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Kinetic study of biosurfactant production with *Pseudomonas aeruginosa* MTCC 424 using residual rice bran oil as substrate

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In this study, the rhamnolipid biosurfactant was produced using *Pseudomonas aeruginosa* MTCC 424 culture with low-cost residual rice bran oil substrate as sole carbon source extracted from the spent bleaching earth discarded by vegetable oil processing industry. Different initial rice bran oil concentrations were used with varying concentrations of carbon-to-nitrogen (C/N) and sodium nitrate ($NaNO_3$), which were utilized for higher cell mass yield and rhamnolipid biosurfactant production. The mathematical Monod model was applied and compared with the experimental data. The modified Monad model was able to predict a brewing profile with a high determination coefficient (R^2) value of 0.9403. The results were found fit in this context and may prove invaluable for developing facilities for production of higher biosurfactant yield by using yield coefficient and specific growth rate data from the study.

Keywords: Biosurfactant, Monod model, *Pseudomonas aeruginosa*, Rhamnolipid biosurfactant, Rice bran oil

Introduction

Surfactants are natural or synthetic organic compounds that possess both hydrophilic (polar and water-loving) and hydrophobic/lipophilic (non-polar and water-repelling or fat-loving) characteristics along with a tendency to adsorb at interfaces[1]. The surfactants constitute the significant part of laundry detergents, household, personal care and other toiletries products. Further applications include enhanced oil recovery, food processing, agrochemicals, pharmaceuticals, mineral beneficiation, fuel and lubricant additives, adhesives, paints and surface coatings. Significant quantities of chemical surfactants are released into the environment every year and this is bound to increase further as the size of surfactant industry is growing with new applications being developed by researchers. Surfactants are particularly toxic to soil, surface water, and aquatic organisms[2].

Biosurfactants are important biotechnology products that have attracted attention of researchers as well as industrial community on account of their wide applicability, ease of preparation, mode of action, high biodegradability, and low toxicity. These are low molecular weight amphiphilic compounds produced by a variety of microorganisms and are effective at low concentrations compared to traditional chemical surfactants[3]. All these beneficial characteristics give them a definite edge over chemical surfactants. Rhamnolipid biosurfactants are attractive because they possess excellent surface activity and are produced in high yields in a relatively short incubation period[4]. Their high demand and high cost has compelled the researchers to develop new methods to get high yield of rhamnolipids under optimum conditions. Rhamnolipids are thus the most thoroughly researched biosurfactants[5].

Pseudomonas aeruginosa is the most common bacteria to produce rhamnolipid biosurfactant. Some carbon and nitrogen sourcesalong with other nurturing conditions are required to grow the bacteria for biosurfactant production[6]. In this work, the kinetic study of rhamnolipid biosurfactant production using *Pseudomonas aeruginosa* MTCC 424 was carried out to get a motivational support for large scale implementation of this process. Biosurfactants produced from industrial waste will be cheaper and cost-competitive with chemical surfactants. For this reason, a low-cost carbon source which is residual rice bran oil obtained from the spent bleaching earth discarded as a waste product by vegetable oil processing industry was used in this study. Results of the study were quite encouraging. The carbon-to-nitrogen (C/N) ratio was found to be the most

significant parameter in genetic system and hence was studied well in this study.

Experimental Section

Theoretical background

Kinetic models are nowadays essential tools for development of laboratory scale processes and for their successful industrial application. A good kinetic model is that one which is effectively and reliably able to predict the profile of key products. Monod kinetic model is one such model that is normally applied to characterize the growth of yeast and substrate profiles; though, Koren and Duvnjak (1993) have mentioned a few processes where the model could not predict the products[7]. Ramana *et al*. (1991) have successfully applied the Monod model for fermentation reaction producing biomass using Pseudomonas aeruginosa CFTR-6[8].

