IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 6 Version. I (June 2018), PP. 54-62

Utilization of Potato peel and Curd whey as the Agro industrial substrates for Biosurfactant production using *Pseudomonas aeruginosa* PBS29

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Abstract: This study elicited the utilization of the cheapest agro-industrial waste as the major substrate for Biosurfactant production. The fermentation medium was formulated by supplementing the substrates, Homogenized Potato peel extract (HPPE) and Curd whey. The utilization of substrates and the biosurfactant activity were accessed. The utilization of the HPPE till 96hrs was found to be significant with the yield of 3.78 ± 0.4 (g/l) and Emulsification index of 60.07± 0.2 (%) respectively. The biosurfactant production was also optimized using the formulated Curd whey medium. The influence of various parameters such as C/P ratio, different metal ions with its synergistic effect, Concentration of Metal ions, Combined C/P ratio with metal ions were determined. The biosurfactant activity was observed to be satisfactory with the surface tension of the optimized Curd whey medium as 33.93 ± 0.3 (mN/m). Thus the formulated production medium using HPPE and Curd whey was suggested for the biosurfactant production using *Pseudomonas aeruginosa* PBS29.

Keywords: Biosurfactant, Homogenized potato peel extract, Curd whey, Surface tension, Emulsification index.

Date of Submission: 28-05-2018 Date of acceptance: 11-06-2018

I. INTRODUCTION

High population and the eco-society setup in this 21st century are generating the huge amount of agricultural waste as well as municipality waste materials. In most developed and developing countries, disposal of those huge waste materials is highly possible with the aid of microorganisms by implementing them as the substrate for different product productions using microorganisms, because the landfills may end up in many environmental and health hazards. As per FAO, the generation of agricultural waste from the European Union is about 700 million tons every year. The recent approaches in many researchers are concentrating on the agro-based substrates. An Agro-industrial waste is believed to be the credible substrate for biosurfactant production and improves the management of waste disposal (Makkar et al., 2011). In fact, the agro-industrial waste has also been recognized as the low-cost substrates with ample nutritive source. These are the renewable resource employed for the biosurfactant production as mentioned in the past (Das and Mukherjee, 2007; Nitschke et al., 2004; Fox and Bala, 2000). In precise, choice of the agro-industrial waste needs to balance the nutritive source for the microorganism during biosurfactant production. The chemical constituent of Potatoes comprises 80% water, 17% carbohydrates, 2% protein, 0.1% fat and 0.9% vitamins, inorganic minerals and trace elements (Makkar and Cameotra, 2002).

The most significant and cost-effective agro-industrial waste include vegetable/ frying oil waste, residual waste from oil processing mill, dairy waste, whey, molasses, cassava wastewater (Das and Mukherjee, 2007; Zhu et al., 2013), Rice water and Potato processing effluent (Banat et al., 2014) Potato peel (Das and Kumar, 2018) etc., to make the biosurfactant production economical. There are several bacteria including *Pseudomonas* (Pacwa-Płociniczak et al. 2014; Sivasubramani and Selvaraj, 2017), *Bacillus* (Joshi et al., 2013), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* (Sneha et al., 2012; Jain et al., 2013), *Serratia rubidaea* (Nalini and Parthasarathi, 2017) and certain fungi like *Candida lipolytica* (Santos et al., 2014; Rufino et al., 2014) have been reported for the biosurfactant production. There are different microorganisms involved in the biosurfactant production and based on which the substrate or the production medium is formulated. This study employed the Potato peel extract and the curd whey to study the optimized conditions for Biosurfactant production using *Pseudomonas aeruginosa* PBS29.

II. MATERIALS AND METHODS

2.1. Microorganism and Fermentation condition

The potent strain selected for the study was *Pseudomonas aeruginosa* PBS29 (Poonguzhali et al., 2017). The cultural condition employed in the study for fermentation was 2% inoculum at pH 7 and incubated at 37̊ C for about 7 days in a rotary shaker with 120 rpm unless specific. The seed culture used for the study was overnight grown culture of *Pseudomonas aeruginosa* PBS29 (GenBank accession number MG273769) in Mineral salt agar medium.

