having various age in hours ranging from 24 hours to 96 hours.
Nitrogen source supplementation in fermentation process is essential for the growth of yeast strain and biosynthesis of nitrogenous compounds such as proteins, vitamins, amino acids and enzymes as well as bioethanol production is depends upon availability of nitrogen in the fermentation medium. However, two inorganic nitrogen sources such as Ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\) and ammonium chloride \((\text{NH}_4)_2\text{Cl}_2\) were studied with various concentrations ranging from 0.1 g.l\(^{-1}\) to 1.0 g.l\(^{-1}\). In the present fermentation process, 0.6 g.l\(^{-1}\) of ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\) was optimum for bioethanol produced was 112.98 g.l\(^{-1}\) at the productivity rate of 2.353 g.l\(^{-1}\).h\(^{-1}\) and percentage of bioethanol was 55.27%, these results indicates that activation of sugar transportation and bioethanol production occurs only at 0.6 g.l\(^{-1}\) of \((\text{NH}_4)_2\text{SO}_4\). It was observed that the supplementation of ammonium chloride \((\text{NH}_4)_2\text{Cl}_2\) in fermentation medium yielded low bioethanol concentration at 0.6 g.l\(^{-1}\) than the ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\).

The metabolic production of bioethanol using statistical optimum concentrations obtained are 0.629813 g.l\(^{-1}\) of ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\), 0.522 g.l\(^{-1}\) of copper chloride \((\text{CuCl}_2\) and 0.061 g.l\(^{-1}\) of manganese chloride \((\text{MgCl}_2.6\text{H}_2\text{O})\). The bioethanol yield obtained was 128.763 g.l\(^{-1}\) at the rate of 2.682 g.l\(^{-1}\).h\(^{-1}\) and the percentage of bioethanol was 61.472% using 5L bioreactor. After statistical optimization was completed, the percentage of bioethanol was improved upto the level of 6.202%.

Additions of copper chloride \((\text{CuCl}_2)\), manganese chloride \((\text{MgCl}_2.6\text{H}_2\text{O})\) to the fermentation medium has a tremendous effect on the production of bioethanol in submerged fermentation. These results shown that the bioethanol productions gained was of 110.18 g.l\(^{-1}\) and 116.64 g.l\(^{-1}\) at 0.5 g.l\(^{-1}\) and 0.06 g.l\(^{-1}\) of copper and manganese, respectively. The
percentage of bioethanol of copper and magnesium were 53.908% and 57.064% respectively. After optimizations of fermentative factors were completed, the bioethanol productions were improved upto 7.564% and 4.408%.

Magnesium chloride (MgCl₂·6H₂O) is very important factor that influence the sequential conversion fermentable sugars to bioethanol in the presence of enzymes such as hexokinase, phosphoglucose isomerase, phosphofructokinase, phosphoglycerate kinase, phosphoglycerate mutase, pyruvate kinase, pyruvate decarboxylase. The optimum concentration of magnesium for yield of bioethanol obtained was 118.0g.l⁻¹ at the concentration of 0.4g.l⁻¹. The percentage of bioethanol was 57.7299% at the bioethanol productivity of 2.458g.l⁻¹.h⁻¹.

Zinc sulphate (ZnSO₄·7H₂O) optimum concentration was also determined using 5L bioreactor. After the completion of fermentations, the bioethanol produced was 114.75g.l⁻¹ at the rate of 2.390g.l⁻¹.h⁻¹ of productivity and the percentage of bioethanol achieved was 56.139 % at 50mg.l⁻¹ of zinc sulphate.

Impact of biotin was investigated to determine optimum concentration for the maximum production of bioethanol. The maximum production of bioethanol gained was 115.68g.l⁻¹ at the rate of productivity of 2.430g.l⁻¹.h⁻¹ and the percentage of bioethanol was 56.5949% at the concentration of biotin of 24mg.l⁻¹. Hence, concentration of biotin at 24mg.l⁻¹ was found to be an optimum concentration.

The higher metabolic bioethanol productions using mahua flower extract were performed using central composite design through response surface methodology. The results have shown that the optimum concentrations for higher bioethanol yield obtained were of magnesium chloride (MgCl₂·6H₂O) of 0.430mg.l⁻¹, zinc sulphate (ZnSO₄·7H₂O) of 54.021 mg.l⁻¹ and biotin of 22.453mg.l⁻¹. After the fermentation with the optimum concentrations, the
yield of bioethanol obtained was 131.28134g.l⁻¹ at the rate of productivity of 2.7350g.l⁻¹.h⁻¹ and the percentage of bioethanol was 62.674%. After the statistical optimization, these results shown that the improved bioethanol productions were 4.9441% with magnesium, 6.353% with zinc and 6.0791% with biotin by Saccharomyces cerevisiae-3190.