Monod model for growth of biomass is given by following equations:

$$\frac{dS}{dt} = \mu S \quad \ldots (1)$$

$$\ln S = \ln S_o + \mu t \quad \ldots (2)$$

$$\mu = {\mu_{max} B}\Big/{(K_m + B)} \quad \ldots (3)$$

where S is cell mass yield (g/L), S_0 is initial cell mass yield (g/L), t is time (h), μ is specific growth rate (h^{-1}), μ_{max} is maximum specific growth rate (h^{-1}), B is substrate concentration, and K_m is Monod constant. Eqs (1) and (2) represent the exponential growth of cell mass, while the substrate B in Eq. (3) is the limiting component.

For better results, the complex microbial reactions with rate of variation of cell mass, substrate and product are used. The modified Monod model is then given by following equation:

$$\frac{ds}{dt} = \mu_{max} S \left(1 - \frac{S}{S_{max}}\right) \quad \ldots (4)$$

where S_{max} is maximum cell mass yield (g/L). At time t=0, S= S_0. The Eq. (4) on integration yields

$$ln\left(\frac{S}{S_{max} - S}\right) = \mu_{max} t + \ln\left(\frac{S_o}{S_{max} - S_o}\right) \quad \ldots (5)$$

Rearrangement of Eq. (5) gives following expression for cell mass:

$$S = \frac{S_o exp\left(\mu_{max} t\right)}{1 - \frac{S_o}{S_{max}}\left(1 - exp\left(\mu_{max} t\right)\right)} \quad \ldots (6)$$

Now the integral method is used to find the solution for Eq. (6), where the value of S_{max}can be assigned from the experimental results.

If the experimental data satisfies the model Eq. (4), the plot of $ln\left(\frac{S}{S_{max} - S}\right)$ *vs t* gives a straight line. With the help of this plot S_0 and μ_{max} can be calculated from the intercept and slope, respectively.

Substrate is utilized for growth of cell mass and biosurfactant production. On assuming further material balance, we can use

$$\frac{dB}{dt} = \frac{1}{Y_{S,B}} \frac{dS}{dt} + biosurfactant \quad \ldots (7)$$

where $Y_{S,B}$ is yield coefficient. Now for the case of non-growth product, the Eq. (7) reduces to

$$- \frac{dB}{dt} = \frac{1}{Y_{S,B}} \frac{dS}{dt} \quad \ldots (8)$$

Integrating Eq.(8) taking an average value for $Y_{S,B}$ with B= B_0 and S=S_0 at t=0,

$$S = (S_0 + Y_{S,B}B_0) - Y_{S,B}B \quad \ldots (9)$$

where B_0 is initial substrate concentration. Eq. (9) is an equation of a straight line. The *S vs. B* plot will give the slope $Y_{S,B}$ and intercept ($S_0 + Y_{S,B}B_0$) for this straight line. The equation gives a distinct straight line for each value of B_0 and $Y_{S,B}$ is obtained by taking the average of slopes for different B_0 values. Though average value of $Y_{S,B}$ can be determined by a single plot of all B_0 values.

Eq. (9) can be further modified to the following form by division with B_0 on both sides:

$$\frac{S - S_o}{B_o} = Y_{S,B} - \frac{Y_{S,B} B}{B_o} \quad (10)$$

Now a plot of $\frac{S - S_o}{B_o}$ *vs* .$\frac{B}{B_o}$ from Eq. (9) will give a straight line for all initial rice bran oil substrate concentrations. The slope and the intercept of this straight line will give the numerical value of $Y_{S,B}$ for the system[8].

Materials

For the purpose of this study, *Pseudomonas aeruginosa* MTCC424 was procured from Institute of Microbial Technology (IMTECH), Chandigarh and standard rhamnolipid biosurfactant was purchased from Sigma Aldrich. The spent bleaching earth containing residual rice bran oil was obtained from a local vegetable oil processing industry named Kanpur Edible Oil. All other chemicals used in investigation

were of analytical grade from Qualikems Fine Chem Pvt. Ltd., Gujarat.

