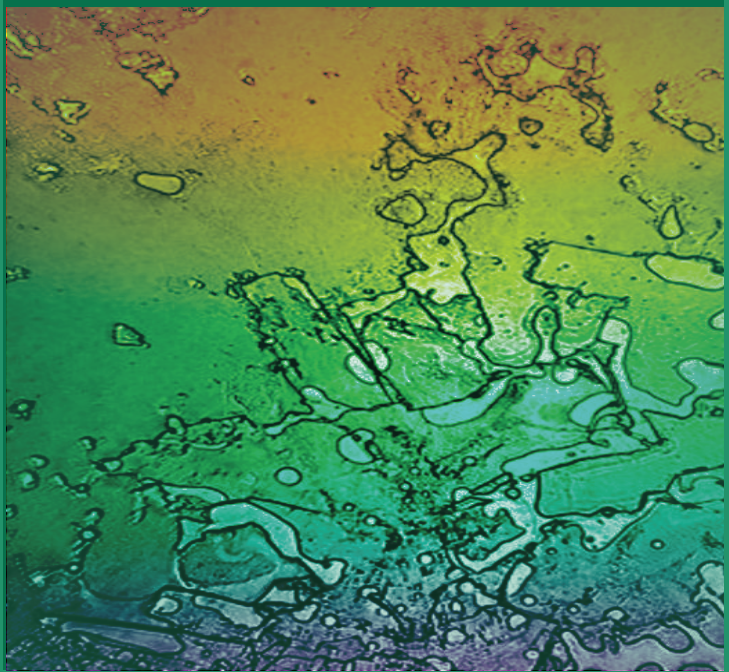
Chemical and Bioprocess Engineering

Trends and Developments



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Edited by

Shirish H. Sonawane, PhD, Y. Pydi Setty, PhD, and Srinu Naik Sapavatu, PhD

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Dr. Shirish H. Sonawane is currently working as Associate Professor in Chemical Engineering Department at the National Institute of Technology, Warangal, Telangana, India. His research interests focus on synthesis of hybrid nanomaterials, cavitation-based inorganic particle synthesis, sonochemical synthesis of nanolatex, process intensification, and microreactor for nanoparticles production. Dr. Sonawane is a recipient of fasttrack young scientist project in the year 2007 from the Department of Science and Technology, Government of India. He has industrial experience from reputed chemical industries such as Bayer Polymers (R&D). After M. Tech he carried out research work in University of Dortmund Germany on emulsion polymerization for four months. Dr. Sonawane has published more than 50 research papers in reputed journals, seven book chapters, and six Indian patents applications. He was a recipient of prestigious the BOYSCAST Fellowship from the Department of Science and Technology Government of India in year 2009. He was a visiting academic and worked in the particle Fluid Processing Center, University of Melbourne, Australia. He is also a Heritage Fellow and worked in the chemical engineering department at Instito Superio Technico Lisbon, Portugal in 2013. He was elected young Associate Fellow from Maharashtra Academy of Science. He has completed six consultancies in the chemical industries and four research projects from the Indian Government. Currently ISRO and Ministry of Environment and Forest projects are going on. He has awarded eight MTech and two PhD, and currently two PhD and two MTech students are working with him. He is reviewer of several international journals, notably in chemical engineering science, industrial engineering chemistry research, chemical engineering journal, materials science, chemical engineering processing, and process intensification. He has a good liaison with industries such as Corning India, Calchem, Roha Carbonates, Felix Industries etc. Recently Corning has donated a €30,000 (approximately Rs. 20 Lacs) reactor to Dr. Sonawane's laboratory for working with pharmaceutical and chemical industries in the area of process intensification. Dr. Sonawane re-

ceived the grant of Rs.80 Lacs for the Minicenter of Excellence in Sonoprocess Engineering for Nanoparticles in 2013 from the Ministry of Human Resources and Development (MHRD).

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He received the Sir Ganga Ram Memorial Award in January 2000 for the paper titled "Study on heat transfer using limpet coil jacket," published in the year 1998 by IEI (India), Chemical Engineering Division, and for the paper titled "Total liquid holdup in a liquid-liquid-solid fluidized bed" he was given a certificate of merit by IEI (India), Chemical Engineering Division. His paper titled "Heat transfer studies using a limpet coil jacket" was selected as the best paper in the technical session at IEI (India) held at Visakhapatnam, India.

Currently he has ISRO Project for preparation of nano Al particles. He also completed the MHRD sponsored project for Curriculum Design and Development and Consultancy from Pilot Scale Production of Nano Additives Argonate Phase-1. He has 16 research articles in SCI journals and one patent, 16 articles in international and national conferences. Four PhD students have completed their theses, and two are working under his guidance.