Amino acids are the rich sources of nitrogen and can make the bioethanol tolerant yeast strains to resists toxic effects during fermentation process. In the present investigation, two amino acids such as proline and glycine were subjected to the batch fermentation process. Proline shown better yield of bioethanol obtained was 114.36g.l⁻¹ at the concentration of 150mg.l⁻¹ with the percentage of 55.949 % than the glycine which produced 112.753 g.l⁻¹ of bioethanol. Hence, proline was used as an osmoprotectant in fermentation process.

Phosphorous is an important factor involved in biosynthesis of de-oxy ribonucleic acid (DNA) and ribonucleic acid (RNA) in yeast cells as well as in alcoholic fermentation. It has a significant effect on bioethanol produced was 119.342g.l⁻¹ at the rate of productivity of 2.486g.l⁻¹.h⁻¹ and the percentage of yield was 58.386%.

The effect of chelating agents such ethylene diamine tetraacetic acid (EDTA) and sodium potassium tartrate (SPT) were applied in alcoholic fermentation. Amongst, EDTA shown a significant effect on bioethanol yield and the bioethanol yield produced was 117.653 g.l⁻¹ at the rate of productivity of 2.451g.l⁻¹.h⁻¹. The percentage was 57.560 %, which is higher than the bioethanol produced (112.623 g.l⁻¹) by sodium potassium tartrate. The rate of bioethanol productivity achieved was 2.346 g.l⁻¹.h⁻¹. The ethylene di-amine tetra acetic acid (EDTA) was found to be the best chelating agent in bioethanol fermentations. Hence, the present fermentation processes were conducted using ethylene diamine tetraacetic acid.
The optimum concentrations for higher metabolic production obtained are proline of 0.163756 g.l⁻¹, phosphorous (NaH₂PO₄) of 5.385 g.l⁻¹ and EDTA of 5.197 g.l⁻¹. The fermentations were performed under the optimum concentrations, and the yield of bioethanol obtained was 129.936 g.l⁻¹ at the rate of productivity of 2.7070 g.l⁻¹.h⁻¹. The percentage of bioethanol was 62.032% using *S. cerevisiae*-3190 in 5L bioreactor. After the batch fermentation process with statistical optimizations, the results shown that the improved bioethanol yields obtained were 6.083 %, 3.646 % and 4.472 % with proline, sodium dihydrogen phosphate and EDTA after 48 hours of fermentation time respectively.

Potassium phosphate (K₂HPO₄) concentration was determined using 5L bioreactor. The results shown that the potassium of 2.0 g.l⁻¹ was optimum for bioethanol and produced bioethanol was 116.981 g.l⁻¹ at the rate of productivity of 2.437 g.l⁻¹.h⁻¹, and the percentage of bioethanol was 57.231 %. The calcium chloride (CaCl₂.2H₂O) concentration at 0.06g.l⁻¹ was found to be optimum for bioethanol produced was 115.947 g.l⁻¹ at the rate of productivity of 2.415 g.l⁻¹.h⁻¹ and the percentage of bioethanol was 56.725%. As well as, cobalt chloride (COCl₂) was found to be optimum concentration at 80mg.l⁻¹. The high yield of bioethanol obtained was 118.635 g.l⁻¹ at the rate of bioethanol productivity of 2.471 g.l⁻¹.h⁻¹ and the percentage of bioethanol was 58.040 %.

The statistically optimized conditions for higher metabolic production were determined as potassium phosphate (K₂HPO₄) of 2.170 g.l⁻¹, calcium chloride (CaCl₂.2H₂O) of 0.6477 g.l⁻¹ and cobalt chloride (COCl₂) of 99.48 mg.l⁻¹. After the fermentation was completed, yield of bioethanol obtained was 132.5151 g.l⁻¹ at the rate of productivity of 2.7607 g.l⁻¹.h⁻¹ and the percentage of bioethanol was 63.263%. The improved percentage of bioethanol yields of potassium, calcium, and cobalt were of 6.032 %, 6.538 %, 5.223 % after 48 hours of fermentation time, respectively.
The important factors that affect bioethanol fermentations in large scale bioethanol productions are ferrous sulphate (Fe₂(SO₄)₃.H₂O), oxygen (O₂) and sodium chloride (NaCl₂). As a result, after 48 hours of fermentation time, the maximum yields of bioethanol obtained were 102.721 g.l⁻¹, 90.530 g.l⁻¹ and 93.641 g.l⁻¹ at the rate of productivity of 2.140 g.l⁻¹.h⁻¹, 1.886 g.l⁻¹.h⁻¹ and 1.950 g.l⁻¹.h⁻¹, and the bioethanol yields obtained were of 50.254 %, 44.290 % and 45.812 % respectively.