Pseudomonas aeruginosa MTCC 424 was transferred to mineral salt medium containing following constituents (g/L): $NaNO_3$ 1.0, KH_2PO_4 1.0, $Na_2HPO_4$2.0, $MgSO_4$ 1.0, $CaCl_2$0.02, $FeSO_4$ 0.002, KCl 1.0 and trace elements 1mL/L. The media components used were as per following concentrations (g/L): KH_2PO_4 0.7, Na_2HPO_4 0.9, $NaNO_3$ 2.0, $MgSO_4.7H_2O$ 0.4, $CaCl_2.2H_2O$ 0.1, trace elements 2 mL/L, CTAB 0.2, Methylene Blue 0.005 and Agar 15.0. Residual rice bran oil was used as sole carbon source or substrate in this study. Rice bran oil in the spent bleaching samples was extracted by soxhlet extraction method using hexane as solvent. The optimum temperature for extraction was found to be 68°C and the optimum time was 2 h. The nutrient bisque was cast-off for grounding to inoculums. The major structure of nutrient bisque was designed with 1.0 g beef extract, 2.0 g yeast extract, 5.0 g peptone, and 5.0 g NaCl dissolved in one liter of distilled water. In the nutrient bisque 15.0 g agar was added to prepare nutrient agar. The cultures were left to flourish in this bisque for about 16-18 h at room temperature. These microbial cultures were than utilized for biosurfactant synthesis under varying substrate and nitrogen source concentrations. The culture was inoculated in basal salt medium with necessary substrate and yeast extract to make sure that there is enough cell mass available for successful rhamnolipid synthesis. They were than incubated in an incubator shaker at 100 rpm and 25°C for 48-72 h. Froth was detected that was removed and then centrifuged for 20 min at 6000 rpm to obtain cell gratis supernatant[9-11].

Results and Discussion

Effect of initial carbon source concentration

In this study, the residual rice bran oil was used as the sole carbon source and its concentration was varied from 10 g/L to 70 g/L and the consecutive effects on cell mass yield and rhamnolipid biosurfactant yield were observed. Fig. 1 shows the effect of initial carbon source (rice bran oil) concentration on kinetic profile of cell mass yield and rhamnolipid biosurfactant yield during the fermentation process with the strain. Table 1 presents the summary of the effect of initial concentration of rice bran oil on cell mass yield and biosurfactant yield. The cell mass increased by about half when the rice bran oil concentration was increased from 10 to 30 g/L. Cell mass yield continued to increase till initial rice bran oil concentration reached the level of 50 g/L. Thereafter cell mass yield decreased with further increase in rice bran oil concentration. Similar pattern was observed with rhamnolipid biosurfactant yield. Highest biosurfactant yield was achieved at 50 g/L initial rice bran oil concentration followed by a drop in yield with further increase in rice bran oil concentration, *i.e.*, from 50 g/L to 70 g/L.

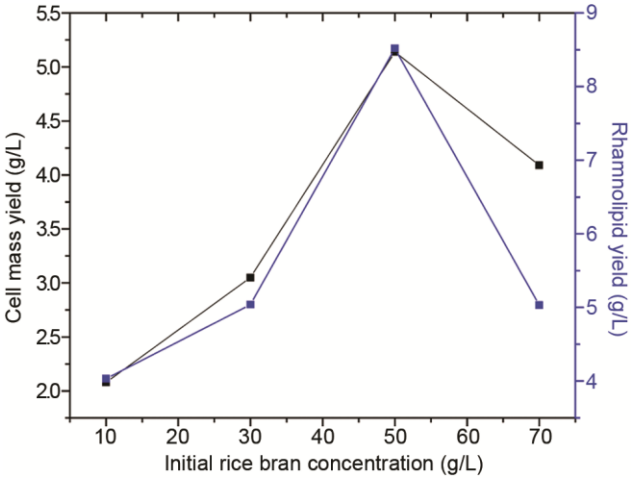

Fig. 1 — Kinetic profile of cell mass yield and rhamnolipid biosurfactant yield using residual rice bran oil as sole carbon source

