Annexure -VIII

University Grants Commission
(Ministry of Human Resource Development, Govt. of India)
Bahadurshah Zafar Marg, New Delhi – 110002

सत्यमेव जयते

ज्ञान-विज्ञान विमुक्तये

Final Report of the work done on the Major Research Project.
(Report to be submitted within 6 weeks after completion of each year)

1.	**Project report No. 1st /2nd /3rd/Final**	Final
2.	**UGC Reference No. & Date**	F.- 43-153/2014(SR) dated 18th September, 2015 (Dispatch date) **MRP-MAJOR-CHEM-2013-26821** FD Diary No.-2229 dated 22nd July, 2015
3.	**Period of report:**	From **01/07/2015** to **30/06/2018**
4.	**Title of research project**	*Biorefining Crotalaria Juncea (Sunn Hemp): Prospects of Utilizing Waste Glycerol for the Production of Succinic Acid*
5.	**(a) Name of the Principal Investigator** **(b) Department** **(c) University/College where work has progressed**	(a) Prof (Dr) Ujjaini Sarkar (b) Department of Chemical Engineering (c) JADAVPUR UNIVERSITY, 188, Raja S. C. Mallick Road, Kolkata-700032.

6.	**Effective date of starting of the project**	01/07/2015 (1st July, 2015)
7.	**Grant approved and expenditure incurred during the period of the report:** **a. Total amount approved Rs.**	**INR 13,01,040/-**
	b. Total expenditure Rs.	**INR 13,01,040/-**
	c. Report of the work done: (Please attach a separate sheet) *See Annex– VIII and Annex A* i. Brief objective of the project ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons iv. Please indicate the difficulties, if any, experienced in implementing the project v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet. vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission. vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any.	

Principal Investigator **(Signatures with seal)**	**Registrar/Principal** **Signature with seal**

Annexure – IX

UNIVERSITY GRANTS COMMISSION BAHADUR SHAH ZAFAR
MARG NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	**Project report No. 1st /2nd /3rd/Final**	Final
2.	**UGC Reference No. & Date**	F.- 43-153/2014(SR) dated 18th September, 2015 (Dispatch date) MRP-MAJOR-CHEM-2013-26821 FD Diary No.-2229 dated22nd July, 2015
3.	**Period of report:**	From **01/07/2015** to **30/06/2018**
4.	**Title of the project**	*Biorefining Crotalaria Juncea (Sunn Hemp): Prospects of Utilizing Waste Glycerol for the Production of Succinic Acid*
5.	**Name and address of the Principal Investigator**	Prof (Dr) Ujjaini Sarkar *Office:* Department of Chemical Engineering JADAVPUR UNIVERSITY, 188, Raja S. C. Mullick Road, Kolkata- 700032 **Telephone #** : +91-33-2457-2695 (Off) **Mobile No**: 8961502639

		e-mail : ujjaini.sarkar@jadavpuruniversity.in (official); abhigyan@hotmail.com (personal) *Residential:* FLAT 2A, MATRI NILAY, 68 A, Motilal Nehru Road, Kolkata 700029. **Telephone #** : +91-33-2485-2975 (Res)
6.	**Name and address of the institution**	JADAVPUR UNIVERSITY, 188, Raja S. C. Mullick Road, Kolkata- 700032 **Telephone #** : +91-(033)-24146666
7.	**UGC approval letter No. & Date**	F.- 43-153/2014(SR) dated 18th September, 2015 (Dispatch date) MRP-MAJOR-CHEM-2013-26821 FD Diary No.-2229 dated22nd July, 2015
8.	**Date of implementation**	01/07/2015 (1st July, 2015)
9.	**Tenure of the Project**	3 years from **01/08/2015** *to* **31/07/2018**
10.	**Total grant allocated**	Total Allocation (Rs.): **13,32,500/-**
11.	**Total grant received**	Total grant received: **13,01,040/-**

		1st installment: **9,30,000/-** 2nd installment: **3,71,040/-**
12.	**Final expenditure:**	**INR 13,01,040/-**
13.	**Title of the project**	*Biorefining Crotalaria Juncea (Sunn Hemp): Prospects of Utilizing Waste Glycerol for the Production of Succinic Acid*
14.	**Objectives of the project**	1. *Batch extraction of Sunn-Hemp oil from Crotalaria juncea seeds* 2. *Modelling the Batch Extraction process* 3. *Trans-esterification of Sunn-Hemp oil already extracted* 4. *Purification of glycerol obtained as a byproduct of trans-esterification* 5. *Batch Fermentation using glycerol as the major carbon source* 6. *Continuous Fermentation using glycerol as the major carbon source: Process optimization* 7. *Catalytic conversion of succinic acid into 1-4, Butanediol.*
15.	**Whether objectives were achieved** **(Give Details)**	***YES*** Initially, oil is extracted from *Crotalaria juncea* using standard Soxhlet apparatus and then the oil is trans-esterified to produce biodiesel. One homogeneous catalyst, potassium hydroxide and three heterogeneous catalysts, calcium oxide powder, capiz and conch shell are used to obtain biodiesel. After production, the biodiesel is physico-chemically characterized using Gas Chromatography Mass Spectrometry (GCMS) for identifying the saturated fatty acids. Some of the basic fuel properties like specific gravity, moisture content, kinematic viscosity, saponification value, iodine value, flash point, fire point, aniline point, cetane number and gross calorific value are determined to characterize the fuel quality of the biodiesel produced. Later, the yield of production is optimized by a response surface design, based on Box–Behnken model and a Factorial design with respect to reaction

		time, oil to methanol mole ratio, catalyst concentration and types of catalysts used. Then the crude glycerol, already separated, is purified, employing various physico-chemical treatments. The purification process is designed on the basis of acidification, neutralization, solvent extraction, adsorption and finally pressure filtration through membrane. Purity of glycerol is measured by Flame Ionization Detector based Gas Chromatography (GC-FID). This work is focused to develop a model of a response surface based on Central Composite Design (CCD), in order to obtain the highest level of purity. Here RSM is used to optimize the parameters affecting the purity of glycerol. Three parameters like type of acid, pH and the amount of adsorbent are chosen as independent factors (variables) for this statistical analysis. In order to remove the colour, activated carbon is used in the range of 0.5g - 1.25g for 10 ml of crude glycerol. Effects of these parameters on the purification process are optimized by Response Surface Methodology (RSM) using Central Composite Design (CCD). From the statistical analysis it is shown that the model is significant with a R^2 value of 0.99. The optimum condition for the production of 90.4% pure glycerol is estimated to be: pH=3.26 and amount of adsorbent =0.933 with the choice of acid being phosphoric acid. This purified glycerol is then used as a carbon source for producing succinic acid using a single culture of *Escherichia coli*. A number of batch fermentations are conducted for different glycerol concentrations to observe the cell growth. Seven growth models like, Monod, Moser, Tessier, Halden-Andrew, Aiba-Edward, Tessier type and Andrews are used to determine the model-specific growth kinetic parameters like, specific growth rate (μ), substrate saturation constant (K_S), and

		substrate inhibition constant ($K_{I,S}$). Considering the inhibition effect, Aiba-Edward model ranked 1 out of 7 in case of two samples and Haldane-Andrew model ranked 1 in case of one sample. Aiba-Edward model gives the best fitment for a large range of concentrations of all the three types of glycerol, crude, purified and laboratory grade. Optimization of process parameters for succinic acid production is done by RSM using Face Centered Central Composite Design (FCCCD) in order to enhance its yield. Maximum yield of succinic acid using purified glycerol is 23.27gL^{-1} and the same is reached with a pH 7 and 37.5°C temperature after adding 2ml inoculum, 1gL^{-1} and 4gL^{-1} of yeast and peptone respectively. This is very close to the optimized yield of 30.76 gL^{-1} using commercial glycerol at a pH 7 and 37.5°C temperature after adding 2ml inoculum, 3gL^{-1} and 4gL^{-1} of yeast and peptone respectively. Statistical analysis of the overall system, in order to investigate the effect of process parameters for the *entire system* on the yield of succinic acid, is done by RSM using Face Centered Central Composite Design. This is followed by a regression analysis, performed in order to obtain the optimum nutrients concentration of the medium, to produce the highest yield of succinic acid. Statistically optimised yield of 21.32 gL^{-1} is validated against an observed yield of 22.82 gL^{-1} with optimised values of process variables being: oil amount= 15.01g, inoculum quantity= 1.05ml, methanol mole= 9.03; fermentation batch time = 72hr; catalyst concentration= 1.26wt%, pH= 5.83; adsorbent= 0.51g; purity of glycerol= 90.4%; glycerol quantity = 14.97g; yeast concentration= 1.44gL^{-1} and peptone concentration = 6gL^{-1}.

16.	**Achievements from the project**	*See Annex A* ■ Process optimization with respect to various innovative combinations of polar and non-polar solvents and co-solvents, extraction time, quantity of seeds and size fractions of the seeds was carried out in a novel extraction apparatus with an annular packed bed inside. This bio-oil has been found to be a prospective biofuel itself [1-2, 7-8]. ■ Batch extraction process was modeled [3]. ■ Trans-esterification was carried out in a batch reactor using some novel catalysts, synthesized and characterized in-house [4]. ■ The waste glycerol, produced during trans-esterification, was purified using sequential desalination [5]. ■ In this study, management and utilization of the glycerol waste stream through the production of microbial value-added metabolites via fermentative processes was substantially evaluated. Succinic acid was produced on a *Batch Scale* [6]. **References** [1] Dutta, R., **Sarkar, U.**, Mukherjee, A., 2014. Extraction of Oil from Crotalaria *juncea* seeds in A Modified Soxhlet Apparatus: Physical and Chemical characterization of A Prospective Bio-Fuel. Fuel *(Elsevier)*. 116, 794–802. [2] Dutta, R., Sarkar, U., Mukherjee, A. 2015. Process optimization for the extraction of oil from Crotalaria juncea using three phase partitioning, 71, 89-96. [3] Dutta, R., **Sarkar, U.**, Mukherjee, A., 2016. Pseudo-kinetics of batch

		extraction of Crotalaria juncea (Sunn hemp) seed oil using 2-propanol. Industrial Crops and Products *(Elsevier)*. 87, 9–13. [4] Sadhukhan, S., **Sarkar, U.**, 2016. Production of Biodiesel from Crotalaria juncea (Sunn-Hemp) Oil Using Catalytic Trans-Esterification: Process Optimisation Using a Factorial and Box–Behnken Design. Waste and Biomass Valorization (*Springer*). 7(2), 343-355. [5] Sadhukhan, S., **Sarkar, U.**, 2016. Production of purified glycerol using sequential desalination and extraction of crude glycerol obtained during trans-esterification of Crotalaria juncea oil. Energy Conversion and Management *(Elsevier)*. 118, 450–458. [6] Sadhukhan, S., Villa, R., **Sarkar, U.** 2016. Microbial production of succinic acid using crude and purified glycerol from a Crotalaria juncea based biorefinery. Biotechnology Reports *(Elsevier)*, 10, 84–93. [7] Dutta, R., Sarkar, U., Mukherjee, A. 2017. Study of transient behaviour of modified Soxhlet apparatus for extraction of a bio-fuel oil from Crotalaria juncea seed. International Journal of Green Energy (*Taylor & Franci*), 14 (8), 675-686. [8] Sadhukhan, S., Bhattacharjee, A., **Sarkar, U.**, Baidya, P.K., Baksi, S.