Statistically optimized concentrations on the bioethanol productions were determined as 0.533 g.l⁻¹ of ferrous sulphate (Fe₂(SO₄)₃.H₂O), 0.330 mg.l⁻¹ of oxygen (O₂) and 1.105 g.l⁻¹ of sodium chloride (NaCl₂). Under these optimized conditions, the maximum yield of bioethanol obtained was 125.929 g.l⁻¹ at the rate of productivity 2.6235 g.l⁻¹ and the percentage of bioethanol production was 60.119 %. After fermentations were completed, the better yields of bioethanol obtained were of 9.865 %, 15.829 % and 14.307 % with Ferrous sulphate, oxygen and sodium chloride after 48 hours of fermentation process.

Amalgamations of organic nitrogen in fermentation that can stimulate fermentation process in large scale production of bioethanol are peptone, urea and yeast extract as source of organic nitrogen were carried out individually. After the batch fermentation process was completed, the maximum yields of bioethanol obtained were 100.634 g.l⁻¹, 114.735 g.l⁻¹ and 118.462 g.l⁻¹ of peptone, urea, and yeast extract at the rate of productivity of 2.090 g.l⁻¹.h⁻¹, 2.390 g.l⁻¹.h⁻¹ and 2.467 g.l⁻¹.h⁻¹ with the percentages were of 49.233 %, 56.132 % and 57.955 %, respectively.

Using the central composite design, the statistical optimization of peptone, urea and yeast extract were carried out using Saccharomyces cerevisiae-3190. The optimized conditions for higher yields of bioethanol were established as peptone of 3.038 g.l⁻¹, urea of 2.566 g.l⁻¹ and yeast extract of 1.573 g.l⁻¹. Under these optimized conditions, bioethanol yield
produced was 135.164 g.l\(^{-1}\) at the rate of productivity of 2.815 g.l\(^{-1}\).h\(^{-1}\) and the percentage of bioethanol was 64.528 %. After the central composite design was performed, the better yields of bioethanol obtained were of 15.295 %, 8.396 %, 6.573 % using peptone, urea and yeast extract.

Medium-I was designed and its impact was examined on bioethanol production using the mixture of optimized conditions which determined substrate concentration of 400 g.L\(^{-1}\), temperature of 30 0C, pH of 5, inoculum volume of 8v/v, agitation of 120 RPM, inoculum age of 48 hours, ammonium sulphate (NH\(_4\))\(_2\)SO\(_4\) of 0.6 g.l\(^{-1}\), copper chloride (CuCl\(_2\)) of 0.5 g.l\(^{-1}\), manganese chloride (MnCl\(_2\).4H\(_2\)O) of 0.06 g.l\(^{-1}\), magnesium chloride (MgCl\(_2\).6H\(_2\)O) of 0.4 g.l\(^{-1}\), zinc sulphate (ZnSO\(_4\).7H\(_2\)O) of 50 mg.l\(^{-1}\), biotin 24 of mg.l\(^{-1}\), proline of 0.150 of g.l\(^{-1}\), sodium di-hydrogen phosphate (NaH\(_2\)PO\(_4\)) of 5.0 g.l\(^{-1}\), ethylene di-amine tetraacetic acid (EDTA) of 5 g.l\(^{-1}\), potassium phosphate (K\(_2\)HPO\(_4\)) of 2.0 g.l\(^{-1}\), calcium chloride (CaCl\(_2\)) of 0.06 g.l\(^{-1}\), cobalt chloride (COCl\(_2\)) of 80 mg.l\(^{-1}\), ferrous sulphate (Fe\(_2\)(SO\(_4\))\(_3\).H\(_2\)O) of 0.5 g.l\(^{-1}\), oxygen (O\(_2\)) of 0.3 mg.l\(^{-1}\), sodium chloride (NaCl\(_2\)) of 0.10 g.l\(^{-1}\), peptone of 3.0 g.l\(^{-1}\), urea of 2.5 g.l\(^{-1}\) and yeast extract of 1.5 g.l\(^{-1}\) were kept constant (shown in table no-4.24.1). Under these optimum concentrations, bioethanol production obtained was 150.562 g.l\(^{-1}\) at the fermentation efficiency of 3.1367 g.l\(^{-1}\).h\(^{-1}\) and the percentage of yield of bioethanol was 73.66 %. The fermentable sugars in medium-I utilized by yeast strain was 90 % and the viable yeast cells were found to be 98 % after 48 hours of fermentation time.