Table 1 — Effect of initial ricebran oil concentration on cell mass yield and biosurfactant yield

Initial rice bran oil (g/L)	Cell mass yield (g/L)	Biosurfactant yield (g/L)
10	2.08	4.03
30	3.05	5.04
50	5.14	8.52
70	4.09	5.03

The decline in both cell mass and rhamnolipid biosurfactant yield at an initial residual rice bran oil concentration of 70 g/L indicates the onset of substrate inhibition and oxygen mass transfer limitations**.** At such high concentrations, excess hydrophobic substrate can impose metabolic stress on *Pseudomonas aeruginosa* due to accumulation of fatty acid intermediates, leading to inhibition of key enzymatic pathways and reduced growth rates. In parallel, increased oil content raises medium viscosity and promotes formation of larger oil droplets or surface layers, which significantly lower oxygen transfer efficiency. Since *P. aeruginosa* is a strictly aerobic microorganism, oxygen limitation directly restricts energy generation and precursor availability

required for biomass formation and rhamnolipid synthesis. Additionally, oil coalescence at higher concentrations reduces effective substrate bioavailability, further aggravating the inhibitory effects.

Effect of C/N ratio

In this study, keeping the concentration of carbon source constant, different molar solutions of $NaNO_3$ were used and its effects on cell mass yield and rhamnolipid biosurfactant yield were observed. Fig. 2 shows the effect of $NaNO_3$concentration on the kinetic profiles of cell mass yield and biosurfactant production during fermentation with *Pseudomonas aeruginosa* MTCC424. Table 2 presents the summary of the effects of $NaNO_3$ concentration on C/N ratio, cell mass yield and biosurfactant yield. The addition of $NaNO_3$ at 0.04 M, 0.05 M, and 0.06 M led to C/N ratios of 13.38, 10.78, and 9.46, respectively. However, optimum biosurfactant production was obtained at the C/N ratio of 10.78 at which the biosurfactant yield was 8.53 g/L.

Heryani and Putra (2017) have reported earlier that higher nitrogen concentration result in moreconsumption of glucose[12]. Here in present study, the optimum cell mass and biosurfactant yield was obtained at C/N ratio of 10.78. It might be due to enhanced performance of *Pseudomonas aeruginosa* strain at a particular nitrogen concentration. The medium with the initial C/N ratio of 10.78 produced the smallest decrease in the C/N ratio (24.86%) at the end of the fermentation process in comparison to the decrease noticed with other two C/N ratios. These results simply indicate towards the higher consumption of nitrogen than carbon at this ratio as compared to other two ratios. Evidently the *Pseudomonas aeruginosa* strain produced more biosurfactant. This is a significant outcome of current study and can be satisfactorily ascribed to affinity pervasion by nitrogen concentration.

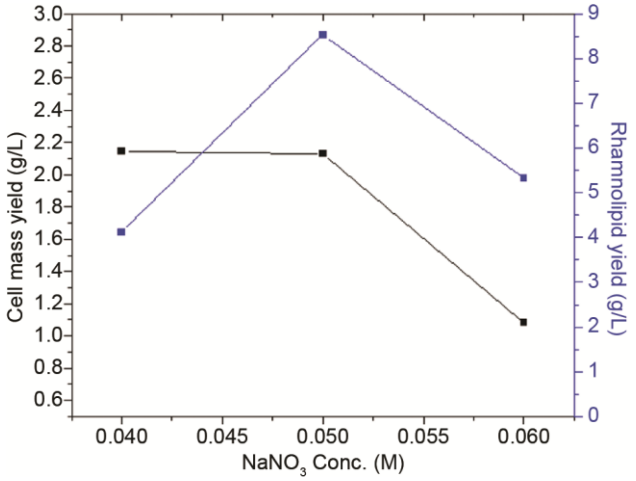

Fig. 2 — Profile of cell mass yield (g/L) and rhamnolipid biosurfactant yield (g/L) at different concentrations of $NaNO_3$ (0.04, 0.05 and 0.06 M)