2.2. Selection of Agro-industrial waste and Substrate Preparation

The agro-industrial waste opted for the study was Potato peel and Curd whey. The homogenized potato peel extract was prepared by modifying the method of Kulkarni et al., (2015). Potato peel was washed with tap water twice and then with distilled water to remove the impurities at the surface. Further, it was blanched with hot water (at 80̊ C for about 15 min) and homogenized using a blender. The content of fine paste (in different percentage described below) obtained was then mixed well with sterile distilled water and filtered through muslin cloth. The extract was further filtered using Whatman filter paper no.1. The Mineral salt broth for fermentation was prepared in the liquidized form of the final extracted material. The curd whey was supplemented in the different ratio in the Mineral salt agar medium and the fermentation was promoted. Similarly, the Curd whey was initially filtered through double layer muslin cloth and then using Whatman filter paper no. 1 and supplemented in the Mineral salt medium for fermentation.

2.3. Biosurfactant production using Potato peel as the substrate

The Mineral salt agar medium was supplemented with the different concentration of homogenized potato peel extract (HPPE) and the biosurfactant production using *Pseudomonas aeruginosa* PBS29 was evaluated employing the fermentation conditions specified above. The sample was drawn at the regular time interval and analyzed the following. The total carbohydrate, reducing and non reducing sugars were analyzed during biosurfactant production. The substrate concentration was estimated as total reducing sugars using the dinitrosalicylic acid method according to Miller, (1959) and expressed in g/l. Emulsification index (E24%), Biosurfactant production (g/l) and the dry cell biomass (g/l) were also determined at different time interval (ranging from 12, 24, 36, 48, 60, 72, 84 and 96 hrs respectively).

2.4. Study on Biosurfactant production using Curd whey

The Mineral salt medium (MSM) was supplemented with different concentrations (2, 4, 6, 8, 10%) of curd whey (v/v). The influence of various factors on the biosurfactant production was also determined for the formulation of curd whey medium. Emulsification index (E24%), Biosurfactant production (g/l), dry cell biomass (g/l), Surface tension and surface tension reduction were evaluated.

2.4.1. Effect of Phosphorous and C/P ratio

The effect of phosphorous source on biosurfactant production was assessed in the medium containing MSM with 4% Curd whey. The phosphorous source such as Potassium dihydrogen phosphate and Dipotassium hydrogen phosphate were supplemented at the concentration range of 1, 1.5, 2, 2.5 and 3 g/l (w/v). The effect of C/P ratio on the biosurfactant activity at the concentration of 10:1, 10:2, 10:3, 10:4 and 10:5 were also determined.

2.4.2. Effect of Metal Ions

The salts of metal ions (w/v) were initially dissolved in deionized water, then filtered sterilized through Millipore filter (0.22 μm) and suspended in the sterilized medium throughout the study to obtain the appropriate concentrations as mentioned below. The influence of different metal ions (at the concentration of 0.01g/l) such as Magnesium, Ferrous, Manganese, Zinc and Calcium ions on the biosurfactant activity were determined by supplementing them in the production medium individually in the form of $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, $MnSO_4.7H_2O$, $ZnSO_4.7H_2O$ and $Cacl_2$ respectively.

2.4.5. Synergistic effect of Metal Ions

The synergistic effect of metal ions such as $FeSO_4$+$MgSO_4$, $FeSO_4$+$MnSO_4$, $FeSO_4$+$ZnSO_4$, $FeSO_4$+$Cacl_2$, $MgSO_4$+$MnSO_4$, $MgSO_4$+$ZnSO_4$, $MgSO_4$+$Cacl_2$, $MnSO_4$+$ZnSO_4$, $MnSO_4$+$Cacl_2$ respectively was assessed by supplementing those combined metal ions in the production medium for evaluating the biosurfactant activity.