Srinu Naik Sapavatu, PhD

Srinu Naik Sapavatu has obtained a PhD in chemical engineering on "Optimization of Parameters for Biological Denitrification of Wastewater using Gas-Liquid-Solid Bioreactor" from the National Institute of Technology, Warangal (2013); an MTech from Chemical Engineering from Osmania University (2003); and a BTech in Chemical Engineering from Nagarjuna University (2001), India. He is an Assistant Professor in the Chemical Engineering

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LIST OF ABBREVIATIONS

AA	acetic acid
AAS	atomic absorption spectrophotometer
ABE	acetone, butanol, ethanol
ABS	acrylonitrile butadiene styrene
AMP	ammonium molybdophosphate
BSA	bovine serum albumin
CA	correspondence analysis
CBD	chitin binding domain
CCD	central composite design
CMC	carboxy methyl cellulose
CTAB	cetyl trimethyl ammonium bromide
CTF	charge transfer functions
DA	degree of deacetylation
DM	demineralized
DNS	di-nitro salicylic acid
EKF	extended Kalman Filter
EU	ethylenic unsaturation
FESEM	field emission scanning electron microscopy
FO	forward osmosis
FTIR	fourier transform infrared
FT-NMR	Fourier transforms nuclear magnetic resonance
GC	gas chromatograph
HPLC	high performance liquid chromatography
IARI	Indian Agricultural Research Institute
ITCC	Indian Type Culture Collection
KF	Kalman filter
LLDPE	low density polyethylene tube
MAP	modified atmosphere packaging
MCA	multi-way correspondence analysis
MCFC	molten-carbonate fuel cell
MG	malachite green
MP	membrane pertraction
MSM	multivariate statistical monitoring
MWCO	molecular weight cut off

NB	nitrobenzene
NLE	non- local environment
NOC	normal operating conditions
NPG	neopentyl glycol
OFAT	one-factor-at-a-time
PAA	peroxyacetic acid
PAN	polyacrylonitrile
PAT	process analytical technology
PCA	principal component analysis
PCD	pitch circle diameter
PDA	potato dextrose agar
PIV	particle image velocimetry
PU	polyurethanes
PVC	poly(vinylchloride)
PVP	polyvinylpyrrolidine
RBD	reactive batch distillation
SCF	super computing facility for bioinformatic
SEM	scanning electron microscopy
SLE	solid liquid equilibrium
SLs	sophorolipids
SOFC	solid-oxide fuel cell
SPR	surface plasmon resonance
SSF	solid state fermentation
TEM	transmission electron microscopy
TMP	trans-membrane pressure
TMP	trimethylol propane
UF	ultrafiltration
VLLE	vapour-liquid-liquid equilibria
WFSFO	waste fried sunflower oil
XRD	X-ray diffraction
YEGS	yeast extracts glucose salt

LIST OF SYMBOLS

A_j	molar holdup on *j*th plate (mol)
D	distillate flow rate (mol/s)
BuAc	butyl acetate
BuOH	butanol
HAc	acetic acid
H_2O	water
L_j	liquid flow rate from *j*th plate (mol/s)
N	no. of stages excluding reboiler and condenser = 31
Q_0	heat duty on condenser-reflux drums (J/s)
Q_{n+1}	heat duty on reboiler (J/s)
R_{ij}	rate of reaction of *i*th component on *j*th plate (s^{-1})
t	time (s)
V	vapour flow rate (mol/s)
x_{ji}	mole fraction of *i*th component in liquid phase on *j*th plate
y_{ji}	mole fraction of *i*th component in vapour phase on *j*th plate
C_s	concentration of surfactant (ppm)
D	pipe diameter (m)
K	consistency of fluid ($Pa.s^n$)
L	length (m)
n	flow behavior index (-)
ΔP	pressure drop (N/m^2)
U_f	fluid velocity (m/s)
ρ_f	density of fluid (kg/m^3)
τ_w	wall shear stress (Pa)
γ_w	wall shear rate (s^{-1})
γ_a	apparent shear rate (s^{-1})

NOMENCLATURE

A	area of heat transfer (m^2)
C_p	specific heat ($kJ\ kg^{-1}\ K^{-1}$)
d	diameter (m)
De	Dean number

g	gravity (m/s^2)
k	thermal conductivity (W m^{-1} K^{-1})
L	length of coil tube
Nu	Nusselt number
Ra	Rayleigh number
R_c	curvature radius of the coil (m)
Re	Reynolds number
T	temperature (K)
v	velocity (m/s)

GREEK LETTERS

ΔT	temperature difference (K)
μ	viscosity (kg m^{-1} s^{-1})
ρ	density (kg m^{-3})
ϕ	volume fraction
β	thermal expansion coefficient (K^{-1})
α	thermal diffusivity (m^2/s)
η	kinematic viscosity

SUBSCRIPT

BF	basefluid
crit	critical
i	inside
NF	nanofluid
NP	nanoparticle
o	outside
s	shell

PREFACE

This book, **Chemical and Bioprocess Engineering: Trends and Development**, is the result of 2013 international conference organized by the Department of Chemical Engineering at the National Institute of Technology, Warangal, India. Out of 165 articles, we have selected 36 articles to publish in this book. We received our inspiration from Professor T. Srinivasa Rao, Director of the National Institute of Technology, Warangal, and the Eminent Scientist Dr. B. D. Kulkarni of National Chemical Laboratory, Pune. They have inspired us to take up the task for writing this book.