Table 2 — Effect of sodium nitrate concentration on C/N ratio, cell mass yield and biosurfactant yield

Parameter	$NaNO_3$		
	0.04M	0.05M	0.06M
C/N at the beginning	13.38	10.78	9.46
C/N at the end	6.76	8.1	4.12
Decrease in C/N at the end (%)	49.48	24.86	56.45
Cell mass yield (g/L)	2.143	5.151	3.078
Biosurfactant yield (g/L)	4.11	8.53	5.31

Kinetic models used for cell mass yield, biosurfactant yield and rice bran oil consumption

A kinetic model for second order biosurfactant production by *Pseudomonas aeruginosa* was investigated. This is a special case of the broader Monod equation. The model was verified both experimentally and theoretically. The modified Monod model was applied to predict the cell mass yield by varying substrate residual rice bran oil concentration. On the basis of experimental results S_{max} is taken and plot of $ln\left(\frac{S}{S_{max}-S}\right)$ *vs. t* gives the value of S_0 and μ_{max}. With the help of B_0, cell mass yield was plotted for each concentration. Slope of each plot gave the individual yield coefficient, which on taking average gives $Y_{S,B}$. Further plot of $\frac{S-S_o}{B_o}$*vs.*$\frac{B}{B_o}$ gave a straight line, which satisfied the Monod mathematical model. A plot of initial rice bran oil concentration *vs.* cell mass yield (Fig. 3) gave a straight line with slope $Y_{S,B}$ and an intercept (S_0+ $Y_{S,B}B_0$). Since this plot gives a different straight line for each B_0 value, the $Y_{S,B}$ was obtained by taking the average of slopes for different B_0 values. The average $Y_{S,B}$ was calculated to be 0.243 (Table 3).

Now $ln[S/(S_{max} - S)]$ *vs. t* Monod plot was drawn to obtain a straight line. The plots for only 10 g/L and 50 g/L initial concentrations are shown here due to space constraints (Figs 4a and 4b). The intercepts and slopes were taken from these plots and S_0 and μ_{max} were mathematically calculated (Table 4).

Kinetics of Monod model

For the reaction order and kinetics of Monod model we followed equation (x) to plot $(S - S_0)/B_0$ *vs.* B/B_0

Table 3 — Slopes of profiles of cell mass yield and substrate consumption

Initial cell mass yield S_0 (g/L)	Yield coefficient ($Y_{S,B}$)
10	0.265
30	0.227
50	0.295
70	0.184
	0.243 (Average)

Table 4 — Calculation of μ_{max}, S_{max} and S_0 for different initial rice bran oil concentrations

Initial Rice bran oil concentration(g/L)	μ_{max}(h^{-1})	S_{max} (g/L)	S_0 (g/L)
10	0.52	2.82	0.18
30	0.38	3.84	0.76
50	0.39	5.91	1.71
70	0.44	4.89	0.86

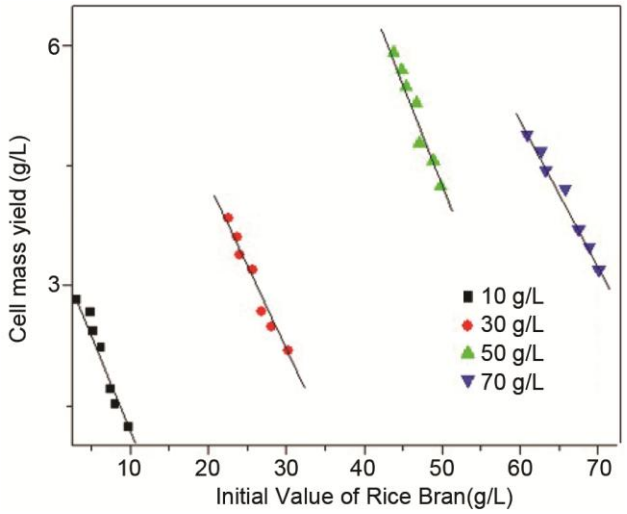

Fig. 3 — Linear profile of cell mass *vs*. different initial rice bran oil concentrations