2.4.6. Effect of Combined C/P ratio with Metal Ions and Concentration of Metal Ions

The various concentrations of metal ions based on the high influence over the biosurfactant production were selected for the further study. For which, metal ions such as MgSO4 and FeSO4 ranging from 0.2, 0.4, 0.8, 1.6

mM were supplemented in the production medium and determined its biosurfactant activity. The combined effect of C/P and the metal ion such as low C/P + $MgSO_4$, High C/P + $MgSO_4$, low C/P + $FeSO_4$ and High C/P + $FeSO_4$ respectively were analyzed for biosurfactant activity by supplementing in the MSM with 4% Curd whey.

2.5. Analysis

The dry cell weight, Emulsification index (E24%), Surface tension and ST reduction were evaluated as previously described (Poonguzhali et al., 2017). The biosurfactant production was determined as specified in the study of Poonguzhali et al., (2018). The growth kinetics of *Pseudomonas aeruginosa* PBS29 during biosurfactant production on the substrates Potato peel and Curd whey were also determined. All the experiments were performed as the batch culture in triplicate and the results were reported in Mean ± Standard deviation using Microsoft Excel 2013.

III. RESULT AND DISCUSSION

The present study has concentrated on the utilization of agro-industrial waste such as Homogenized Potato peel extract (HPPE) and Curd whey as the cheapest substrate for the biosurfactant production using the strain *Pseudomonas aeruginosa* PBS29. The Homogenized potato peel substrate used in the study was starchy rich material and an inexpensive source, while the Curd whey was lactose rich material. The increase in demand for the biosurfactant improved the necessity for the economical production which can possible with the cheapest sources. When concentrating on the low-cost substrate, agro-industrial waste plays a promising role in biosurfactant production.

In the current investigation, the optimized concentration of HPPE was identified based on the levels of the substrate to be supplemented in the media constituents. When assessing the concentration (ranging from 2%, 4%, 6%, 8% and 10%) of HPPE supplemented in the Mineral salt medium, 2% HPPE (Figure 1) found to exhibit the better biosurfactant activity compared with other concentrations. Utilization of potato peel (at 2%) by *Pseudomonas aeruginosa* PBS29 (Figure 2) has shown the significant increase in biosurfactant yield of 3.78 ± 0.4 (g/l) and Emulsification index of 60.07± 0.2 (%) respectively till 96 hrs (Table 1) and not much difference thereafter. This investigation demonstrated the growth kinetics of *Pseudomonas aeruginosa* PBS29 when grown on the substrates HPPE and Curd whey (Table 2). The present findings revealed the growth kinetics of *Pseudomonas aeruginosa* PBS29 on the biosurfactant yield may vary based on the substrate utilization ($Y_{P/S}$) and production to dry cell biomass ($Y_{P/X}$), which was in accordance to the suggestion of Rane et al., (2017).

However, the study of Noh et al., (2014) reported that the product yield on biomass was calculated as the ratio of biosurfactant concentration to the corresponding biomass and not by the highest biomass obtained. Moreover, it was inferred from literature that not much study was carried out the biosurfactant (rhamnolipid) production using Potato peel as substrate by *Pseudomonas aeruginosa* rather biosurfactant production was obtained by *Bacillus* species implemented Potato peel as substrate (Sharma et al., 2015; Das and Mukherjee, 2007; Fox and Bala, 2000). Only few studies reported the rhamnolipid type of biosurfactant using Potato peel (Das and Kumar, 2018; Ansari et al., 2014).

According to (Kaskatepe and Yildiz, 2016), Whey was the most abundant byproduct generated from the dairy industries constituting of 75% lactose, 15% protein and other components like vitamins and minerals. In the present study, Curd whey was supplemented in the Mineral salt medium (Figure 3) in the varied ratio (2%, 4%, 6%, 8% and 10% respectively) and confirmed the ratio of 4% to be used for further optimization of the Curd whey medium. The phosphorous source such as Potassium dihydrogen phosphate and Di-potassium hydrogen phosphate in the production medium was altered from 1 to 3 g/l and observed its influence on the biosurfactant activity. It was observed that there was the difference in the biosurfactant activity by altering the phosphorous source and the concentration of 2g/l was found to have an optimal biosurfactant activity (Figure 4). The Phosphate limitation led to the decreased cell dry biomass and also exhibited a variation in the biosurfactant activity too. In coherence, Mulligan et al. (1989) demonstrate the reduction in surface tension when the phosphorous source has been depleted.

