Modulation of biosynthesis of bacosides by cell suspension culture of *Bacopa monnieri* in novel bioreactor

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Bacosides are pharmaceutically important compounds that have immense importance for the treatment of memory disorders and Alzheimer's disease. At NIT Rourkela we attempted to perform initial docking simulations using the molecular structure of Bacoside A and other phytopharmaceuticals and commercial drug for the inhibition of acetyl cholinesterase. The results suggested bacoside A showed good anti-Alzhiemer's drug properties like high binding energy value, good drug likeness property and group 4 toxicity. Central composite design was utilized to predict the best model for prediction of optimal production of bacosides in cell suspension cultures. The model predicted values were compared with experimental values for biomass production and bacoside A concentration in cell suspension cultures which showed high correlation for both the parameters. The bioprocess strategy was also optimized systematically for optimal callus induction, followed by callus proliferation. The kinetics of growth and product formation were studied under optimized conditions to find the values of kinetic parameters under experimental conditions. The production of bacoside A was mainly non-growth associated and found to be maximum during the stationary phase of the cell suspension culture. The production of bacosides was scaled up to 5-l stirred tank bioreactor in the batch and repeated batch mode. The production of bacosides was much higher in the repeated batch mode. These bioprocess strategies can be helpful for the enhanced production of various other valuable triterpenoid saponins.

Biography

Dr. Nivedita Patra completed PhD program at the Department of Biotechnology and Medical Engineering at IIT Delhi and published 26 papers from her PhD and postdoctoral work as first author or corresponding author. She received best paper award in 3 conferences and Early Career Award by Government of India in 2017. She is currently working as Assistant Professor at the Department of Biotechnology and Medical Engineering at National Institute of Technology Rourkela since 2014. Her work interest is to utilize microbial fermentation based methods of recovery in scaled-up bioreactor and the production of herbal drugs in plant bioreactor.

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Introduction

- ***Bacopa monnieri*** (Brahmi/Jal brahmi) is an ayurvedic herb.
- Its medicinal properties are accredited to the presence of secondary metabolites, called bacoside A, B, C, D, E and F (Sivaramakrishna et al., 2005).
- To enhance the production of biomass as well as secondary metabolites in vitro, it is necessary to optimize the culture conditions and medium composition (Bansal et al., 2017).
- After optimization, the culture can be transferred to a bioreactor for producing the targeted metabolite in a large scale for commercialisation.

Objectives

- ✓ Optimization of phytohormone concentration in the medium and callus induction from different explants.
- ✓ Study of substrate inhibition kinetics in cell suspension culture of *Bacopa monnieri*.
- ✓ Study of batch cultivation of *Bacopa monnieri* in stirred tank bioreactor.
- ✓ Study of repeated-batch cultivation of *Bacopa monnieri* in stirred tank bioreactor.

Review of Literature

1. Effect of the concentration of plant growth regulators on callus induction and biomass productivity

Sl. No.	Review of Literature	Reference
1	The maximum callus biomass of 15.20 g was obtained from *Bacopa monnieri*-derived shoot-tip explants on MS media containing 2.0 mg/l 2,4-D in 4 weeks.	Talukdar et al., 2014
2	For *Bacopa monnieri*-derived leaf explants, when MS media contained higher concentration of NAA, callus induction initiated first followed by shoot formation; and vice-versa, when the concentration of NAA was lower or equal to that of BAP.	Karatas et al., 2013

Review of Literature (contd.)

2. Optimization of influential variables using statistical methods

Sl. No.	Review of Literature	Reference
1	Inoculum density, concentrations of KH_2PO_4, KNO_3 and glucose were optimized using RSM for maximizing the production of biomass and bacoside A in *Bacopa monnieri*-cell suspension culture.	Bansal et al., 2017
2	The concentrations of NAA, 2, 4-D and kinetin were optimized using RSM for enhancing the yield of biomass and isoflavone in soybean cell suspension culture.	Devi and Giridhar, 2014

Review of Literature (contd.)