(Fig. 5) for all initial concentrations of rice bran oil. Following these concentrations, a straight line appeared that led us to calculate $Y_{S,B}$ by way of slope of straight line which verified the model as well as the predicted kinetics. Profile shows the best matching values of model for the initial rice bran concentration of 50 g/L.

Model verification

The predicted model was verified by making comparison between the experimental and theoretical data to satisfy the present work. With the help of experimental value S_0= 1.71 g/L and μ_{max}= 0.39 for initial rice bran oil concentration of 50 g/L, the *S vs*. *t* plot exhibited the same profile (Fig. 6) as theoretically predicted by the Monod model using Eq. (6). It can be seen from Fig. 5 that the best suited initial rice bran

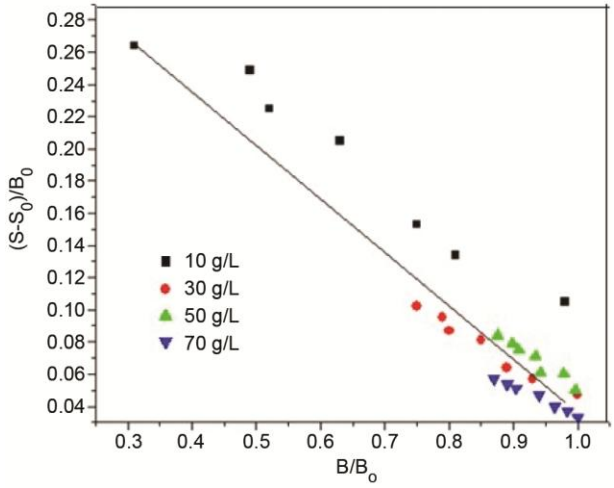

Fig. 5 — Identification of biomass yield coefficient - Profile of (S-S_0/B_0 *vs*.B/B_0) at different initial rice bran oil concentration (B_0)