The present findings correlated with the suggestion of Clarke et al., (2010), who also insisted the limitation of phosphorous source triggered the rhamnolipid production by lowering the cell growth. Thereby the analysis of the C/P ratio at 10:2 had an impact on the biosurfactant production (Figure 5). In a similar investigation of Batista et al., (2010), demonstrated the low C/Mg with the low C/P in the production media influenced the biosurfactant production using *Candida tropicalis* but also suggested that the biosurfactant yield found to be unchanged.

Among the various metal ions, Magnesium sulfate ($MgSO_4$) and then Ferrous sulfate ($FeSO_4$) found to have a significant effect over the biosurfactant production (Figure 6). The effect of metal ions varied based on the metal ion employed, concentration (Figure 7) and the synergistic effect of the metal ions in the medium (Figure 8). The metal ions as trace elements in the medium enhanced the biosurfactant production to a great extent (Wei et al., 2007). Certain study remarked that iron and other metal ions need to be supplemented in the production medium for an increased biosurfactant production (Zhi et al., 2013). In fact, Joshi et al., (2015) declared that maximum cell growth was observed with $MgSO_4$ at 5mM concentration and also insisted on the doubling of the cell growth with the metal ion, $ZnSO_4$ but not much with $FeSO_4$ and $Cacl_2$. The variation in the present findings may be due to the production strain and the fermentation conditions.

Furthermore, the enhance biosurfactant production was also reported by the fungi *Aspergillus ustus* and *Pleurotus djamor* by implementing ferrous sulfate in the medium (Velioglu and Urek, 2015). The report of Venkataramana & Karanth (1989) revealed the increase in C/P ratio stimulated the cell growth and rhamnolipid production as well as established that the divalent iron decreased the rhamnolipid production. In this study, as for the growth of the microbial cell concern, increase by its cell biomass (dry weight) was observed till a specific concentration of the phosphorous and then decreased further, which was in acceptance by Guerra-Santos et al., (1984).

Despite the report stating that the supplementation of metals to the medium has not provided evidence to influence the production of biosurfactants by *Torulopsis bombicola* (Cooper and Paddock, 1984), the current investigation prescribed the low C/P ratio along with the metal ion ($MgSO_4$) exhibiting the decreased surface tension (33.93 ± 0.3 mN/m) of all the parameters tested using Curd whey as the substrate (Figure 9). The similar interesting finding was reported by Oren and Litchfield (1994) insisting on the magnesium ions for the credible increase in the biosurfactant production. However, Kanna et al., (2014) reported the effect of metal ions such as Ca^+, Mg^+ and Fe^+ in reducing the surface tension to an appreciable amount. The analysis regarding the C/P ratio combined with the metal ion, Magnesium in the form of $MgSO_4.7H_2O$ influencing the biosurfactant production (3.67 ± 0.2 g/l) using the Curd whey as substrate and thereby this study provided a clear-cut investigation of those parameters individually as well as the combined effect.

IV. CONCLUSION

This study has provided the better understanding of the utilization of Agro-industrial waste such as potato peel and Curd whey as the cheapest source for the effective biosurfactant production employing the production strain *Pseudomonas aeruginosa* PBS29. The biosurfactant production of 3.78 ± 0.4 g/l and was significant till 96hrs using the potato peel medium. While the Curd whey resulted in 3.67 ± 0.2 g/l of biosurfactant production. The influence of the phosphorous source at 2g/l and the metal ion $MgSO_4$ (at 0.4 mM) was identified with its potent combined activity of low C/P ratio with $MgSO_4$. The formulated medium using potato peel and Curd whey has proved its efficacy in biosurfactant production and hence recommended for large scale production. These agro-industrial wastes are surplus, cost-effective and abundantly available feedstock that would better provide the economical biosurfactant in the global market.