The medium-II was designed with statistical optimization of various physicochemical and nutritional factors using central composite design (CCD) for bioethanol production and its impact was studied using batch fermentation process in 5l bioreactor. The fermented samples were analyzed during the time of intervals using gas chromatography. After optimization process was completed, the optimized conditions were established as substrate concentration of 409.916 g.l\(^{-1}\), temperature of 31.43 0C, pH of 4.9, inoculum...
volume of 9.0003 v/v, agitation of 117.286 RPM, inoculum age of 53.660 hours, ammonium sulphate (NH$_4$)$_2$SO$_4$) of 0.629 g.l$^{-1}$, copper chloride (CuCl$_2$) of 0.522 g.l$^{-1}$, manganese chloride (MnCl$_2$.4H$_2$O) of 0.061 g.l$^{-1}$, magnesium chloride (MgCl$_2$.6H$_2$O) of 0.43 g.l$^{-1}$, zinc sulphate (ZnSO$_4$.7H$_2$O) of 54.021 mg.l$^{-1}$, biotin 22.453 of mg.l$^{-1}$, proline of 0.163 of g.l$^{-1}$, sodium Di-hydrogen phosphate (NaH$_2$PO$_4$) of 5.3859 g.l$^{-1}$, ethylene di-amine tetraacetic acid (EDTA) of 5.197 g.l$^{-1}$, potassium phosphate of 2.170 g.l$^{-1}$, calcium chloride of 0.064 g.l$^{-1}$, cobalt chloride (COCl$_2$) of 99.43 mg.l$^{-1}$, ferrous sulphate (Fe$_2$(SO$_4$)$_3$.H$_2$O) of 0.533 g.l$^{-1}$, oxygen (O$_2$) of 0.330, sodium chloride (NaCl$_2$) of 1.105 g.l$^{-1}$, peptone of 3.038 g.l$^{-1}$, urea of 2.566 g.l$^{-1}$ and yeast extract of 1.572 g.l$^{-1}$. Under these optimized fermentative conditions, the highest yield of bioethanol achieved was 195.284 g.l$^{-1}$ at the rate of productivity of 4.068 g.l$^{-1}$h$^{-1}$ and the theoretical bioethanol yield achieved was 93.22 %. The total fermentable sugars utilized for bioethanol production by *S.cerevisiae*-3190 were 99.53 % and it was also found that viable yeast cells were 97 % after 48 hours of fermentation.

As a result of statistical experiments, the fermentative medium-II has exhibited a property of osmoprotectant and higher bioethanol yields by yeast cells. The bioethanol tolerance by yeast cells and biomass content were achieved under stress conditions like substrate concentration, temperature, pH, and agitation. Hence, the statistical design of medium-II for the bioethanol production using batch fermentation process could be novel fermentative method.

The present fermentation process results in the reduction of the production cost of bioethanol, labour cost, and contamination risk. There by checks bioethanol production cost in industrial scale using response surface methodology (RSM). The highest bioethanol productions can be achieved using a potential substrate Mahua flower (*Madhuca indica*) with *Saccharomyces cerevisiae*- 3190. More over bioethanol could be an alternative fuel owing to the depletion of petroleum fuels and their increase in prices day by day.
5.2. Economic impact of bioethanol production in India:

According to Ministry of Petroleum and Natural Gas (MPNG), 5% blend in petrol would require 500 million litres of ethanol per annum in India and the blending percentage would go up to 10% or higher in the incoming year. Thus, ethanol substitute 500 million litres of petrol per year, which can save up to Rs.830 crores of foreign exchange (Andhra Pradesh Pollution Control Board, (APPCB), (2004).

Padma Vasudevan, et al, (2005) reported that the exploitation of potential biomass is needed for converting modern energy carriers at competitive prices. Therefore, Mahua flower could be a potential substrate for bioethanol production with the present fermentation method. The present bioethanol fermentation process offers promising alternative method as it can be produced from mahua flower extract.