This book covers different areas of chemical and biochemical engineering. Some of the important and recent topics in this field include CFD simulation, statistical optimization, process control, wastewater treatment, microreactors, fluidized bed drying, and hydrodynamic studies of gas liquid mixture in pipe. Some additional important and special topics include ultrasound-assisted extraction, process intensification, polymers and coatings, etc. Modeling of bioreactor and enzyme systems and biological nitrification are notable topics from biochemical engineering. We, the editors, are proud to bring out this special type of book that reports on these areas.

All the chapters went through a crucial process of peer review and were improved through the addition of the experts' comments and suggestions.

We would also like to acknowledge the team of Apple Academic Press, Ms. Sandra Jones Sickels, Vice President (Editorial and Marketing), Mr. Ashish Kumar, President and Publisher, and Mr. Rakesh Kumar from Apple Academic Press. Inc., USA, for their prompt and supportive attention to all our queries related to editorial assistance.

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INTRODUCTION

This book is divided into two parts. The first part contains 22 chapters covering different areas of chemical and bioprocess engineering. Selected articles from the 2013 International Conference on Chemical and Biochemical Engineering in India, held at the Chemical Engineering Department of the National Institute of Technology, Warangal, are organized in two parts. The two parts cover a wide variety of interdisciplinary topics from nanoscience with emphasis on particle production to applications in wastewater treatment, energy materials, etc.

In Part I, we attempted to highlight recent trends in chemical and biochemical engineering, such as integration of processes syrup and concentrate, uniflow cyclone design and its optimization, batch fermentation process and para multivariate statistical MCA model analysis, extended Kalman filter (EKF) based state and parameter estimation for reactive batch distillation process, statistical optimization, newer feedstock for ethanol production, and optimization of these processes. Some of the reaction kinetics problems of aniline nitration reaction are also addressed in Chapter 8. Detailed experimental rheological studies of microbubbles and suspension are also reported as are several thermodynamics studies of petroleum feed-stock butanol decomposition. A novel method developed to increase the cleavage efficiency of recombinant bovine enterokinase using magnesium ion as cofactor is also included as part of the coverage of biochemical engineering. A detailed study of the development of an optimum condition for production of chitosan from fish scales is also reported in Chapter 15. CFD study of mixing in shear thinning fluid in stirred tanks is also reported in this book. Also included is heat transfer analysis, CuO in helical coil. Homology modeling of a 3-D structure of L-asparaginase from *Enterobacter aerogenes* KCTC2190 using Swiss-Model is also discussed. The hydrodynamic behavior of mixing of binary mixture in a spout-fluid bed is strongly influenced by the difference in properties of the respective particles and is also covered. The effect of various parameters of continuous fluidized bed drying is reported in Chapter 20. Flow behavior in microchannels by using CFD simulation and velocity profile at outlet of the microchannels is also reported. Also in this book the experimental study and work that involves mass transfer without chemical reaction and mass transfer with chemical reaction is examined.

The second section of this book consists of chapters related to energy, the environment, and nanotechnology and allied chemical engineering themes.

Energy generationand storage is one of the important challenges today, and chemical engineering will play an important role in this area; hence we felt it was important to include some coverage of subject area of materials engineering and energy. Chapter 23 reports on the finding of the use of proper equipment and optimization of air fuel mixture that can save significant energy and can be helpful for the environment also. Membrane technology is a very important area in terms of clean drinking water, and separation technology is a concern, which is explained in Chapter 24. Another chapter reports on the performance of the hollow fiber (HF) membrane synthesized by using PVDF as a semicrystalline polymer. Chapter 25 recounts a study on epoxidation of vegetable oil that can be carried out *in situ* with formed or preformed peroxyacid in the presence of an acidic catalyst. Chapter 30 deals with the removal of toxic heavy ions metals, mainly copper and their ions from pollutant industrial wastewater.

The development of a kinetic model for the production of acetone, butanol, and ethanol (ABE) by using fermentation of starch is also related in Chapter 27. In Chapter 28 vegetable biomass used for production of holocellulolytic enzymes is examined. The removal of ammonium from wastewater by using method of biological nitrification is reported in Chapter 29, and in Chapter 30, the removal of chromium from aqueous solution by using sawdust, fly ash, and bagasse as an adsorbent is reported. In Chapter 31 the preparation of PANI-calcium zinc phosphate nanocomposite by ultrasound assisted *in situ* emulsion polymerization of aniline is covered. The performance of 2K epoxy coatings in presence of nanoclay filler is related in Chapter 32. In Chapter 33 the biosynthesis of silver nanoparticles using natural reducing agents is described. Degradation and adsorption of azo dye Malachite Green (MG) that is widely used in textile dyeing by means of ultrasonic cavitation is also reported in Chapter 34. Synthesis of nanoparticles of iron oxide is examined in Chapter 35. In addition, a comparative study of the production of nanoparticles in microreactor and advanced flow reactor is presented in Chapter 36.