Acknowledgment

We express our sincere thanks to the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram for providing the laboratory facility. We also articulate our gratefulness to the Department of Microbiology, M.R. Government Art College, Mannargudi for their valuable support right through the work.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

[1]. Banat IM, Satpute SK, Cameotra SS, Patil R and Nyayanit NV. Cost effective technologies and renewable substrates for biosurfactants' production. Front Microbiol 2014; 5: 1-18.

[2]. Clarke KG, Ballot F and Reid SJ. Enhanced rhamnolipid production by *Pseudomonas aeruginosa* under phosphate limitation. World J Microbiol Biotechnol 2010; 26: 2179–2184. DOI 10.1007/s11274-010-0402-y

[3]. Cooper GD and Paddock AD. Production of a Biosurfactant from *Torulopsis bombicola*. Appl. Environ. Microbiol 1984; 47: 173–176.

[4]. Das AJ and Kumar R. Utilization of agro-industrial waste for biosurfactant production under submerged fermentation and its application in oil recovery from sand matrix. Bioresource Technology 2018; 260: 233-240. https://doi.org/10.1016/j.biortech.2018.03.093

[5]. Das SK and Mukherjee AK. Comparison of lipopeptide biosurfactants production by Bacillus subtilis strains in submerged and solid state fermentation systems using a cheap carbon source: Some industrial applications of biosurfactants. Process Biochem 2007; 42(8): 1191-1199. https://doi.org/10.1016/j.procbio.2007.05.011

[6]. Ansari FA, Hussain S, Ahmed B, Akhter J and Shoeb E. Use of Potato peel as cheap carbon source for the bacterial production of Biosurfactants. Int. J. Biol. Res 2014; 2(1): 27-31.

[7]. FAO. Food Loss and Food Waste. 2016 [cited 2018 March 28]; Available from: http://www.fao.org/food-loss-and-food-waste/en/.

[8]. Fox SL and Bala GA. Production of surfactant from *Bacillus subtilis* ATCC 21332 using potato substrates. Biores. Technol 2000; 75(3): 235-240. https://doi.org/10.1016/S0960-8524(00)00059-6

[9]. Guerra-Santos L, Kappeli O and Fiechter A. *Pseudomonas aeruginosa* Biosurfactant Production in Continuous Culture with Glucose as Carbon Source. Applied and Environmental Microbiology 1984; 48(2): 301-305.

[10]. Jain RM, Mody K, Joshi N, Mishra A and Jha B. Production and structural characterization of biosurfactant produced by an alkaliphilic bacterium, *Klebsiella* sp.: Evaluation of different carbon sources. Colloids and Surfaces B: Biointerfaces 2013; 108: 199– 204.

[11]. Joshi PA, Singh N and Shekhawat DB. Effect of metal ions on growth and biosurfactant production by Halophilic bacteria. Adv. Appl. Sci. Res 2015; 6(4): 152-156.

[12]. Joshi SJ, Geetha SJ, Yadav S and Desai AJ. Optimization of Bench-Scale Production of Biosurfactant by *Bacillus licheniformis* R2. APCBEE Procedia 2013; 5: 232 – 236.

[13]. Kaskatepe B and Yildiz S. Rhamnolipid Biosurfactants Produced by *Pseudomonas* Species. Braz. Arch. Biol. Technol 2016; 59: e16160247. http://dx.doi.org/10.1590/1678-4324-2016160786

[14]. Kulkarni SO, Kanekar PP, Jog JP, Sarnaik SS and Nilegaonkar SS. Production of copolymer, poly (hydroxybutyrate-co-hydroxyvalerate) by *Halomonas campisalis* MCM B-1027 using agro-wastes. Int. J. Biol. Macromol 2015; 72: 784–789. doi: 10.1016/j.ijbiomac.2014.09.028

[15]. Makkar RS and Cameotra SS. An update on the use of unconventional substrates for biosurfactant production and their new applications. Appl. Microbiol. Biotechnol 2002; 58(4): 428-34. DOI: 10.1007/s00253-001-0924-1

[16]. Makkar RS, Cameotra SS and Banat IM. Advances in utilization of renewable substrates for biosurfactant production. AMB Express 2011; 1: 5.