David., et al. (2012) reported that the industrial scale-up of low-valuable products and an increase of 1% production yield could be highly rewarding in financial terms. Ethanol has been a good energy source of automobiles and industries (Udhayaraja, et al., (2012). Saravana Murugan., (2013) reported that bioethanol can be blended up to 20% with diesel or petrol. Balat., et al., (2009) reported that the bioethanol blend of gasoline for automobiles can significantly reduce petroleum fuels use and green house gas emissions.

At present, one million tons of mahua flowers (Madhuca indica) are available in India. Therefore, one million tons of mahua flowers could produce 7,31,300 tons of total fermentable sugars thus bioethanol yield can be produced up to 3,484,32 tons with the present fermentative method. When the bioethanol used as blend in petrol, thus bioethanol production replaces not less than 3,484,32 tons of petrol.
5.3. Conclusion

✓ Mahua flower (*Madhuca indica*) was chosen as a substrate for the production of bioethanol. Mahua flower is cheap in price and found abundantly in nature. It is rich in sugars, vitamins, and minerals.

✓ The biochemical data of mahua flower indicated that the flower can be directly used as a substrate for bioethanol production without any pretreatment and it is also interesting to note that it has antibiotic properties. Mahua flower extract (MFE) is novel and it is also extremely suitable for the highest bioethanol productions at pilot plant scale level.

✓ Culture medium composition using mahua flower extract could be suitable for yeast cell growth and the cost fermentation medium can also be reduced by replacing pure sugars like glucose, sucrose, maltose, fructose and xylose with mahua flower extract in large scale industrial bioethanol productions.

✓ Initial optimization of physico-chemical and nutritional parameters using response surface methodology for the bioethanol production such as substrate concentration, pH, temperature, agitation, fermentation time, inoculum volume and inoculum age were found to have significant effect on bioethanol production. Inorganic nitrogen source, trace metals, chelating agents, phosphate source, amino acids, vitamins, oxygen, and organic nitrogen sources yielded high ethanol concentrations at optimum conditions.

✓ Using initially optimized fermentative conditions, a fermentative medium-I was designed for bioethanol productions and their impacts on bioethanol productions were investigated. From the experiments, it was found that optimizations of initial standardization
of physico-chemical and nutritional conditions for alcoholic fermentations in the fermenter using yeast cells are crucial for rapid fermentative process through submerged fermentations.

✓ Bioethanol fermentations using initial fermentative conditions of medium-I, yielded 73.66 % of bioethanol (150.66 g.l\(^{-1}\)) and utilized 90 % of total fermentable sugars at 48 hours of fermentation period. The viable yeast cells count was 98 % at 48 hours of fermentations.

✓ The sequential statistical optimization of physico-chemical and nutritional parameters using response surface methodology for the bioethanol production such as substrate concentration, pH, temperature, agitation, fermentation time, inoculum volume and inoculum age were found to have significant effect on bioethanol production. Inorganic nitrogen source, trace metals, chelating agents, phosphate source, amino acids, vitamins, oxygen and organic nitrogen sources for bioethanol productions are necessary for large scale industrial fermentations.

✓ The present downstream fermentation process is rapid with statistically optimum fermentative conditions of medium-II. The metabolic production of bioethanol yield achieved was 195.284g.l\(^{-1}\). The theoretical yield of bioethanol achieved was 93.22 %. The total sugars converted to bioethanol production achieved were 99.53 % by yeast strain \textit{S.cerevisiae}-3190. The results indicated that the fermentation efficiency is high with the yeast strain \textit{S.cerevisiae}-3190, because it is resistant to stress conditions like osmotic stress and high ethanol concentration. It can also reduce production cost in large scale industrial fermentation process.
✓ “Three factors at a time” of fermentation experiments would be useful for optimization of fermentation fermentative conditions to achieve highest bioethanol concentrations using Box-Wilson statistical response surface method (RSM).

✓ Central composite designs of eight medium designs were formulated with S.cerevisiae-3190 in 5 litre batch bioreactor. Using the optimum conditions of each medium design, a potential fermentative medium-II was formulated and their impacts were studied on improvement of bioethanol yields. The experimental results indicated that optimizations of fermentative conditions through response surface methodology (RSM) could enhance bioethanol yields.

✓ The present fermentative method indicated that non-renewable energy sources could be replaced by the mahua flower extract (MFE) as a substrate on bioethanol productions under statistical optimum fermentative conditions. Thus, bioethanol replaces 3,48,432 tons of petrol.