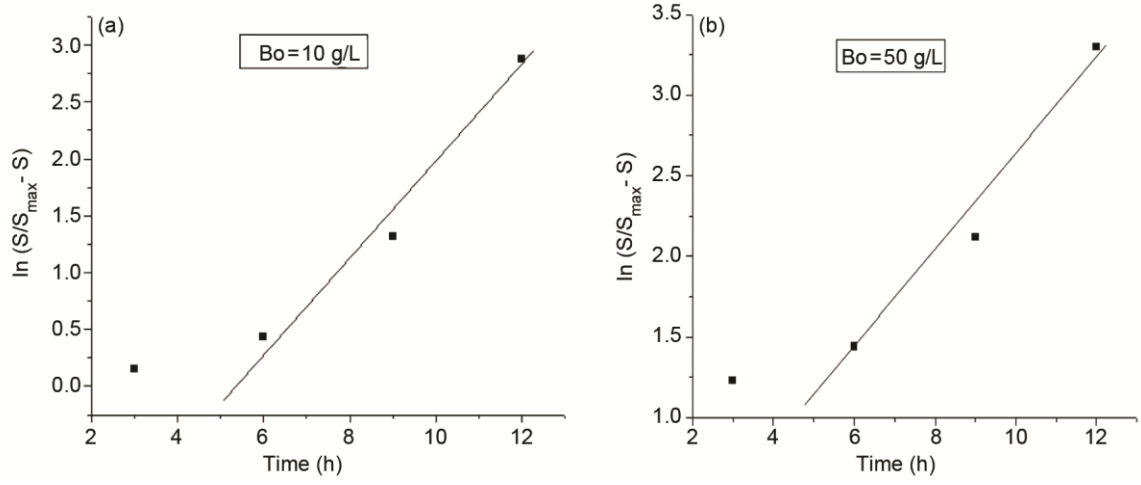

Fig. 4 — Plots of $ln\left(\frac{S}{S_{max}-S}\right)$ *vs*. *t* for initial concentration of (a) 10 g/L and (b) 50 g/L

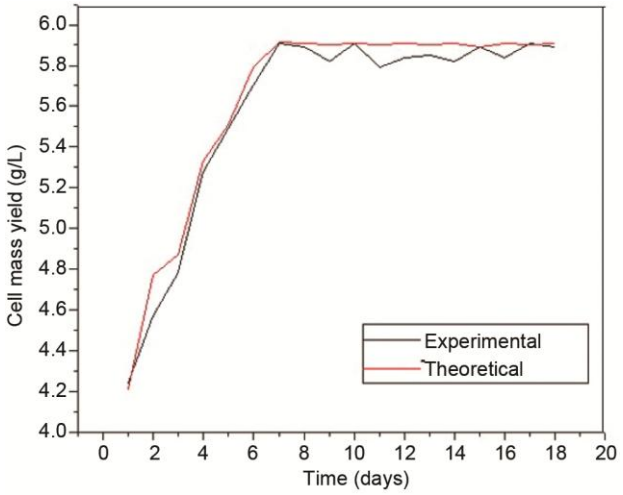

Fig. 6 — Profile of predicted model *vs*. experimental data for initial rice bran oil concentration B_0= 50 g/L

oil concentration for this study was 50 g/L which gave higher cell mass yield and rhamnolipid biosurfactant production. The result confirmed that the predicted model was quite similar to our experimental study for the production of rhamnolipid biosurfactant.

Overall, the enhanced performance observed in the present study is the result of **a** synergistic interaction between substrate characteristics, nutrient optimization, and controlled process kinetics[13,14]. The use of residual rice bran oil recovered from spent bleaching earth as the sole carbon source provided a hydrophobic, fatty-acid-rich substrate that inherently promotes rhamnolipid synthesis, as biosurfactant production facilitates emulsification and cellular uptake of such substrates. Systematic variation of the initial carbon concentration revealed an optimal value of 50 g/L, at which both cell mass (5.14 g/L) and biosurfactant yield (8.52 g/L) were maximized, indicating efficient substrate utilization without the onset of substrate inhibition or mass transfer limitations observed at higher concentrations[15].

In addition, optimization of the C/N ratio to 10.78 ensured sufficient nitrogen availability for biomass formation while inducing a metabolic shift toward secondary metabolite production, thereby enhancing rhamnolipid yield. The robustness of this optimized system was further confirmed through successful application of the modified Monod kinetic model, which showed close agreement between experimental and predicted growth profiles, demonstrating predictable and stable process behaviour[16,17]. Operating at 25°C contributed to sustained growth and biosurfactant synthesis under energy-efficient conditions, aligning with the low-cost and sustainable objectives of the study[15]. Collectively, these factors explain the superior biosurfactant production performance achieved in the present work compared to non-optimized or empirically designed systems. These biosurfactant may be used in various industrial applications[18,19].

Conclusion

Rhamnolipid biosurfactant was successfully produced using *Pseudomonas aeruginosa* MTCC 424 culture. This study emphasized on effect of initial residual rice bran oil concentration applied here as sole carbon source. Rice bran oil concentration was varied from 10–70 g/L and biosurfactant concentrations of 1–8.5 g/L were produced. The C/N ratio of 10.78 was found optimum for the production of maximum biosurfactant yield. The cell mass and biosurfactant concentrations were effectively predicted using the modified Monod equation. The experimental values were quite in agreement with those predicted by Monod Model and the maximum cell mass was achieved at initial rice bran concentration of 50 g/L.