We trust that the varied chapters in this book will stimulate new ideas, methods, and applications in ongoing advances in this growing area of chemical and biochemical engineering.Through this book we have provided an exploration of the emerging areas in this field, such as water treatment, CFD simulation, nanotechnologies, polymer engineering, drying,fluidization, mass transfer, biochemical engineering, fermentation technologies, reactive distillation, microreactor, and more, that are poised to make the new revolution become a reality.

PART I

CHEMICAL AND BIOPROCESS ENGINEERING

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CHAPTER 1

STATISTICAL OPTIMIZATION OF CULTURE MEDIA COMPONENTS FOR ENHANCED PRODUCTION OF SOPHOROLIPIDS FROM *STARMERELLA BOMBICOLA* MTCC 1910 USING WASTE FRYING SUN FLOWER OIL

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CONTENTS

1.1 INTRODUCTION

Surfactants constitute an important class of industrial chemicals used widely in almost every sector of modern industry. Present worldwide production of surfactants is around 12.5 million tons per year worth approximately US$28 billion, growing at the rate of about 0.5 million tons per year. Around 60 percent of surfactant production is used in household detergents, 30 percent in industrial and technical applications, 7 percent in industrial and institutional cleaning, and 3 percent in personal care. Asia accounts for largest share of global detergents consumption at 33 percent, whereas Eastern Europe's soaps and detergents market is reported to grow at a faster rate than Asia [1]. Almost all of these surfactants are petroleum based and are produced by chemical processes, which often damaging to environment are leading to significant ecological problems. Hence interest in biosurfactants has been steadily increasing in recent years due to their eco-friendly nature [2].

Microbially produced surfactants which are referred to as biosurfactants must compete with surfactants in three aspects—cost, functionality, and production capacity to meet the needs of intended applications [3]. Biosurfactants like glycolipids, rhamnolipids that are environmental friendly, nontoxic, and active over wide range of temperature and pH, can be produced with reasonable yields by microorganisms. One class of biosurfactants that is being currently produced industrially is glycolipids [4]. They are made up of disaccharide sophorose linked to a hydroxy fatty acyl moiety. The hydroxy fatty acid itself counts in general 16 or 18 carbon atoms and can have one or more unsaturated bonds [5]. They possess good surface active properties and show excellent skin compatibility, a property that is very important for cosmetic and personal care applications [6, 7].

The cost of raw materials makes up approximately 40–50 percent of the whole expense for biosurfactants production [8]. To overcome this problem, processes should be coupled to utilization of waste substrates combating at the same time their polluting effect which balances the overall costs [9]. Frying oil is produced in large quantities for use in both food industry and at the domestic scale. After usage, cooking oil changes its composition and contains more than 30 percent of polar compounds depending on the variety of food, the type of frying, and the number of times it has been used [10]. In the present work along with glucose, waste fried sunflower oil (WFSFO) has been used as carbons substrate, to synthesize sophorolipids from *Starmerella bombicola*, with two objectives. Earlier researchers have already studied the synthesis of sophorolipids using glucose and sunflower oil from *Starmerella bombicola* and reported and yield of 38.6 g/l [11].

Starmerella bombicola a teleomorph of *Candida bombicola* is a less reported species capable of producing sophorolipids (SLs). Sophorolipids fermentation is a two-step process in which production occurs after growth when one of the nutrients, such as the nitrogen source, has been exhausted [12, 13]. Any attempt to increase the yield of a biosurfactants demands optimal addition of media components and selection of the optimal culture conditions that will induce the maximum or the optimal production [14]. Hence for maximizing the production of sophorolipids, the concentrations of different media components are critical. A very efficient way to enhance the value and quality of research and to cut the process development time and cost is through statistically designed experiments. The full factorial central composite design (CCD) consists of a complete 2^K factorial design, where K is the number of test variables, n_o center points ($n_o \geq 1$) and two axial points on the axis of each design variable at a distance α ($=2^{K/4}$) from the design center [15]. A full factorial composite design is used to acquire data to fit an empirical second-order polynomial model. For four factors, the quadratic model takes the following form,

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 \\ \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 \quad (1.1)$$

where x_1, x_2, x_3, and x_4 represent four selected factors, concentration of glucose, WFSFO, yeast extract, and urea. Y is the response value, concentration of sophorolipids produced at the end of incubation period. β_i and β_{ij} are the model coefficient parameters estimated from regression results.

1.2 MATERIALS AND METHODS

1.2.1 MICROORGANISM

Starmerella bombicola MTCC 1910 was procured from Institute of Microbial Technology, Chandigarh, India. The organism was maintained on dextrose—peptone—yeast extract agar slants and subcultures every 4–6 weeks. Seed culture was developed from frozen *Starmerella bombicola* in 250 ml Erlenmeyer flasks containing sterile 50 ml medium. The composition of the inoculum medium was (g/l): glucose 100 and yeast extract 10. This was inoculated with one loop of the strain from agar slant and incubated for 48 hr at 30°C on a rotary shaker at 200 rpm [15, 16].