		2018. Simultaneous degumming and production of a natural gum from Crotalaria juncea seeds: Physicochemical and rheological characterization. International Journal of Biological Macromolecules *(Elsevier)*, 111, 967-975.
17.	**Summary of the findings** (*in 500 words*)	**Method I** *Batch Extraction of Sunn-Hemp Oil from Crotalaria juncea seeds* Process optimization with respect to various types of solvents and co-solvents, extraction time, quantity of parent seeds, size fractions of the seeds and shape of the extraction volume was carried out. **Method II** *Trans-esterification of the extracted oil using homogeneous and heterogeneous catalysts* Trans-esterification was carried out using the traditional methanol-potassium hydroxide route in a batch reactor. Also, some natural heterogeneous catalysts (conch shell, capiz etc.) were tried. *In this process lots of glycerol would be produced as a byproduct.* **Method III** *Purification of waste glycerol* In this research work, several methods would be used to purify the waste glycerol, namely sequential desalination followed by extraction, adsorption of colour and finally filtration through membrane under vacuum.

		1. *Removal of alcohol*: Excess alcohol from the trans-esterification step can be removed by evaporation in a rotary evaporator. 2. *Acidification*: Initially, glycerol would be acidified by dilute sulphuric acid or phosphoric acid to split the soap and purify the glycerol. The charred substances thus produced would be filtered off. The samples would then be decanted to recover the crude fatty acids. 3. *Neutralization*: The aqueous glycerine solutions are neutralized by 50% sodium hydroxide. The salt crystallizing out would be removed by decantation. 4. *Solvent extraction*: In order to purify and concentrate the solutions further, these would be solvent extracted and filtered in order to remove the residual salt. Finally, the same would be evaporated to obtain the partially purified glycerine. **Method IV** *Batch Fermentation of glycerol into Succinic Acid* • Bacterial fermentation of the purified glycerol would produce succinic acid in batches under specific process conditions. *Here, glycerol would act as the main carbon source.* • Various types of *succini-producen* strains would be used for the batch fermentation process. • Growth kinetics of the engineered strains would be modeled using various appropriate inhibitory and non-inhibitory models. **Method V** *Continuous Fermentation of glycerol into Succinic Acid* • To start with the particular *succini-producen* strain, whose growth

		has been already optimized in batch scale, under *Method IV*, would be used for the continuous fermentation process. • Continuous fermentation of the purified glycerol would be carried out under various process conditions of pH, temperature, purity of glycerol, inoculum quantity, yeast concentration, peptone concentration etc. • Process parameters would be optimized in order to maximize the production of succinic acid in a continuous fermenter. **Method VI** *Downstream processing of the fermented broth to purify the crude succinic acid* Crude succinic acid would be cleaned using ionic liquids/membrane filtration/crystallisation etc. **Method VII** *High pressure hydrogenation of succinic acid into 1-4, Butanediol.* • Couple of novel bi-metallic catalysts would be synthesized and characterized to carry out the hydrogenation process under high pressure and temperature in a batch reactor. • Effects of doping ratios for various pairs of metallic/metallic-non-metallic/non-metallic catalysts would be investigated with respect to the overall catalyst activity. Tests would be carried out at various operating temperatures and pressures along with changes in the mixing ratios of various precursor catalysts.

18.	**Contribution to the society** *(Give details)*	Global emissions of CO_2 and other harmful gases such as CO, SOx, NOx and particulates generated by fossil fuel combustion have led to serious greenhouse effects, acid rain, remarkable human health problem and deterioration of the global environmental condition as a whole. Thus identification of environment friendly and renewable sources of alternative energy is one of the thrust areas of the scientific community. Biodiesel from bio oil may mitigate the above problem to a good extent. Normally biofuel is produced from soybeans, canola oil, animal fat, palm oil, corn oil, waste cooking oil and jatropha oil etc. However, attempts are now made towards biofuel production from non-food lignocellulose, microalgae (i.e. phytoplankton), macro algae (i.e. sea wood) etc. Recent studies indicate that the aromatic content and type, sulfur content, extraction temperature, and density are important factors for emission control. Biomass based agricultural oils have a great potential towards production of cleaner bio-diesel substitute. These bio-oils are better than diesel fuel in terms of sulfur content, flash point, aromatic content and biodegradability. Recently, Succinic acid has attracted great interest because *it can be used as a precursor for different chemical reagents, synthetic resins, biodegradable polymers, herbicides, fungicides, inks, and detergents* among other products. This acid also increases the metabolic efficiency in farm cattle. Commercially, succinic acid can be produced petrochemically from butane and maleic anhydride; however, the high conversion cost of maleic anhydride to succinic acid by the chemical process, limits the use of succinic acid as a precursor. The interest in producing succinic acid by fermentative processes from renewable resources has increased during the last decade owing to

		environmental and economic concerns. Crotalaria *juncea* could be a new source for the production of Biodiesel and India is one of the largest producers in the world. With this background we intend to produce Succinic Acid from the waste glycerol produced by the trans-esterification of Sunn-Hemp Oil. The strong potential of crude glycerol for the development of biorefinery may be demonstrated by the production of several chemicals using microbial route.
19.	**Whether any PhD enrolled/produced out of the project**	▪ *Dr (Ms) Suvra Sadhukhan (Degree awarded, CSIR funded)* ▪ *Mr Pabitra Kumar Baidya (enrolled, earlier funded from UGC-MRP, now being funded by UGC-RGNF)* ▪ *Dr Debopam Banerjee (last Project Fellow for UGC-MRP)*
20.	**No. of publications out of the project** (*Please attach*)	[1] Dutta, R., **Sarkar, U.**, Mukherjee, A., 2014. Extraction of Oil from Crotalaria *juncea* seeds in A Modified Soxhlet Apparatus: Physical and Chemical characterization of A Prospective Bio-Fuel. Fuel *(Elsevier)*. 116, 794–802. [2] Dutta, R., **Sarkar, U.**, Mukherjee, A. 2015. Process optimization for the extraction of oil from Crotalaria juncea using three phase partitioning, 71, 89-96. [3] Dutta, R., **Sarkar, U.**, Mukherjee, A., 2016. Pseudo-kinetics of batch extraction of Crotalaria juncea (Sunn hemp) seed oil using 2-propanol. Industrial Crops and Products *(Elsevier)*. 87, 9–13. [4]

		Sadhukhan, S., **Sarkar, U.**, 2016. Production of Biodiesel from Crotalaria juncea (Sunn-Hemp) Oil Using Catalytic Trans-Esterification: Process Optimisation Using a Factorial and Box–Behnken Design. Waste and Biomass Valorization (*Springer*). 7(2), 343-355. [5] Sadhukhan, S., **Sarkar, U.**, 2016. Production of purified glycerol using sequential desalination and extraction of crude glycerol obtained during trans-esterification of Crotalaria juncea oil. Energy Conversion and Management *(Elsevier)*. 118, 450–458. [6] Sadhukhan, S., Villa, R., **Sarkar, U.** 2016. Microbial production of succinic acid using crude and purified glycerol from a Crotalaria juncea based biorefinery. Biotechnology Reports *(Elsevier)*, 10, 84–93. [7] Dutta, R., **Sarkar, U.**, Mukherjee, A. 2017. Study of transient behaviour of modified Soxhlet apparatus for extraction of a bio-fuel oil from Crotalaria juncea seed. International Journal of Green Energy (*Taylor & Franci*), 14 (8), 675-686. [8] Sadhukhan, S., Bhattacharjee, A., **Sarkar, U.**, Baidya, P.K., Baksi, S. 2018. Simultaneous degumming and production of a natural gum from Crotalaria juncea seeds: Physicochemical and rheological characterization. International Journal of Biological Macromolecules *(Elsevier)*, 111, 967-975.

Principal Investigator
(Signatures with seal)

Registrar/Principal
Signature with seal

Annex A

Summary of work done

In this Annex, we will summarize the entire research work carried out and conclude with the important outcomes of each part of research.