Conflict of Interest

The authors declare no conflict of interest.

References

1 Dave N & Joshi T, A concise review on surfactants and its significance, *Int J Appl Chem*, 13 (2017) 663.

2 Schramm L L, Stasiuk E N & Marangoni D G, Surfactants and their applications, *Annu Rep Prog Chem Sect*, 99 (2003) 3.

3 Mukherjee A K & Das K, Microbial surfactants and their potential applications: An overview in: Biosurfactants, Sen R (Ed), *Landes Biosci*, (2010) 54.

4 Abdel-Mawgoud M, Hausmann R, Lépine F, Müller M M & Déziel E, Rhamnolipids: detection, analysis, biosynthesis, genetic regulation, and bioengineering of production in: Biosurfactants from genes to applications, Soberón-Chávez G (Ed), Springer, (2011) 13.

5 Kumar R & Das A J, Rhamnolipid Biosurfactant, Springer, Singapore, (2018).

6 Kaskatepe & S Yildiz, Rhamnolipid biosurfactants produced by Pseudomonas species, *Braz Arch Biol Technol*, 59 (2016) 1.

7 Koren W & Duvnjak Z, Kinetics of the selective fermentation of glucose from glucose/fructose mixtures using Saccharomyces cerevisiae ATCC 36859, *Acta Biotechnol*, 13 (1993) 311.

8 Ramana K V, Charyulu N C L N & Karanth N G, A mathematical model for the production of biosurfactants by Pseudomonas aeruginosa CFTR-6: Production of biomass, *J Chem Tech Biotechnol*, 51 (1991) 525.

9 Mishra A, Trivedi R K, Synthesis and characterization of biosurfactant using waste from oil processing industry as substrate by Pseudomonas aeruginosa (MTCC 424), *Rasayan J Chem*, 12 (2019) 1011.
10 Benincasa M, Abalos A, Oliveira I & Manresa A, Chemical structure, surface properties and biological activities of the biosurfactant produced by Pseudomonas aeruginosa LBI from soapstock, *Antonie van Leeuwenhoek*, 85 (2004) 1.
11 Weber A & Zeiner T, Purification of biosurfactants in: Biosurfactants production and utilization-processes, technologies, and economics, Kosaric N & Sukan F V (Eds), CRC Press, (2015) 129.
12 Heryani H & Putra M D, Kinetic study and modeling of biosurfactant production using Bacillus sp., *Electron J Biotechnol*, 27 (2017) 49.
13 Banat I M, Franzetti A, Gandolfi I, Bestetti G, Martinotti M G, Fracchia L, Smyth T J & Marchant R, Microbial biosurfactants production, applications and future potential, *Appl Microbiol Biotechnol*, 87 (2010) 427.
14 Banat I M, Satpute S K, Cameotra S S, Patil R & Nyayanit N V, Cost effective technologies and renewable substrates for biosurfactants' production, *Front Microbiol*, 5 (2014) 697.
15 Marchant R & Banat I M, Microbial biosurfactants: Challenges and opportunities for future exploitation, *Trends Biote*chnol, 30 (2012) 558.
16 Monod J, The growth of bacterial cultures, *Annual Review Microbiol*, 3 (1949) 371.
17 Shuler M L & Kargi F, Bioprocess engineering: Basic concepts (3rd Edn.). Prentice Hall, (2017).
18 Mishra A & Trivedi R K, Deinking of old used papers using crude rhamnolipid biosurfactant, *Pollut Res*, 40 (2021) 250
19 Mishra A & Trivedi R K, A comparative study of biosurfactant preparation by *Pseudomonas aeruginosa* MTCC 424 using rice bran oil and soybean oil substrates. *J Indian Chem Soc*, 97(11b) (2020) 2501.