1.2.2 EXPERIMENTAL DESIGN

A 2^4 full factorial CCD with 7 replicates at the center point, 16 cube points and 8 axial points was used to optimize the production of sophorolipids. The upper

and lower limits of range of variables covered in the present study are shown in Table 1.1.

TABLE 1.1 Experimental range and levels of independent variables

	−2	−1	0	1	2
Glucose (g/l)	0	50	100	150	200
WFSFO (g/l)	0	50	100	150	200
Yeast extract (g/l)	0	5	10	15	20
Urea (g/l)	0	0.5	1.0	1.5	2.0

The details of the experimental conditions generated by the software are shown in Table 1.2.

TABLE 1.2 F.F.C.composite design matrix of four variables in coded and natural units with the observed responses

Obs No	x_1	x_2	x_3	x_4	Glucose (g/l)	WFSFO (g/l)	Y. Ext (g/l)	Urea (g/l)	SLs Prod (g/l)
1	−1	−1	−1	−1	50	50	5	0.5	30.06
2	+1	−1	−1	−1	150	50	5	0.5	33.21
3	−1	+1	−1	−1	50	150	5	0.5	32.12
4	+1	+1	−1	−1	150	150	5	0.5	33.02
5	−1	−1	+1	−1	50	50	15	0.5	31.19
6	+1	−1	+1	−1	150	50	15	0.5	28.41
7	−1	+1	+1	−1	50	150	15	0.5	26.10
8	+1	+1	+1	−1	150	150	15	0.5	19.24
9	−1	−1	−1	+1	50	50	05	1.5	23.66
10	+1	−1	−1	+1	150	50	05	1.5	21.52
11	−1	+1	−1	+1	50	150	05	1.5	21.12
12	+1	+1	−1	+1	150	150	05	1.5	17.12
13	−1	−1	+1	+1	50	50	15	1.5	15.23
14	+1	−1	+1	+1	150	50	15	1.5	10.12
15	−1	+1	+1	+1	50	150	15	1.5	9.52
16	+1	+1	+1	+1	150	150	15	1.5	12.62
17	−2	0	0	0	0	100	10	1	13.24

TABLE 1.2 *(Continued)*

Obs No	x_1	x_2	x_3	x_4	Glucose (g/l)	WFSFO (g/l)	Y. Ext (g/l)	Urea (g/l)	SLs Prod (g/l)
18	+2	0	0	0	200	100	10	1	17.25
19	0	−2	0	0	100	0	10	1	25.21
20	0	+2	0	0	100	200	10	1	18.25
21	0	0	−2	0	100	100	0	1	30.12
22	0	0	+2	0	100	100	20	1	24.85
23	0	0	0	−2	100	100	10	0	33.31
24	0	0	0	+2	100	100	10	2	15.92
25	0	0	0	0	100	100	10	1	33.12
26	0	0	0	0	100	100	10	1	33.52
27	0	0	0	0	100	100	10	1	32.14
28	0	0	0	0	100	100	10	1	32.56
29	0	0	0	0	100	100	10	1	33.29
30	0	0	0	0	100	100	10	1	32.92
31	0	0	0	0	100	100	10	1	33.10

All the experiments were carried out in duplicate and the amount of sophorolipids produced was estimated based on the average value of two experiments, which are also listed in Table 1.2. The optimal concentrations of the media components were obtained by solving the regression equation which was developed by fitting the process variables against the concentration of the sophorolipids produced.

1.2.3 RECOVERY, QUANTIFICATION, AND CHARACTERIZATION OF SOPHOROLIPIDS

The volume of entire culture medium after fermentation was measured and centrifuged at 10,000 rpm at 4°C for 20 min. The supernatant was extracted twice with equal volumes of ethyl acetate. Since the recovery and concentration of biosurfactants from fermentation broth largely determines the production cost, ethyl acetate was identified to be a better choice than the highly toxic chloro-organic compounds [17]. The pure sophorolipids were recovered from the hexane by vacuum evaporation and then accurately weighed. Sophorolipids synthesized

using glucose and WFSFO was characterized by Fourier transforms infrared spectroscopy (FT-IR) and Fourier transforms nuclear magnetic resonance (FT-NMR) techniques.

1.3 RESULTS AND DISCUSSION

1.3.1 RESPONSE SURFACE METHODOLOGY

The amount of sophorolipids (g/l) produced, under each of the experimental conditions is listed in Table 1.2 and are related to the process variables using the regression equation by surface response methodology as,

$$Y = 3.65 + 0.37x_1 + 0.21x_2 + 1.3x_3 + 7.77x_4 - 0.002x_1^2 - 0.001x_2^2 - 0.051x_3^2 - 8.00x_4^2 - 0.0005x_1x_2 - 0.002x_1x_3 - 0.06x_1x_4 - 0.003x_2x_3 - 0.006x_2x_4 - 0.31x_3x_4 \quad (1.2)$$

Where Y is the amount of sophorolipids and x_1, x_2,x_3, and $x_4$are the coded values of the test variables glucose, WFSFO, yeast extract, and urea concentrations respectively. The significance of each coefficient was determined by student's *t* test and *p* values which are listed in Table 1.3.