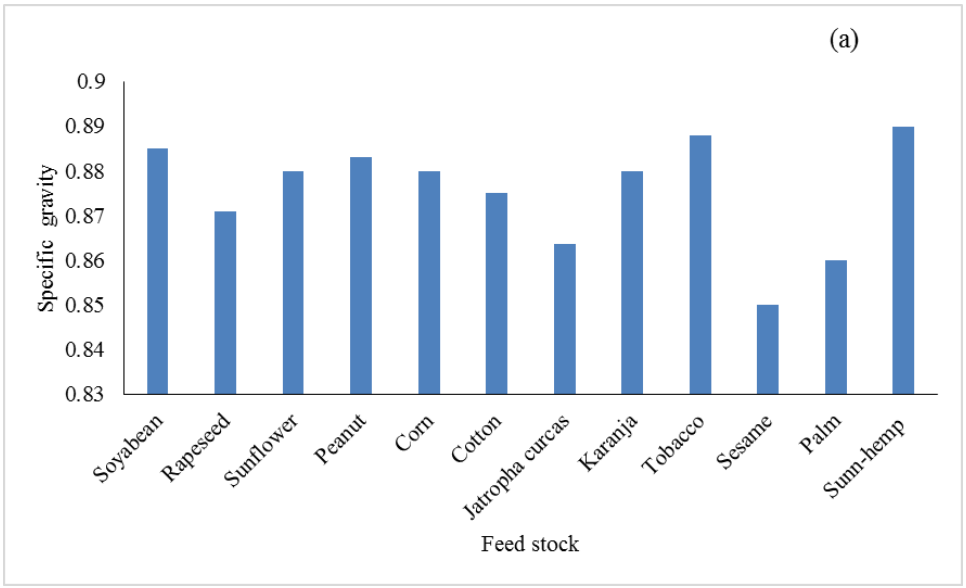
Activity I

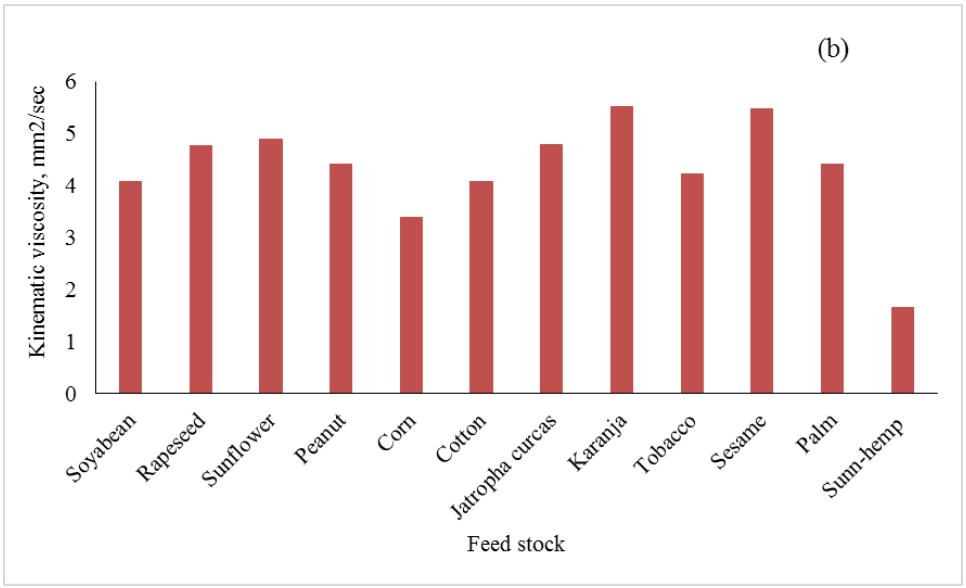
- *Trans-esterification of the Sunn-hemp oil is carried out in order to obtain methyl esters using various types of catalysts, both homogeneous and heterogeneous.*

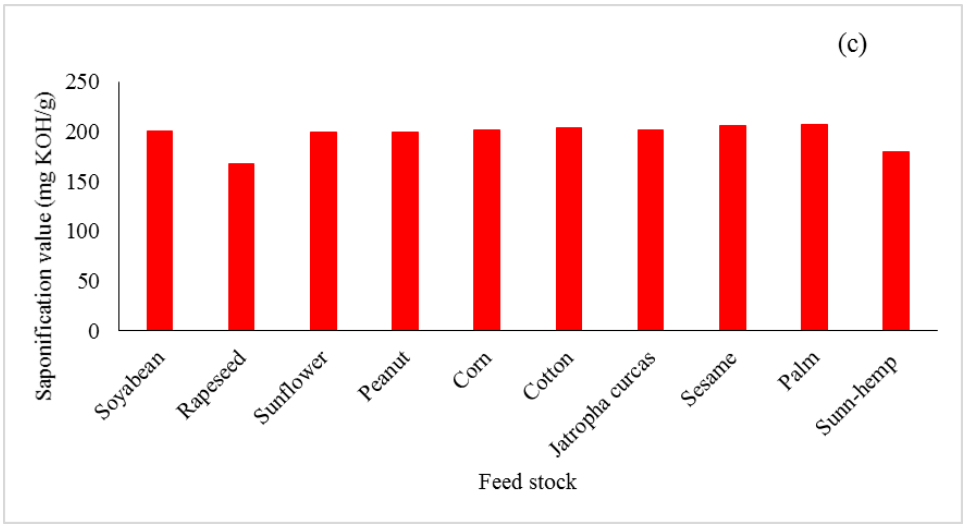
  - Two natural sources of $CaCO_3$ enriched shells; capiz and conch are calcined to convert $CaCO_3$ to CaO to be used as solid catalysts.
  - The heterogeneous catalysts are physically characterized in order to locate the active sites using X-Ray powder Diffraction (XRD).
  - The methyl esters are analytically identified and estimated using GCMS and the biodiesel on the whole is characterised for various fuel properties.
  - The process is statistically optimized using a Response Surface Methodology (RSM) to maximize the yield of biodiesel.
  - The effects of various parameters like time of reaction, moles of methanol, catalyst concentration and type of catalyst are investigated on the biodiesel yield.
  - The feasibility of trans-esterification of *Crotalaria juncea* oil is validated by the statistical support of the agreement of the modeled yield with the experimental data.

- *Physico-chemical characterization of the biodiesel*

Comparison of fuel properties of the Sunn-hemp biodiesel produced using KOH catalyst, under the optimum conditions along with other vegetable biodiesels are given in Figure 1.


(a)
Specific gravity
0.9
0.89
0.88
0.87
0.86
0.85
0.84
0.83
Soyabean
Rapeseed
Sunflower
Peanut
Corn
Cotton
Jatropha curcas
Karanja
Tobacco
Sesame
Palm
Sunn-hemp
Feed stock


(b)
Kinematic viscosity, mm2/sec
6
5
4
3
2
1
0
Soyabean
Rapeseed
Sunflower
Peanut
Corn
Cotton
Jatropha curcas
Karanja
Tobacco
Sesame
Palm
Sunn-hemp
Feed stock


(c)
Saponification value (mg KOH/g)
250
200
150
100
50
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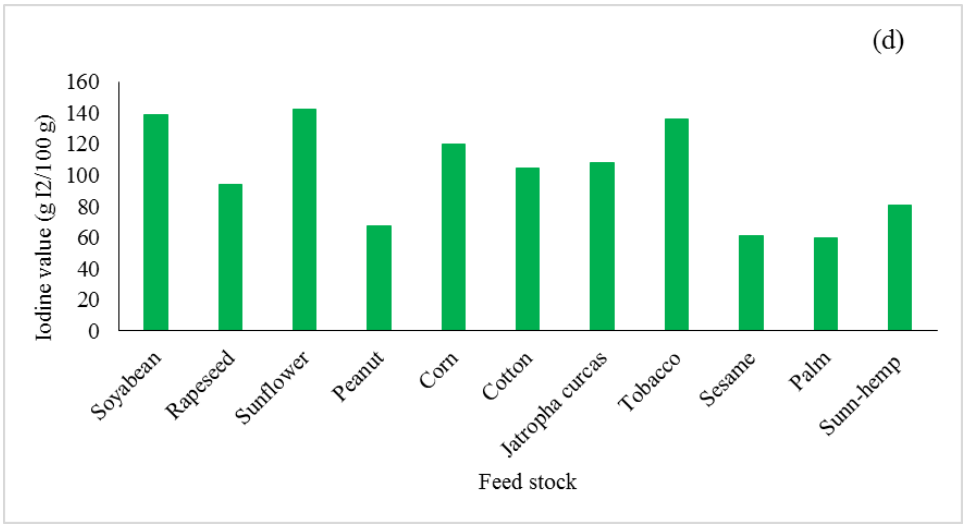
Rapeseed
Sunflower
Peanut
Corn
Cotton
Jatropha curcas
Sesame
Palm
Sunn-hemp
Feed stock


(d)
Iodine value (g I2/100 g)
160
140
120
100
80
60
40
20
0
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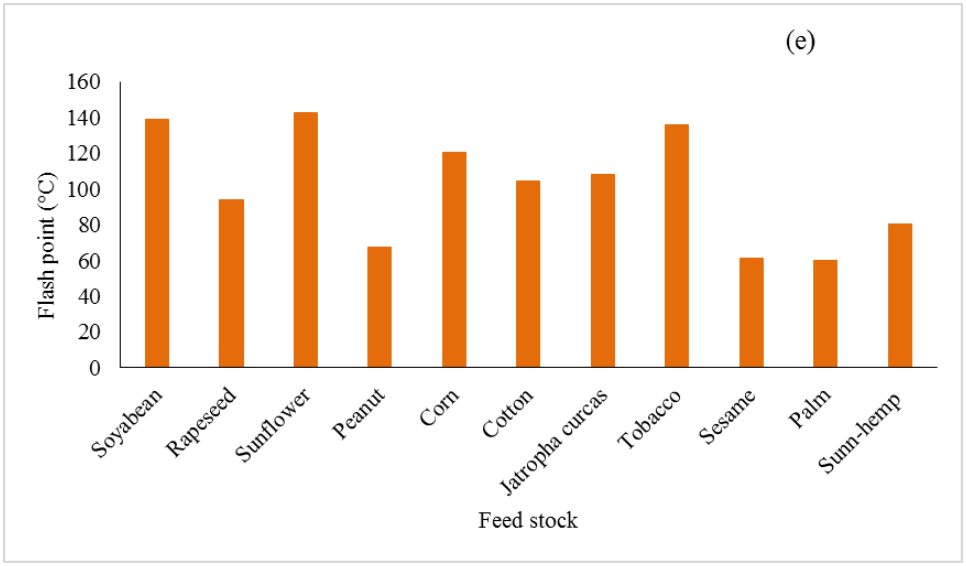
Sunflower
Peanut
Corn
Cotton
Jatropha curcas
Tobacco
Sesame
Palm
Sunn-hemp
Feed stock