3. Effect of increasing substrate concentration on cell growth

Sl. No.	Review of Literature	Reference
1	The growth of the cells was found to get completely inhibited at the concentrations of 162.27 g/l sucrose, 26.35 g/l potassium nitrate and 0.63 g/l potassium dihydrogen phosphate in cell suspension culture of *Azadirachta indica.*	Prakash and Srivastava, 2006
2	Substrates at inhibitory concentration may interrupt the control functions within the cell, modify physiochemical parameters, dissociate enzyme complexes, inhibit reaction cascades, or chemically react with one or more components of cell, resulting in inhibition of growth and metabolism.	Edwards, 1970

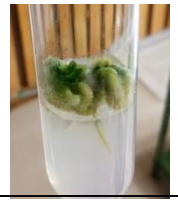
Review of Literature (contd.)

4. Large-scale production of plant secondary metabolites using bioreactor

Sl. No.	Review of Literature	Reference
1	Production of leucosceptoside A from the cell suspension culture of *Harpagophytum procumbens* improved by 44 % when cultivated in a stirred tank reactor.	Georgiev et al., 2011
2	Podophyllotoxin was produced from the callus of *Podophyllum hexandrum* in a stirred tank reactor and the overall productivity showed an improvement of 27 % as compared to that in shake flask cultures.	Chattopadhyay et al., 2002
3	Alkaloid production from cell suspension culture of *Holarrhena antidysenterica* in stirred tank reactor was 160 times greater than that produced by the field grown plants.	Panda et al., 1992

Materials and Methods

Surface sterilization of explants



Induction of roots and callus

Optimization of explant type

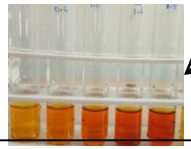
Optimization of phytohormone concentration using RSM

Inhibition kinetics study

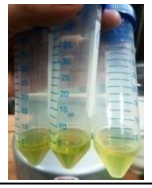
Batch and repeated-batch culture

Analytical studies

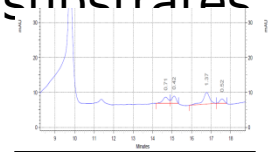
Biomass



Residual substrates



Bacoside



HPLC

Figure 2: Flow diagram depicting the mass production of bacoside using modified stirred tank reactor

Materials and Methods (contd.)

1. **Standardization of the surface sterilisation method for explants and callus and root induction using explants derived from in vitro and field-grown *Bacopa***

Surface sterilization method was standardized by treating the explants with different combinations of bavistin and NaOCl

↓

Root induction

↓

Culturing of shoots on MS media supplemented with 0.9 % agar and 1 mg/l IAA at pH 5.80.

↓

Callus induction

↓

Culturing of leaves on MS media supplemented with 0.8 % agar, 0.5 mg/l NAA and 0.1 mg/l BAP at pH 5.80 .

↓

Incubation in white fluorescent light of intensity 90-100 µE/m^2/s at 25-28 °C and 14 hours photoperiod and 10 hours dark period.

Materials and Methods (contd.)

2. Optimization of callus induction from different explants

Preparation of MS media

↓

Inoculation of explants (excised nodes, internodes and leaves)

↓

Incubation in white fluorescent light of intensity 90-100 µE/m^2/s at 25-28 °C and 14 hours photoperiod and 10 hours dark period, for 27 days

↓

Harvesting of callus

↓

Statistical analysis

Materials and Methods (contd.)

3. Optimization of phytohormone concentration in the cu[illegible] using PCM

BAP (0.1-2.0 mg/l) and NAA (0.2-1.0 mg/l) were used in Design Expert 5.0.6 to design the experiment and 13 experimental runs were obtained

Induction medium for induction of callus from leaf explants

Maintenance medium for maintenance of subcultured callus

Incubation of culture in dark condition at 25-28 °C for 33 days

Incubation of culture in dark condition at 25-28 °C for 15 days

Harvesting of callus

Statistical analysis

Materials and Methods (contd.)