TABLE 1.3 Analysis of variance for sophorolipids production

Source	Degrees of Freedom	Sum of Squares	Adjusted Sum of Squares	Adjusted Mean of Squares	*t* Value	*p* Value
Regression	14	1,816.04	1,816.036	129.717	14.57	0.000
Linear	4	1,045.12	292.427	73.107	8.21	0.001
Square	4	745.16	745.163	186.291	20.93	0.000
Interaction	6	25.75	25.749	4.291	0.48	0.812
Residual error	16	142.44	142.439	8.902		
Lack of fit	10	141.14	141.138	14.114	65.09	0.000
Pure error	6	1.30	1.301	0.217		
Total	30	1,958.47				

$R^2 = 0.927$; adj $R^2 = 0.864$; and $R = 0.96$.

The *p* values were used as a tool to determine the significance of each of the coefficients, which in turn, are necessary to understand the pattern of mutual interactions between the test variables [18]. The larger of the magnitude of the *t*-value and smaller of the value of *p*-value, the more significant is the corresponding coefficient [15]. Based on the *p*-values, interactions of glucose-

WFSFO, glucose-urea, and WFSFO-urea are insignificant. After removing the insignificant parameters, Eq. (1.2) can be simplified as,

$$Y = 3.65 + 0.37x_2 + 0.21x_3 + 1.3x_3 + 7.77x_4 - 0.002x_1^2 - 0.001x_2^2 - 0.051x_3^2 - 8.00x_4^2 - 0.002x_1x_3 - 0.003x_2x_3 - 0.31x_3x_4 \quad (1.3)$$

A high R^2 value of 0.927 and R of 0.96 indicate the close proximity of the model equation with the experimental data. Among the first order terms, glucose has the lowest p value, which implies the first order effects of glucose is very significant, and it has to be maintained at zero level for the maximum production of sophorolipids. WFSFO has also got low p value (0.007). Increasing the concentration of oil resists the growth of microorganism as it creates stress to the microorganism and retards the production of sophorolipids.

The quadratic main effects of glucose and sunflower oil are also more pronounced than the others. This suggests that any minor change in these variables from their zero level values would cause a second-order positive or negative shift in the production of sophorolipids. The initial concentration of glucose is very important as *Starmerella bombicola* grows by consuming glucose, while the stress created by the presence of WFSFO after the depletion of glucose, produces extracellular product sophorolipids. Sophorolipids produced acts as emulsifier which facilitates the consumption of lipidic carbon source by microorganism. As concentration of the sunflower oil has to be maintained around zero level, concentration of the yeast extract can be minimized which increases the production of sophorolipids. Process economics favors such a reduction as yeast extract is costlier than sunflower oil. It is also well known that sophorolipids production occurs under nitrogen limiting conditions [12–16]. The summary of the results of the ANOVA analysis of the quadratic response surface model is presented in Table 1.4.

TABLE 1.4 The least squares fit and parameter estimates(significance of regression coefficients)

Term	Factor Errors	Standard Errors	t	p
Intercept	3.650	8.760	0.417	0.682
x_1	0.373	0.069	5.377	0.000
x_2	0.213	0.069	3.067	0.007
x_3	1.304	0.069	1.880	0.078
x_4	7.767	6.94	1.120	0.279
$x_1 \times x_1$	−0.001	0.000	−7.784	0.000
$x_2 \times x_2$	−0.001	0.000	−4.879	0.000

TABLE 1.4 *(Continued)*

Term	Factor Errors	Standard Errors	t	p
$x_3 \times x_3$	−0.051	0.022	−2.300	0.035
$x_4 \times x_4$	−8.003	−8.003	−3.586	0.002
$x_1 \times x_2$	0.000	0.000	0.002	0.999
$x_1 \times x_3$	−0.002	0.003	−0.801	0.435
$x_1 + x_4$	−0.006	0.030	−0.214	0.833
$x_2 \times x_3$	−0.003	0.003	−1.039	0.314
$x_2 \times x_4$	0.006	0.030	0.188	0.853
$x_3 \times x_4$	−0.312	0.298	−1.044	0.312

Regression Eq. (1.3) was solved to get optimal design conditions. The optimum levels of glucose, WFSFO, yeast extract, and urea were found to be 100 g/l, 87.6 g/l, 6.9 g/l, and 0.3 g/l respectively. Under the optimized experimental conditions the model predicts a highest concentration sophorolipids corresponding to 38.67 g/l. To ensure the repeatability of the optimum conditions, experiments were conducted at the optimized conditions, which resulted in a sophorolipids concentration of 37.44 g/l in close agreement with the model prediction.

^{1}H-NMR spectrum of the sophorolipid taken in chloroform is as shown in the Figure 1.1.