(e)
Flash point (°C)
160
140
120
100
80
60
40
20
0
Soyabean
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Sunflower
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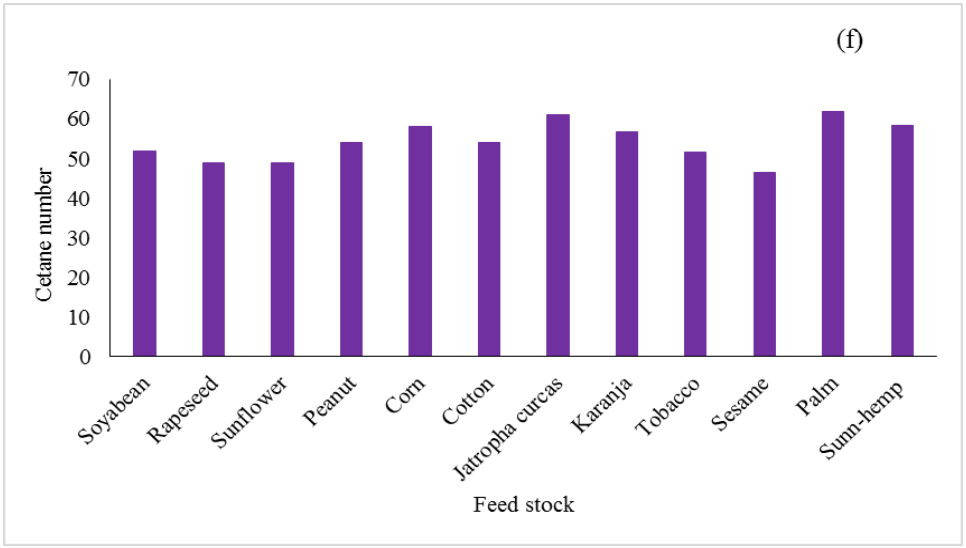
Corn
Cotton
Jatropha curcas
Tobacco
Sesame
Palm
Sunn-hemp
Feed stock


(f)
Cetane number
70
60
50
40
30
20
10
0
Soyabean
Rapeseed
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Peanut
Corn
Cotton
Jatropha curcas
Karanja
Tobacco
Sesame
Palm
Sunn-hemp
Feed stock

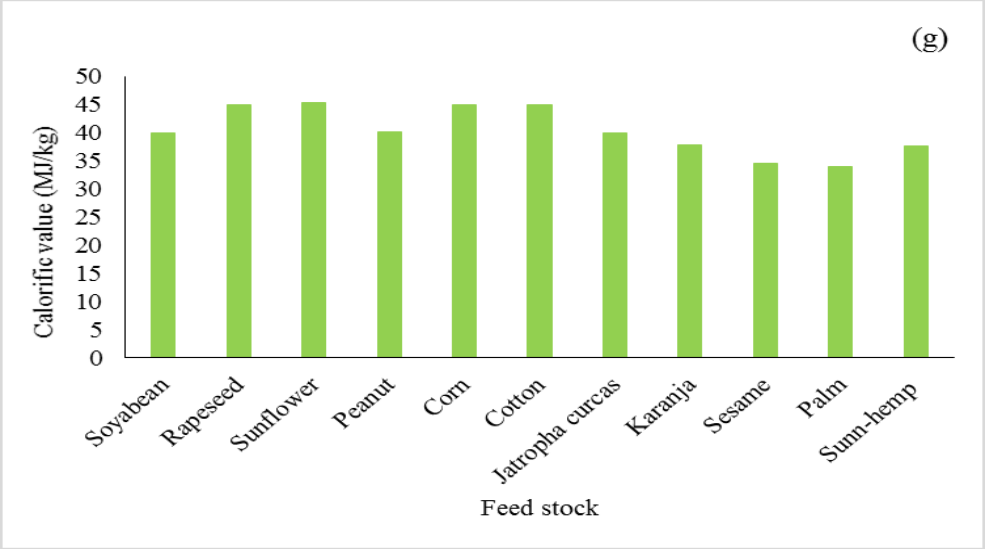


Figure 1 Fuel characteristics of the biodiesels produced from Sunn-hemp and other vegetable oils: (a) specific gravity, (b) kinematic viscosity, (c) saponification value, (d) iodine number, (e) flash point, (f) cetane number, (g) calorific value.

Activity II

❖ *Separation and purification of crude glycerol*

In this research work, several methods have been used to purify the waste glycerol, namely sequential desalination followed by extraction, adsorption of colour and finally separation through membrane under vacuum.

- Removal of alcohol: Excess alcohol from the trans-esterification step can be removed by evaporation in a rotary evaporator.
- Acidification: Initially, glycerol is acidified by dilute sulphuric acid or phosphoric acid to split the soap and purify the glycerol. The charred substances produced were filtered off. The samples were then decanted to recover the crude fatty acids.
- Neutralization: The aqueous glycerine solutions are neutralized by 50% sodium hydroxide. The salt crystallizing out was removed by decantation.
- Solvent extraction: In order to purify and concentrate the solutions further, they were solvent extracted and filtered to remove the residual salt. Finally, they were evaporated to obtain the crude glycerine.

Figure 2 differentiates between the standard sequence of purification and the specific sequence of methods that has been applied in this study.

❖ *Characterisation of glycerol obtained from Sunn-hemp seeds*

Table 1 shows an appreciable decrease in the quantities of fatty acids, *as relative percentages*, with crude glycerol as the reference. From this table it is seen that removal of fatty acids can be achieved by filtration under vacuum using suitable membranes (cellulose acetate).

Table 1 Components of fatty acids and their derivatives present in crude and purified glycerol obtained from Sunn-hemp seed-oil along with the corresponding peak areas, as relative %.

Components	Peak Areas (%) (RT, min)		
	Crude glycerol	Purified glycerol (*before membrane separation*)	Purified glycerol (*after membrane separation*)
hexadecanoic acid	3.5 *(16.75)*	0.74	0.32
octadecenoic acid	3.12 *(18.69)*	-	-
octadecadienoic acid	15.68 *(19.08)*	2.22	-
pentadecanoic acid	17.72 *(22.03)*	16.23	8.24
eicosatetraenoic acid	10.94 *(24.08)*	10.39	3.17
octadecadienoic acid	39.59 *(24.68)*	28.49	-
octadecatrienoic acid	2.18 *(25.51)*	-	-

❖ Optimization of the process parameters in order to obtain the maximum purity in the production of glycerol.

➢ Effects of various process parameters like type of acid, pH and amount of adsorbent used for the purification process are statistically optimized by Response Surface Methodology (RSM) using a Central Composite Design (CCD).

➢ After getting the optimum value, a new experiment is performed with the modelled conditions. The result shows a close agreement between experimental observation and model prediction. Optimum condition for a 90.4% pure glycerol is estimated to be attained at a pH of 3.26, an adsorbent quantity of 0.933g with phosphoric acid.

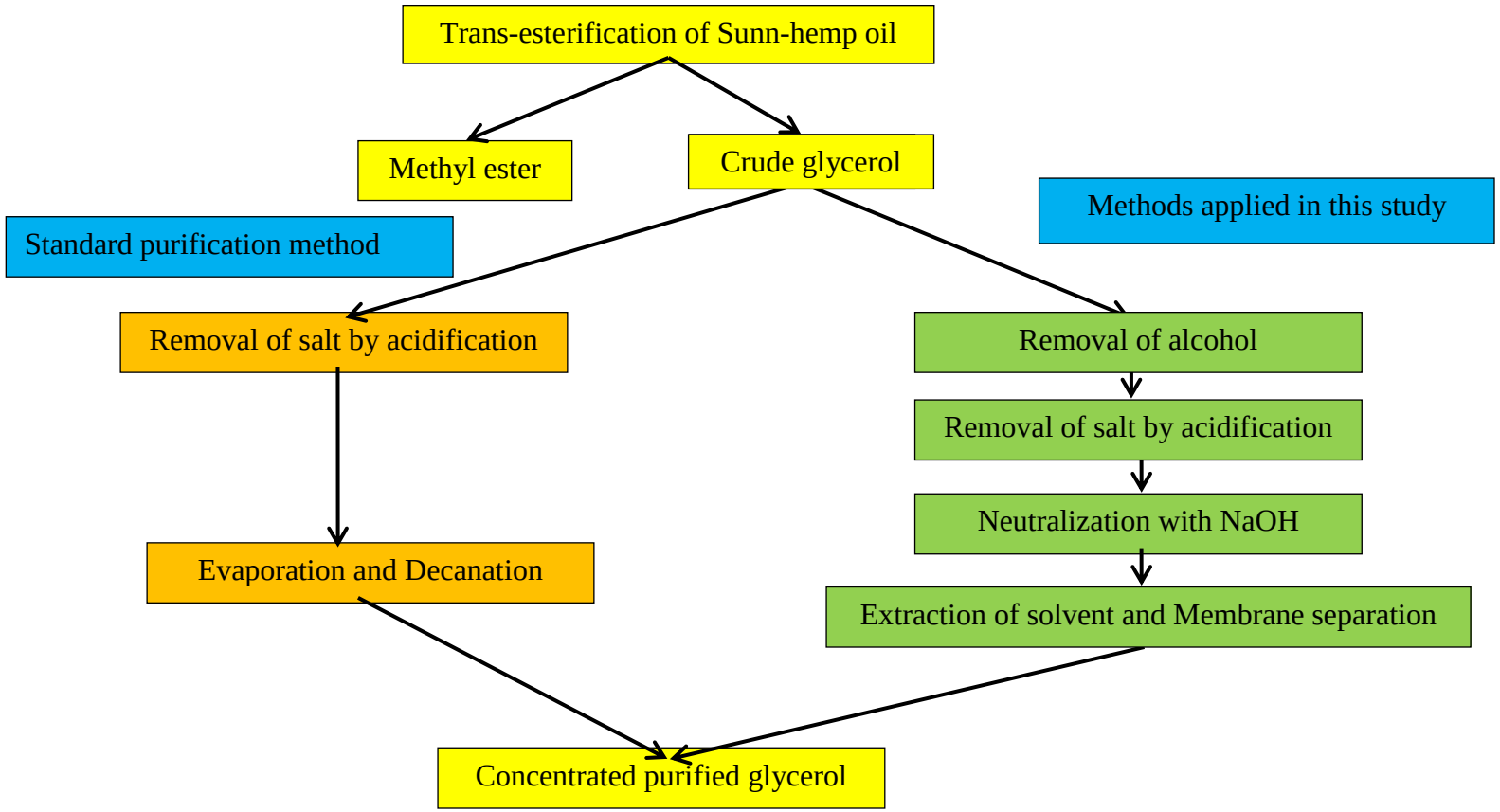


Figure 2 Schematic diagram of standard glycerol purification method and the novel method applied in this study.