4. Substrate Inhibition Kinetics

Carbon

↓

Preparation of liquid MS medium with varying concentration of sucrose from **20-120 g/l**

Phosphate

↓

Preparation of liquid MS medium with varying concentration of KH_2PO_4 from **0.18-1.0 g/l**

↓

Inoculation of callus

↓

Incubation in dark at 27 °C and 100 rpm for 8 days

↓

Harvesting of culture on Day-4, 6 and 8

↓

Statistical analysis

Materials and Methods (contd.)

5. Batch cultivation of *Bacopa monnieri* in stirred tank bioreactor

Using cell suspension culture

Using pre-established callus culture

↓

Establishment of callus culture

↓

Establishment of cell suspension culture

↓

Cultivation in reactor at 125 rpm, 25 °C and pH 5.80 in dark for 6 days and sampling was done in every 12 hours

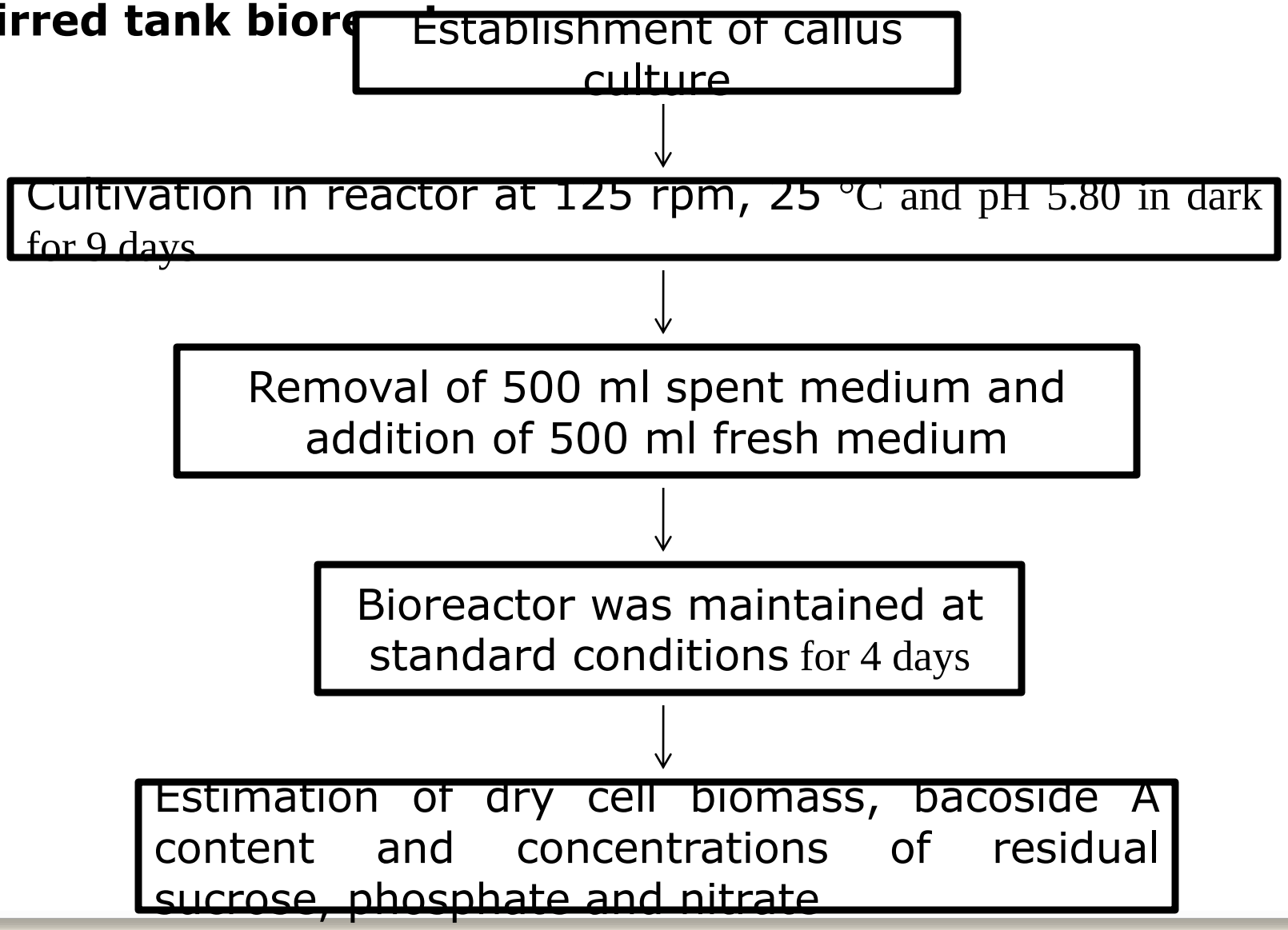
Cultivation in reactor at 125 rpm, 25 °C and pH 5.80 in dark for 9 days