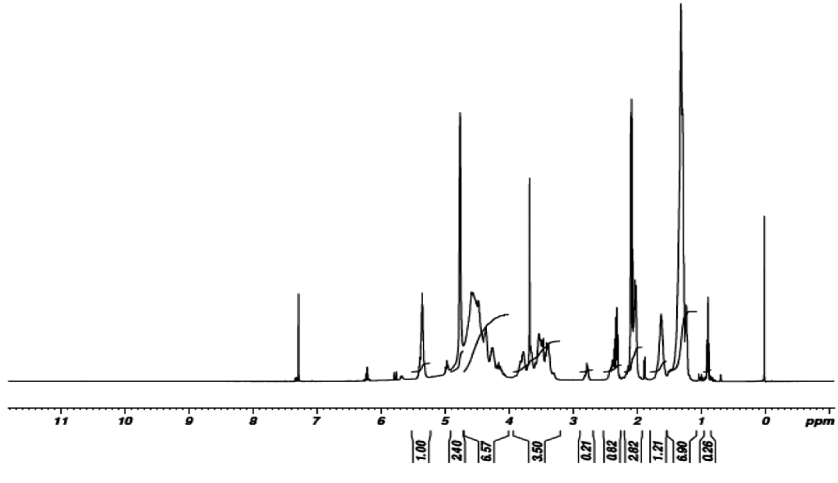

FIGURE 1.1 ^{1}H NMR spectra of sophorolipids synthesized from glucose and WFSFO.

It was assigned to a typical glycolipid type structure. The protons of glucose-H-1′ and glucose-H-2″ were resonated at 4.46 and 4.48, and 4.56 and 4.58 ppm respectively. The other protons of two glucoses were resonated at 3.50–4.40 ppm. Multiple signals of protons at 1.23–1.32 revealed the existence of a fatty acid chain moiety and signals at 5.35–5.36 revealed –CH=CH– group in the fatty acid chain. A signal at 2.09 ppm revealed the presence of ($-COCH_3$) group in the sophorolipid.

1.4 CONCLUSIONS

In the present work mixed carbon source glucose and WFSFO was employed for the synthesis of sophorolipids from *Starmerella bombicola.* It can be concluded that sunflower oil can be replaced by WFSFO without any major reduction in the amount of sophorolipids synthesized. Gas chromatographic analysis of WFSFO revealed that, there was an increase in the oleic acid level from 31.38 to 41.28 percent, which is desirable for the synthesis of sophorolipids. In the present work culture media components were optimized for the maximum production sophorolipids by using 2^4—full factorial response surface model based on central composite design. The regression model developed relates sophorolipids produced to the process variables which enable to analyze the individual, cumulative, and interactive effects of the medium components. The optimum condition for maximizing the sophorolipids production was obtained by solving the regression equation. Based on the model the optimum concentrations of glucose, WFSFO, yeast extract, and urea were found to be 100 g/l, 87.6 g/l, 6.9 g/l, and 0.3 g/l respectively, with the amount of sophorolipids produced being 38.67 g/l. The study identified the interaction between sunflower oil and yeast extract to be critical for the maximum production of sophorolipids. FT-IR and ^{1}H-NMR analysis confirmed the structure of glycolipid for the product formed.

KEYWORDS

- **Biosurfactant**
- **Mixed carbon substrate**
- **Response surface methodology**
- **Sophorolipids**
- ***Starmerella bombicola***

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CHAPTER 2

OPTIMIZATION OF ETHANOL PRODUCTION FROM MIXED FEEDSTOCK OF CASSAVA PEEL AND CASSAVA WASTE BY COCULTURE OF *SACCHAROMYCOPSIS FIBULIGERA* NCIM 3161 AND *ZYMOMONAS MOBILIS* MTCC 92

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CONTENTS

2.1 INTRODUCTION

The overall decline in the conventional oil production, emission of carbon monoxide due to burning of fossil fuels and threatening global climatic change highlights the need for an alternative energy fuel [1]. Ethanol serves this purpose well as it possesses the following advantages over conventional fuel: it reduces CO emissions, it can easily blend with petrol, ensures cleaner environment, and also renewable. Bioethanol can be produced from domestically abundant sources of biomass including agricultural and forestry residues, waste papers and other sizeable portions of municipal solid wastes [2].

Cassava is cultivated in tropical and subtropical regions of Africa, Asia, Latin America, and the Caribbean by poor farmers, many of them women, often on marginal land. It is the third largest source of food carbohydrates in the tropics after rice and maize—providing a basic diet for around 500 million people, 200 million of them in sub-Saharan Africa. The cassava plant gives the highest yield of carbohydrates per cultivated area among crop plants, only surpassed by sugarcane and sugar beets [3]. Cassava peel and waste are the wastes generated during the processing of their tuber to starch. They pose serious threat to environment because they are highly organic in nature [4]. Starch and total sugars present in cassava peel and waste are hydrolyzed to fermentable sugars by amylolytic yeast and then fermented to ethanol by yeasts or bacteria.

The objectives of the present study was to demonstrate the fitness of mixed feedstock of cassava peel and cassava waste for ethanol production through one step fermentation by employing one-factor-at-a-time (OFAT) and to optimize the process parameters for maximum ethanol production.