Activity III

E Coli fermentation using glycerol as the major carbon source: Process optimization

- Kinetic models are developed for the microbial conversion of crude, laboratory grade and purified glycerol obtained in the process of biorefining *Crotalaria juncea* to produce succinic acid using *Escherichia coli*. Batch tests are performed for nine different substrate concentrations of commercial, purified and crude glycerol, to observe cell growth and substrate utilization rate.
  - Seven different models (inhibitory and non-inhibitory) are parameterized using non-linear least-square routines, in MATLAB, and then validated with a large range of glycerol concentrations to find out the inhibitory effects of the substrate on the growth rate of *Escherichia coli*.
- Inhibitory (Halden-Andrew, Aiba-Edward, Tessier type and Andrews) as well as non-inhibitory (Monod, Moser and Tessier) models are fitted to the relationship between specific growth rate and substrate concentration obtained from the growth curves.
- Considering the inhibition effect, Aiba-Edward model ranked 1 out of 7 in case of two samples and Haldane-Andrew model ranked 1 in case of one sample.
  - The best fit model can be used for design and optimization of batch fermentation processes to achieve a maximum yield of succinic acid.

Table 2 gives the details of overall performance of all the seven models.

Table 2 Overall model performance.

Rank	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
1				1	2		
2	1	1				1	
3	1				1	1	
4				2		1	
5	1						2
6			2				1
7		2	1				

- Aiba-Edward model gave the best fitment for *a large range of concentrations* of all the three types of glycerol, crude, purified and laboratory grade.

- Fermentation will not be effective with low nutrient levels. There should be a balance of trace minerals and other micronutrients which are optimal for the particular fermentative strain. If nitrogen levels are low, or trace minerals are missing, bacterial growth is

affected. On the other hand, toxic chemicals are inhibitory towards the growth of the microorganism.

- Crude glycerol, a by-product of biodiesel production, has many impurities which inhibit the growth of *E. coli*. In fermentation, impurities present in this material influence biochemical pathways in bacteria cells and can limit the efficiency of metabolite production.

Impurities in crude glycerol and their effects on the biochemistry of microorganisms: In general

Crude glycerol consists of glycerol and some impurities like methanol, inorganic salts, free fatty acid, unreacted mono-, di-and triacylglycerols, methyl esters, and water. The presence of these impurities has a pronounced negative effect on the morphology and biochemical processes of bacterial cells. As a result, lower concentrations of metabolites are obtained from crude glycerol as compared to those from pure glycerol.

Impurities in glycerol interact with each other and can have a synergic effect.

Inhibition of microbial growth and methanol

Although alcohol influences a cell membrane and increases its permeability, methanol, an aliphatic alcohol, in low concentrations with one carbon atom, does not influence the cell membrane or decrease metabolite production.

Inhibition of microbial growth by salts

There are several ways in which salts inhibit microbial growth. High concentrations of monovalent salts decrease van der Waals force in a lipid membrane and cause swelling of the cell membrane. This swelling exerts a negative effect on the energetic barrier in a lipid layer of the membrane and changes the course of biochemical processes in the cells. It influences the transportation of nutrition factors through the cell membrane. The acidity or alkalinity of the medium resulting from the addition of some of the salts can have profoundly adverse effects on bacterial growth. The free fatty acids (linoleic, stearic, oleic acids) have a major influence on the synthesis of metabolites. Fatty acids are component of the cell membrane and are incorporated into the acyl chains and alk-1-enyl chains of cellular lipids; their presence thus disturbs the correct process of metabolite synthesis.

- Stearic acid, a saturated fatty acid with an 18-carbon chain present, aligns with the fatty acid tails of the membrane in the fermentation medium.

- Oleic acid causes a kink in the molecule which hampers the diffusion of nutrient factors and metabolites through the membrane.

- Linoleic acid, a compound with a high degree of unsaturation, has two kinks which cause the inhibition of nutrient factors and thereby limit the synthesis of some products.

- The unsaturated fatty acids with two or more double bonds which are present in crude glycerol have strong influence on the diffusion of the substrate along the membrane.

Activity IV

Optimization of succinic acid production

❖ Statistical analysis for optimization of succinic acid production
  - ➢ This work is focused to develop a model of a response surface based on Face Centered Central Composite Design (FCCCD), in order to obtain the highest yield.
  - ➢ The operating parameters like, temperature, pH of the media, inoculum quantity and the composition of the nutrients like, yeast and peptone concentrations are changed.
  - ➢ Three types of glycerol, namely laboratory grade, crude and purified, are used for each experiment.

❖ Statistical analysis for the overall biorefining system

Statistical analysis of the overall system investigates the effect of process parameters for the *entire system* on the yield of succinic acid. Optimisation of process parameters for succinic acid production is done by Response Surface Methodology (RSM) using Face Centered Central Composite Design (FCCCD). Then a regression analysis is performed in order to obtain the optimum nutrients concentration of the medium to produce the highest yield of succinic acid.

Experimental methods

Statistical analysis of eleven independent variables with the Design Expert Software is performed to suggest a high goodness of fit of the model. The ranges of eleven variables selected for this study are enlisted in Table 3 and the design matrix along with the coded

value for the selected process variables is presented in Table 4. A set of ninety six experiments are performed where the response, (Yield of succinic acid) is correlated with the input factors of the process (oil amount, methanol mole, time of trans-esterification, KOH concentration, pH of glycerol purification medium, amount of adsorbent for colour removal, purity of glycerol, glycerol amount for fermentation, inoculum quantity, yeast concentration and peptone concentration). The model was justified by the coefficients of determination (R^2) and analysis of variance (ANOVA) along with the contour plots for the independent variables developed from the experimental data, given in Table 5. The design of experiment builds up an equation (6.1) for the yield of succinic acid with the coded variables.

Yield of succinic acid =

16.73+0.076A+0.16B-0.14C+0.17D+0.11E-0.092F+0.35G+3.63H+0.23J-0.18K+0.14L (6.1)

Table 3 Experimental ranges of the independent variables for the overall RSM.

Independent variables		Ranges of parameters	
Code	Name	Minimum	Maximum
A	oil amount	15	25
B	methanol mole	9	13
C	time of trans-esterification	2	6
D	KOH concentration	1	3
E	pH of glycerol purification medium	1	6
F	amount of adsorbent for colour removal	0.5	0.875
G	purity of glycerol	90.4	90.58
H	glycerol amount for fermentation,	5	15
J	inoculum quantity	1	3
K	yeast concentration	1	5
L	peptone concentration	2	6

Table 4 Experimental data and results of FCCCD for overall RSM for the production of succinic acid.

Run	Oil amount, g	Methanol mole	Time, hr	Catalyst concn., wt%	pH	amount of adsorbent, g	Purity of glycerol, %	Glycerol amount, g	Inoculum quantity ml	Yeast concn., gL^{-1}	Peptone concn., gL^{-1}	Yield of succinic acid gL^{-1}
1	15	13	6	1	6	0.5	90.58	5	1	1	2	14.21
2	25	13	6	3	6	0.875	90.4	5	1	1	6	13.02
3	15	13	2	1	1	0.5	90.58	5	3	1	2	14.57
4	25	13	2	3	6	0.875	90.58	15	1	5	6	20.52
5	20	11	4	3	3.5	0.6875	90.49	10	2	3	4	16.88
6	15	9	6	3	6	0.5	90.4	5	1	1	2	12.99
7	25	13	2	3	1	0.5	90.58	5	3	5	2	13.44
8	20	11	4	2	3.5	0.6875	90.58	10	2	3	4	20.58
9	15	9	6	1	1	0.5	90.58	5	1	1	6	13.2
10	25	13	2	3	1	0.5	90.4	5	3	1	6	13.49
11	15	9	2	1	1	0.875	90.58	15	1	1	2	17.89
12	20	9	4	2	3.5	0.6875	90.49	10	2	3	4	18.12
13	20	11	4	2	3.5	0.6875	90.49	10	2	3	4	16.51
14	25	9	6	1	1	0.875	90.4	5	3	1	6	14.02
15	20	13	4	2	3.5	0.6875	90.49	10	2	3	4	16.9
16	15	9	2	3	6	0.875	90.58	5	1	5	6	14.87
17	20	11	6	2	3.5	0.6875	90.49	10	2	3	4	18.02
18	15	9	2	3	1	0.875	90.58	15	3	5	6	22.35
19	20	11	4	2	6	0.6875	90.49	10	2	3	4	15.64
20	15	9	2	3	1	0.5	90.4	15	3	1	2	18.51
21	15	9	2	3	6	0.875	90.4	5	3	1	6	13.23
22	15	13	2	3	1	0.875	90.58	5	1	1	6	13.78