↓

Estimation of dry cell biomass, bacoside A content and concentrations of residual sucrose, phosphate and nitrate

Materials and Methods (contd.)

6. Repeated-batch cultivation of *Bacopa monnieri* in stirred tank bioreactor



Materials and Methods (contd.)

7. Analytical techniques

- **Sucrose** concentration was estimated by using DNS method (Miller, 1959).

- **Nitrate** concentration was determined using salicylic acid method (Cataldo et al., 1975).

- **Phosphate** concentration was determined by a method reported by Murphy and Riley (1962).

- The content of total **bacoside A** was estimated using HPLC (Deepak et al., 2005).

RESULTS (contd.)

Table 10: Results of the *Bacopa monnieri* cell suspension culture on Day 0 and Day 6 of the cultivation in the stirred tank reactor

Factor	Day 0 of the culture	Day 6 of the culture
Total biomass (g/l DW)	1.10	3.37
Sucrose concentration (g/l)	26.99 ± 0.61	5.51 ± 0.86
Nitrate concentration (g/l)	0.94 ± 0.04	0.09 ± 0.04
Phosphate concentration (g/l)	0.11 ± 0.003	0.03 ± 0.003
Total bacoside A (mg/g DW)	0	18.05

RESULTS (contd.)

7.2. Batch cultivation performed using callus cultures

Results	Day 0 of the culture	Day 9 of the culture
Total biomass (g/l DW)	0.45	3.04
Sucrose concentration (g/l)	30.00	14.90 ± 0.08
Nitrate concentration (g/l)	3.55	0.04 ± 0.003
Phosphate concentration (g/l)	0.17	0.008 ± 0.00
Total bacoside A (cellular)	20.04 mg/l	27.36 mg/g DW
Total bacoside A (extracellular)	0	28.05 ± 4.8 mg/l

The bacoside production increased by 1.52 times compared to the bacoside produced by cultivating suspension culture in stirred tank reactor.

RESULTS (contd.)

Table 12: Summarized results for batch cultivation of *Bacopa monnieri* in stirred tank reactor (Run-2)

Results	**Day 0 of the culture**	**Day 7 of the culture**
Total biomass (g/l DW)	0.40	0.78
Sucrose concentration (g/l)	30.00	15.54 ± 0.14
Nitrate concentration (g/l)	3.55	0.05 ± 0.002
Phosphate concentration (g/l)	0.17	0.009 ± 0.00
Total bacoside A (cellular)	20.04 mg/l	263.21 mg/g DW
Total bacoside A (extracellular)	0	226.28 mg/l

The production of bacoside increased by 14.58 times compared to the bacoside produced by cultivating suspension culture in stirred tank reactor.

RESULTS (contd.)

Table 13: Summarized results for repeated-batch cultivation of *Bacopa monnieri* in stirred tank reactor

Results	**Day 0 of the culture**	**Day 9 of the culture**	**Day 13 of the culture**
Total biomass (g/l DW)	0.46	Not estimated	1.40
Sucrose concentration (g/l)	30.00	17.84 ± 0.06	16.12 ± 0.17
Nitrate concentration (g/l)	3.55	0.14 ± 0.002	0.13 ± 0.00
Phosphate concentration (g/l)	0.17	0.02 ± 0.0003	0.02 ± 0.0004

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Thank You