2.2 MATERIALS AND METHODS

2.2.1 MATERIALS

Cassava peel and waste were collected from starch processing industry in Namakkal district, Tamil Nadu. *Saccharomycopsis fibuligera* NCIM 3161 was received from National Collection of Industrial Microroganisms, National Chemical Laboratory, Pune, Maharashtra and *Zymomonas mobilis* MTCC 92 was received from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, Punjab. *Saccharomycopsis fibuligera* NCIM 3161 was grown on 100 ml sterile broth in 250 ml Erlenmeyer flask containing 3% (w/v) cassava starch, 0.05% (w/v) yeast extract and 0.05% (w/v) peptone by incubating at 28°C. *Zymomonas mobilis* MTCC 92 was grown separately on 100 ml serile broth in 250 ml Erlenmeyer flask containing yeast extract glucose salt (YEGS) medium (20% (w/v) glucose, 1.11% (w/v) yeast extract, 1 g/10 ml

$MgCl_2$, 1 g/10 ml $(NH_4)_2SO_4$, 1 g/10 ml KH_2PO_4) by incubating at 30°C. Both cultures were subcultured every fourth week.

2.2.2 BATCH FERMENTATION STUDIES

The batch fermentation studies were carried out in 250 ml Erlenmeyer flasks by mixing substrate and inoculum providing optimum environmental conditions for bioconversion. The flasks were kept on a thermostat incubated shaker for different periods of time. The supernatant was used for measurement of ethanol concentration.

2.2.2.1 EFFECT OF CASSAVA PEEL-TO-CASSAVA WASTE RATIO ON ETHANOL PRODUCTION

In single-step fermentation, the effect of cassava peel-to-cassava waste ratio was studied with different ratios of mixed feedstock of cassava peel-to-cassava waste (1:1, 2:1, and 3:1) maintaining constant substrate concentration of 50 g/L, 4.5 pH, temperature of 37°C, inoculum size of 10% (v/v), and agitation of 100 rpm for 72 h. The quantity of ethanol produced was quantified using dichromate method [5].

2.2.2.2 EFFECT OF SUBSTRATE CONCENTRATION ON ETHANOL PRODUCTION

The effect of substrate concentration was studied by varying from 10 to 90 g/L maintaining constant cassava peel-to-cassava waste ratio of 2:1, 4.5 pH, and temperature of 37°C, inoculum size of 10% (v/v) and agitation of 100 rpm for 72 h in single-step fermentation.

2.2.2.3 EFFECT OF REACTION TIME ON ETHANOL PRODUCTION

Single-step fermentation was studied by varying reaction time from 72 to 168 h to study the effect of reaction time on ethanol concentration maintaining constant cassava peel-to-cassava waste ratio of 2:1, substrate concentration of 50 g/L, 4.5 pH, temperature of 37°C, inoculum size of 10% (v/v), and agitation of 100 rpm.

2.2.2.4 EFFECT OF TEMPERATURE ON ETHANOL PRODUCTION

In single-step fermentation, the effect of temperature was studied by varying from 27 to 47°C maintaining constant cassava peel-to-cassava waste ratio of 2:1, substrate concentration of 50 g/L, 4.5 pH, inoculum size of 10% (v/v) and agitation of 100 rpm for 120 h.

2.2.2.5 EFFECT OF PH ON ETHANOL PRODUCTION

The effect of pH was studied by varying from 3.5 to 5.5 using 0.1 N HCl or 0.1 N NaOH. The cassava peel-to-cassava waste ratio of 2:1, substrate concentration of 50 g/L, temperature of 37°C, inoculum size of 10% (v/v) and agitation of 100 rpm for 120 h were maintained constant in single-step fermentation.

2.2.2.6 EFFECT OF INOCULUM SIZE ON ETHANOL PRODUCTION

Single-step fermentation was studied by varying inoculum size from 5 to 15% (v/v) with *Saccharomycopsis fibuligera* to *Zymomonas mobilis* ratio of 1:1 to study the effect of inoculum size on ethanol concentration maintaining constant cassava peel-to-cassava waste ratio of 2:1, substrate concentration of 50 g/L, 4.5 pH, temperature of 37°C, and agitation of 100 rpm for 120 h.

2.2.2.7 EFFECT OF AGITATION SPEED ON ETHANOL PRODUCTION

A mixed feedstock of cassava peel-to-cassava waste in the ratio of 2:1 with concentration of 50 g/L was inoculated with 10% (v/v) of inoculum for 120 h at a constant temperature of 37°C on shaker varying speed from 50 to 150 rpm for bioconversion studies for 120 h with pH of 4.5.

2.2.3 ANALYTICAL METHODS

Cell growth was measured by measuring optical density at 660 nm [6]. The total reducing sugars were determined in the broth after centrifuging at 5,000 rpm for 10 min by 3,5-dinitrosalicylic acid method [7]. Starch concentration was estimated colorimetrically by anthrone method [8].