23	20	11	4	2	3.5	0.6875	90.49	10	2	3	4	16.32
24	15	9	6	1	1	0.5	90.4	15	3	5	6	18.42
25	20	11	2	2	3.5	0.6875	90.49	10	2	3	4	17.47
26	25	9	2	3	1	0.5	90.58	15	1	5	2	18.73
27	25	9	6	1	6	0.875	90.4	15	1	1	2	19.45
28	15	13	6	1	6	0.875	90.4	15	1	5	6	20
29	15	9	6	1	6	0.875	90.4	5	3	5	2	11
30	25	9	2	1	1	0.875	90.4	15	3	5	2	19.6
31	25	9	2	1	6	0.5	90.58	5	3	5	2	10.87
32	20	11	4	1	3.5	0.6875	90.49	10	2	3	4	17.06
33	15	13	6	1	1	0.5	90.4	15	1	1	2	17.89
34	15	9	2	1	6	0.5	90.4	15	1	5	2	23.51
35	25	13	2	1	6	0.5	90.4	15	1	1	2	18.47
36	25	9	6	1	6	0.5	90.4	5	1	5	6	12.54
37	15	9	6	1	1	0.5	90.58	5	1	5	2	11.4
38	25	9	2	1	1	0.875	90.58	5	1	5	6	12.09
39	15	13	6	3	1	0.875	90.4	15	3	1	6	18.25
40	15	11	4	2	3.5	0.6875	90.49	10	2	3	4	18.52
41	20	11	4	2	3.5	0.6875	90.49	10	2	3	4	15.45
42	25	9	2	1	6	0.875	90.4	5	1	5	2	11.46
43	25	11	4	2	3.5	0.6875	90.49	10	2	3	4	17.97
44	25	13	6	3	1	0.5	90.4	15	1	5	6	19.54
45	20	11	4	2	3.5	0.6875	90.49	10	2	3	4	14.78
46	20	11	4	2	3.5	0.6875	90.49	10	2	3	4	17.42
47	20	11	4	2	1	0.6875	90.49	10	2	3	4	15.49
48	15	13	2	3	1	0.875	90.4	15	1	5	2	20.55
49	20	11	4	2	3.5	0.6875	90.49	10	3	3	4	17.89
50	25	13	2	3	6	0.5	90.4	15	3	5	6	23.21
51	25	9	6	3	6	0.5	90.4	15	3	1	6	20.03

52	25	13	2	3	1	0.875	90.4	5	3	1	2	13.26
53	25	13	6	3	1	0.5	90.58	5	1	1	2	12.6
54	15	9	6	3	6	0.875	90.4	15	3	5	2	18.06
55	15	13	2	1	6	0.875	90.58	15	3	5	2	18.91
56	25	13	2	1	6	0.875	90.4	5	3	5	6	13.44
57	20	11	4	2	3.5	0.6875	90.4	10	2	3	4	18.89
58	20	11	4	2	3.5	0.6875	90.49	15	2	3	4	19.42
59	20	11	4	2	3.5	0.5	90.49	10	2	3	4	15.35
60	20	11	4	2	3.5	0.6875	90.49	10	2	3	6	19.46
61	20	11	4	2	3.5	0.6875	90.49	10	2	5	4	20.26
62	15	9	2	1	1	0.5	90.4	5	3	5	6	10.21
63	25	9	6	1	6	0.875	90.58	15	3	5	6	18.02
64	15	13	2	1	1	0.5	90.58	15	1	5	6	18.87
65	25	13	6	1	1	0.875	90.4	15	1	5	2	19.12
66	20	11	4	2	3.5	0.6875	90.49	5	2	3	4	12
67	15	13	6	3	6	0.5	90.58	5	3	5	6	12.14
68	15	13	6	1	1	0.875	90.58	5	3	5	6	13.64
69	25	13	6	1	6	0.5	90.58	15	3	5	6	20.97
70	25	9	2	1	1	0.5	90.58	15	3	1	6	23.27
71	15	13	2	1	1	0.875	90.4	5	1	1	6	10.86
72	25	9	2	3	6	0.5	90.58	5	1	1	6	13.01
73	15	13	2	1	6	0.875	90.58	15	3	1	6	20
74	20	11	4	2	3.5	0.6875	90.49	10	1	3	4	16.16
75	15	9	6	3	1	0.875	90.4	5	1	5	6	13.68
76	25	9	2	1	1	0.5	90.4	5	1	1	2	13.44
77	25	9	6	3	6	0.875	90.58	5	1	5	2	11.02
78	15	13	6	3	1	0.5	90.58	15	1	5	2	23.2
79	25	9	2	3	6	0.875	90.58	15	3	1	2	22.89
80	20	11	4	2	3.5	0.6875	90.49	10	2	3	2	19.77

81	25	9	6	3	1	0.5	90.58	5	3	5	6	14.21
82	25	13	2	3	6	0.5	90.4	5	1	5	2	10.465
83	15	13	2	1	6	0.5	90.4	5	3	1	2	14.75
84	20	11	4	2	3.5	0.6875	90.49	10	2	1	4	16.46
85	25	13	6	3	6	0.5	90.58	15	3	1	2	22.35
86	15	9	6	1	6	0.5	90.58	15	3	1	2	21.51
87	15	13	6	3	1	0.5	90.4	5	3	5	2	12.05
88	25	9	6	3	1	0.875	90.58	15	3	1	2	19.45
89	15	13	2	3	6	0.5	90.4	15	1	1	6	20.45
90	20	11	4	2	3.5	0.6875	90.49	10	2	3	4	15.46
91	15	13	6	3	6	0.875	90.58	5	3	1	2	14
92	25	13	6	1	6	0.875	90.58	5	3	1	2	14.7
93	15	9	6	3	6	0.875	90.58	15	1	1	6	18.45
94	20	11	4	2	3.5	0.875	90.49	10	2	3	4	19.7
95	25	13	6	1	1	0.875	90.58	15	1	1	6	21.23
96	25	9	2	3	1	0.875	90.4	15	1	1	6	20.4

Table 5 Analysis of variance (ANOVA) for the overall experimental results of the FCCCD.

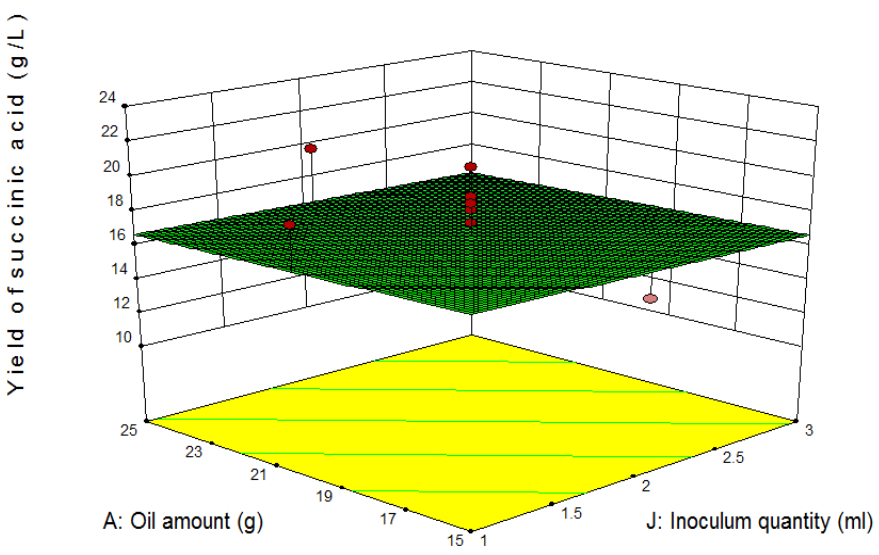
Source	Sum of squares	Degree of freedom (df)	Mean square	F value	P value Prob > F	Remarks
Model	944.48	11	85.86	32.30	< 0.0001	significant
A-Oil amount	0.40	1	0.40	0.15	0.6997	
B-Methanol mole	1.85	1	1.85	0.69	0.4070	
C-Time	1.41	1	1.41	0.53	0.4685	
D-Catalyst concentration	2.10	1	2.10	0.79	0.3763	
E-pH	0.84	1	0.84	0.32	0.5746	
F-amount of adsorbent	0.57	1	0.57	0.22	0.6435	
G-Purity of glycerol	8.42	1	8.42	3.17	0.0788	
H-Glycerol amount	920.04	1	920.04	346.08	< 0.0001	
J-Inoculum quantity	3.55	1	3.55	1.33	0.2513	
K-Yeast concentration	2.37	1	2.37	0.89	0.3482	
L-Peptone concentration	1.39	1	1.39	0.52	0.4709	
Residual	223.31	84	2.66			
Lack of Fit	218.85	79	2.77	3.11	0.1017	not significant
Pure Error	4.46	5	0.89			
Cor Total	1167.80	95				

The statistical significance of the model is established after obtaining ANOVA results, from P-values or probability of error values, which demonstrates the interaction strength of each parameter. In this model, a *P*-value <0.0001, corresponding to the F value of 32.3 along with high coefficient of determination (R^2 *=0.81*), adjusted coefficient of determination (*adjusted* R^2 *= 0.78*), predicted coefficient of determination (*Predicted* R^2*=0.75)* and a substantial low value of the coefficient of variation (CV= 9.75%) indicate high significance in predicting the response values and the suitability of the deduced model. Using design expert software the response surface plots, representing the effects of independent variables on the yield of succinic acid (dependant variable) for various fixed parameters, are obtained and shown in Figure 3.

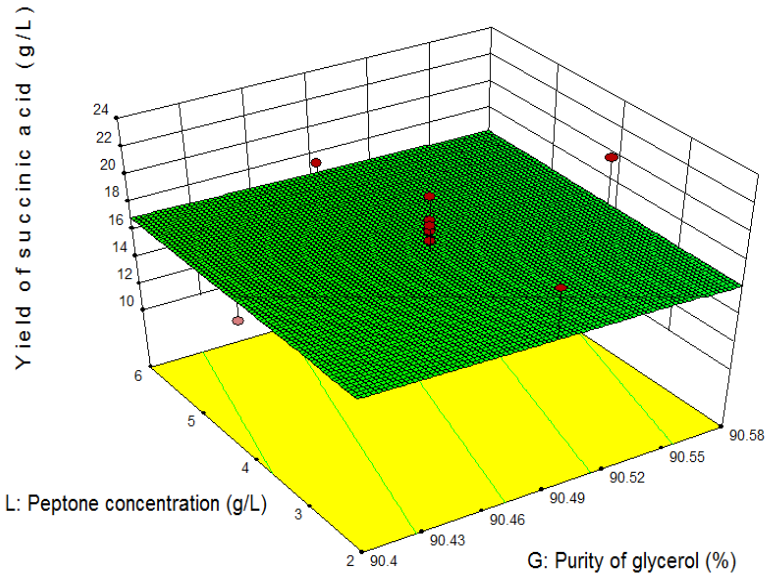
Figure 3a shows the influence of *interaction between oil amount and inoculum quantity* keeping other parameters constant, such as: methanol mole=11, time = 4 hr, catalyst

concentration= 2wt%, pH=3.5, adsorbent= 0.69g, purity of glycerol=90.49%, glycerol amount= 10g, yeast concentration= 1.54 gL^{-1} and peptone concentration =4 gL^{-1}. Figure 3b shows the influence of *interaction between peptone concentration and purity of glycerol* keeping other parameters constant, such as: oil amount= 20g, methanol mole=9.48, time = 4 hr, catalyst concentration= 2wt%, pH=3.5, adsorbent= 0.69g, glycerol amount= 10g, inoculum quantity = 2 ml and yeast concentration= 1.54 gL^{-1}. Figure 3c shows the influence of *interaction between glycerol amount and methanol mole* keeping other parameters at constant values, such as: oil amount= 20g, time = 4 hr, catalyst concentration= 2wt%, pH=3.5, adsorbent= 0.69g, purity of glycerol=90.49%, inoculum quantity = 2 ml, yeast concentration= 1.54 gL^{-1} and peptone concentration =4 gL^{-1}. Figure 3d shows the influence of *interaction between reaction time and methanol mole* keeping other parameters fixed, such as: oil amount= 20g, catalyst concentration= 2wt%, pH=3.5, adsorbent= 0.69g, purity of glycerol=90.49%, glycerol amount= 10g, inoculum quantity = 2 ml, yeast concentration= 1.54 gL^{-1} and peptone concentration =4 gL^{-1}. Figure 3e shows the influence of *interaction between inoculum quantity and glycerol amount* keeping other parameters at constant value, such as: oil amount= 20g, methanol mole=11, time = 4 hr, catalyst concentration= 2wt%, pH=3.5, adsorbent= 0.69g, purity of glycerol=90.49%, yeast concentration= 1.54 gL^{-1} and peptone concentration =4 gL^{-1}. Figure 3f shows the influence of *interaction between yeast concentration and pH of purification media* keeping other parameters at constant values, such as: oil amount= 24.55 g, methanol mole=12.77, time = 2.28 hr, catalyst concentration= 2.99wt%, adsorbent= 0.5g, purity of glycerol=90.57%, glycerol amount= 14.98g, inoculum quantity = 2.9 and peptone concentration =5.99 gL^{-1}. Figure 3g shows the influence of *interaction between amount of adsorbent and catalyst concentration* keeping other parameters constant, such as: oil amount= 24.55 g, methanol mole=12.77, time = 2.28 hr, pH=3.09, purity of glycerol=90.57%, glycerol amount= 14.98g, inoculum quantity = 2.9, yeast concentration= 1.05 gL^{-1} and peptone concentration =5.99 gL^{-1}. Figure 3h shows the influence of *interaction between inoculum quantity and purity of glycerol* keeping other parameters constant, such as: oil amount= 16.35 g, methanol mole=12.77, time = 2.28 hr, catalyst concentration= 2.99wt%, pH=3.09, adsorbent= 0.5g, glycerol amount= 14.98g, yeast concentration= 1.05 gL^{-1} and peptone concentration =5.99 gL^{-1}.

(a)

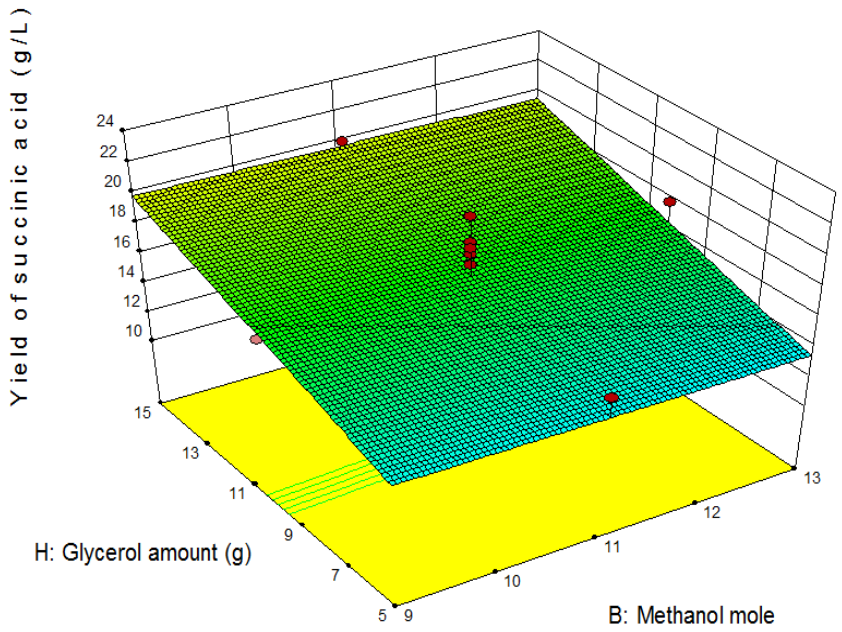

Yield of succinic acid (g/L)
24
22
20
18
16
14
12
10
25
23
21
19
17
15
A: Oil amount (g)
1
1.5
2
2.5
3
J: Inoculum quantity (ml)

(b)

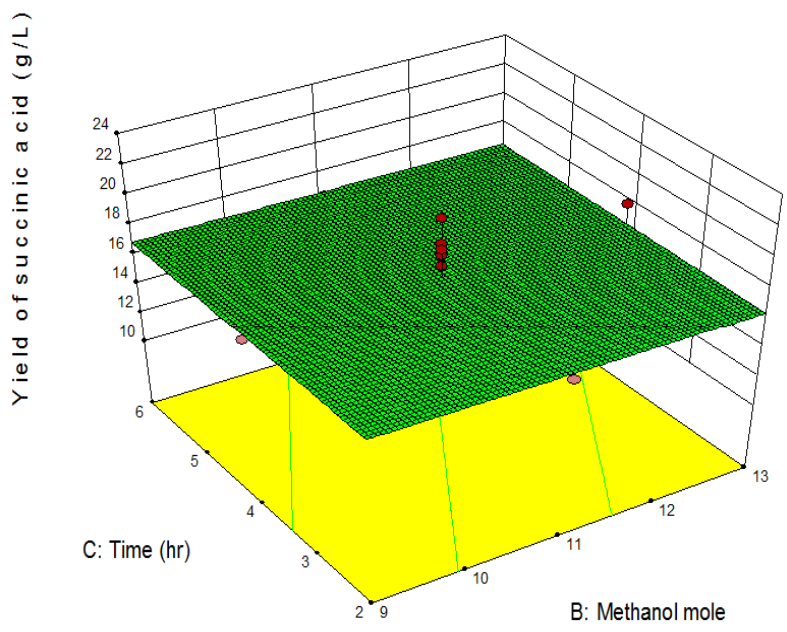

Yield of succinic acid (g/L)
24
22
20
18
16
14
12
10
6
5
4
3
2
L: Peptone concentration (g/L)
90.4
90.43
90.46
90.49
90.52
90.55
90.58
G: Purity of glycerol (%)

(c)

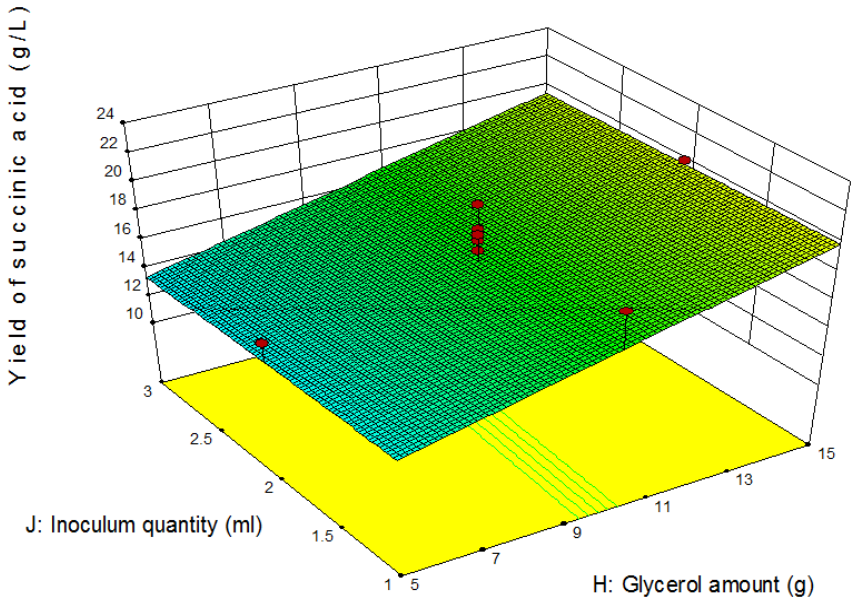
(d)


Yield of succinic acid (g/L)
24
22
20
18
16
14
12
10
15
13
11
9
7
5
H: Glycerol amount (g)
9
10
11
12
13
B: Methanol mole

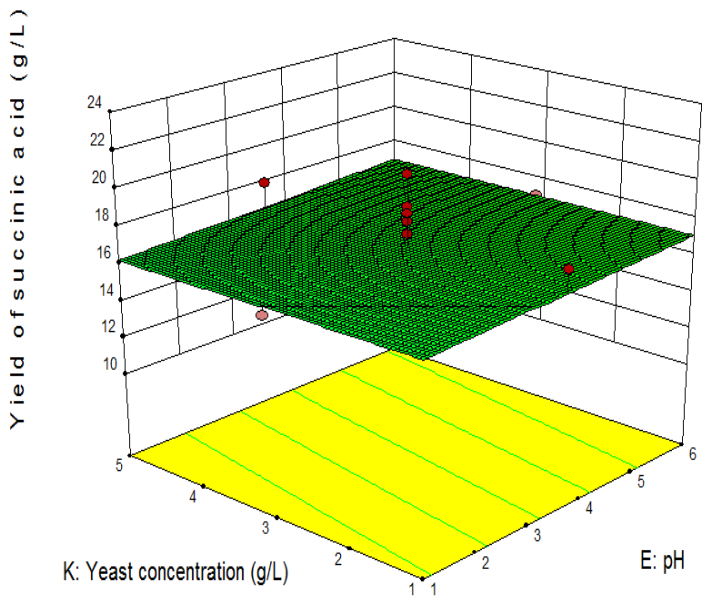
(e)


Yield of succinic acid (g/L)
24
22
20
18
16
14
12
10
6
5
4
3
2
C: Time (hr)
9
10
11
12
13
B: Methanol mole

(f)


Yield of succinic acid (g/L)
24
22
20
18
16
14
12
10
3
2.5
2
1.5
1
J: Inoculum quantity (ml)
5
7
9
11
13
15
H: Glycerol amount (g)

(g)


Yield of succinic acid (g/L)
24
22
20
18
16
14
12
10
5
4
3
2
1
K: Yeast concentration (g/L)
1
2
3
4
5
6
E: pH

(h)

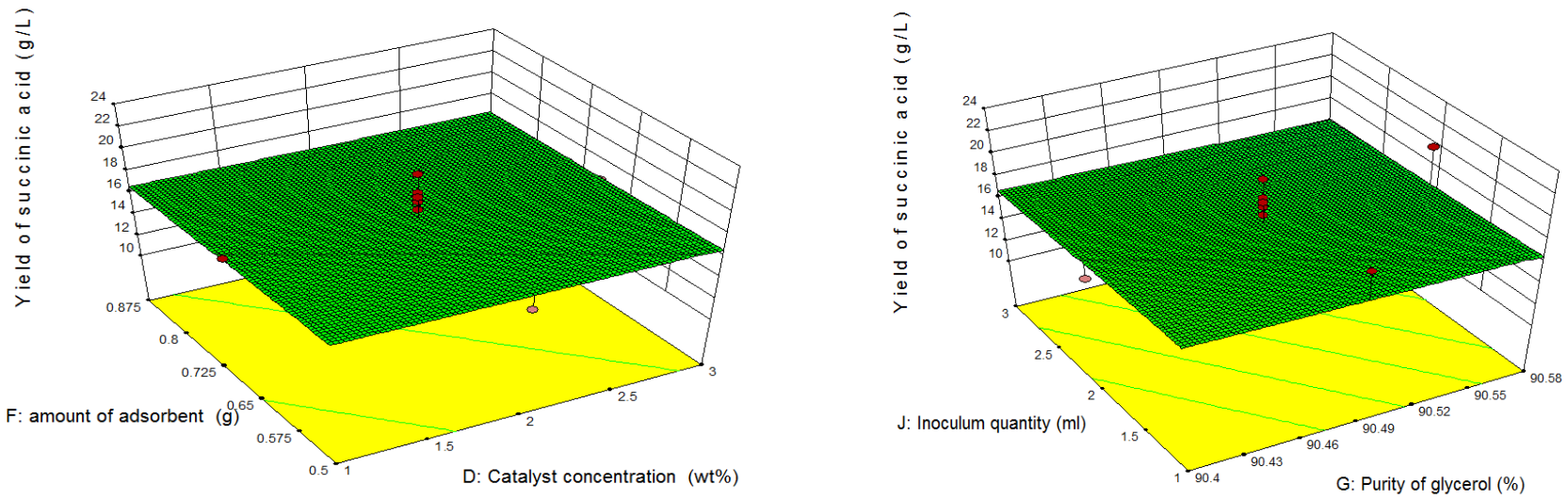
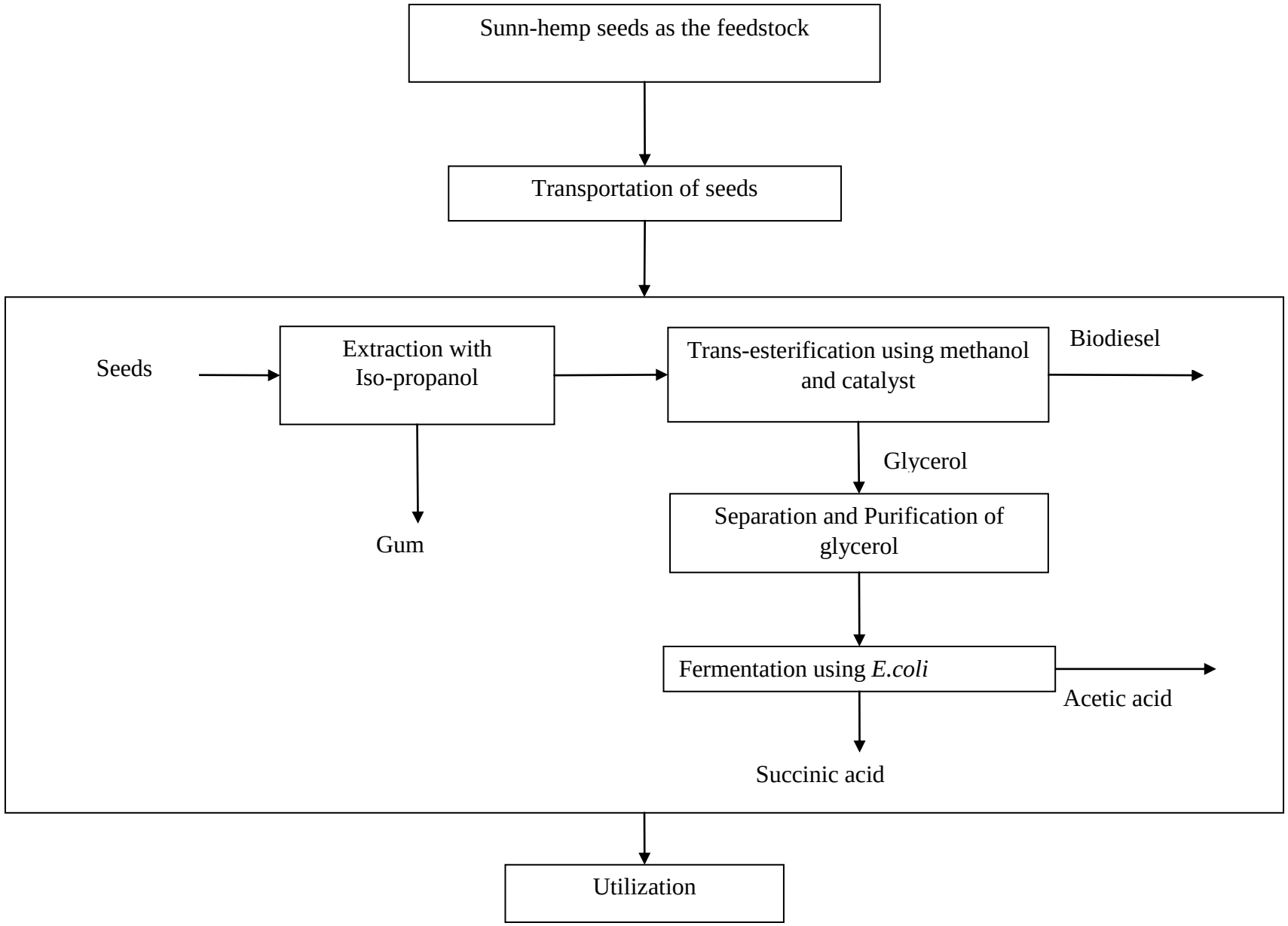


Figure 3 Response surfaces for production of succinic acid with varying process parameters: (a) oil amount and inoculum quantity; (b) peptone concentration and purity of glycerol; (c) glycerol amount and methanol mole; (d) reaction time and methanol mole; (e) inoculum quantity and glycerol amount; (f) yeast concentration and pH of purification media; (g) amount of adsorbent and catalyst concentration; (h) inoculum quantity and purity of glycerol.

- An integrated bio-refinery consists of all the steps starting from raw material handling and processing to utilization of products. The same is shown in the following schematic diagram.


Sunn-hemp seeds as the feedstock
Transportation of seeds
Seeds
Extraction with Iso-propanol
Gum
Trans-esterification using methanol and catalyst
Biodiesel
Glycerol
Separation and Purification of glycerol
Fermentation using E.coli
Acetic acid
Succinic acid
Utilization

Concluding remarks

- *Trans-esterification of the Sunn-hemp oil*:
  - Optimised statistical value of yield (60.92 wt%) was verified by performing trans-esterification reaction of *juncea* oil to form a biodiesel using KOH catalyst with 2 wt% catalyst and methanol to oil ratio 11:1 and the maximum yield of biodiesel was found to be 91.25 wt%.
  - After certain modifications or blending with petroleum derived diesel, this biodiesel could be used as a promising bio-fuel and *Crotalaria juncea* could be a new non edible feedstock for a technically feasible biorefinery.

- *Separation and purification of crude glycerol:*
  - A novel purification sequence, using acidification, neutralization, solvent extraction, decolourisation and finally pressure filtration through a membrane, was followed in order to remove impurities like soap, free fatty acids and fatty acid methyl esters.
  - Neutralization is carried out with four strong concentrated inorganic acids with corresponding purity being: phosphoric (*purity*: 94.4%), sulphuric (purity: 84.4%), hydrochloric (purity: 66.6%), perchloric acid (purity: 62.3%) and acetic acid (organic) with corresponding purity achieved was 63.6%.
  - As, there is not much difference in the properties of purified and commercial glycerol, purified one can be used as a replacement of commercial glycerol.
- *Microbial fermentation with E.Coli with purified glycerol: Production of succinic acid:*

  - It is evident that, purification of crude glycerol is necessary as impurities like soap, free fatty acids (FFAs) and Fatty Acid Methyl Esters (FAMEs) are harmful for the growth of *E.Coli*.
  - As, there is not much difference in the growth kinetics of *E.Coli* with purified and commercial glycerol as the main carbon source, first one can be used as a replacement of commercial glycerol.

  - Production of succinic acid: *Process optimization:*
  - Statistically optimised yield of 21.32 gL^{-1} is validated against an observed yield of 22.82 gL^{-1} with optimised values of process variables being: oil amount= 15.01g, inoculum quantity= 1.05ml, methanol mole= 9.03; fermentation batch time = 72hr; catalyst concentration= 1.26wt%, pH= 5.83; adsorbent= 0.51g; purity of glycerol= 90.4%; glycerol quantity = 14.97g; yeast concentration= 1.44gL^{-1} and peptone concentration = 6gL^{-1}.
  - It is thus concluded that *Crotalaria juncea* could be a new feedstock for a biorefinery from which various important building blocks can be obtained.