[17]. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem. 1959; 31 (3):426–428. DOI: 10.1021/ac60147a030

[18]. Mulligan CN, Mahmourides G and Gibbs BF. The influence of phosphate metabolism on biosurfactant production by *Pseudomonas aeruginosa*. J Biotechnol 1989; 12: 199–210.

[19]. Nalini S and Parthasarathi R. Optimization of rhamnolipid biosurfactant production from *Serratia rubidaea* SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant. Annals of Agrarian Science 2017; (In Press) 1-8. https://doi.org/10.1016/j.aasci.2017.11.002

[20]. Nitschke M, Ferraz C and Pastore GM. Selection of microorganisms for biosurfactant production using agro industrial wastes. Braz J Microbiol 2004; 35: 81–85. http://dx.doi.org/10.1590/S1517-83822004000100013

[21]. Noh NAM, Salleh SM and Yahya ARM. Enhanced rhamnolipid production by *Pseudomonas aeruginosa* USM-AR2 via fed-batch cultivation based on maximum substrate uptake rate. Letters in Applied Microbiology 2014; 58: 617—623.

[22]. Oren A and Litchfield CD. A procedure for the enrichment and isolation of *Halobacterium* FEMS Microbiology Letters 1994; 173: 353-358. DOI: 10.1016/S0378-1097(99)00095-6

[23]. Pacwa-Płociniczak M, Płaza GA, Poliwoda A and Piotrowska-Seget Z. Characterization of hydrocarbon-degrading and biosurfactant producing *Pseudomonas* sp. P-1 strain as a potential tool for bioremediation of petroleum-contaminated soil. Environ Sci Pollut R 2014; 21(15): 9385–9395. doi: 10.1007/s11356-014-2872-1

[24]. Poonguzhali P, Rajan S, Parthasarathi R. Screening and characterization of Biosurfactant producing bacteria from Hydrocarbon and Pesticides contaminated soils from Cuddalore district. Life Science Archives. 2017; 3(6): 1207 – 1217. DOI: 10.22192/lsa.2017.3.6.3

[25]. Poonguzhali P, Rajan S, Parthasarathi R. Antimicrobial and stability study of Biosurfactant produced from *Pseudomonas aeruginosa* PBS29. Indo - Asian Journal of Multidisciplinary Research. 2018; 4(1): 1347 – 1358. DOI: 10.22192/iajmr.2018.4.1.3

[26]. Kanna R, Gummadi SN and Kumar GS. Production and Characterization of Biosurfactant by *Pseudomonas putida* MTCC 2467. Journal of Biological Sciences 2014; 14: 436-445.

[27]. Rane AN, Baikar VV, Ravi Kumar V and Deopurkar RL. Agro-Industrial Wastes for Production of Biosurfactant by *Bacillus subtilis* ANR 88 and Its Application in Synthesis of Silver and Gold Nanoparticles. Front. Microbiol 2017; 8: 492. doi: 10.3389/fmicb.2017.00492

[28]. Batista RM, Rufino RD, Luna JM, Souza JEG, Sarubbo LA. Effect of Medium Components on the Production of a Biosurfactant from *Candida tropicalis* Applied to the Removal of Hydrophobic

Contaminants in Soil. Water Environment Research 2010; 82(5):418-25. doi:10.2175/106143009X12487095237279

[29]. Rufino RD, Luna JM, Takaki GMC, Sarubbo LA. Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. Electronic Journal of Biotechnology. 2014; 17(1): 34-38. https://doi.org/10.1016/j.ejbt.2013.12.006

[30]. Santos DKF, Brandao YB, Rufino RD, Luna JM, Salgueiro AA, Santos VA, Sarubbo LA, Optimization of cultural conditions for biosurfactant production from *Candida lipolytica*, Biocatal. Agric. Biotechnol. 3 (2014) 48–57. https://doi.org/10.1016/j.bcab.2014.02.004

[31]. Santos DKF, Rufino RD, Luna JM, Santos VA and Sarubbo LA. Biosurfactants: Multifunctional Biomolecules of the 21st Century. Int. J. Mol. Sci 2016; 17: 401-431.

[32]. Sharma D, Ansari MJ, Gupta S, Ghamdi AA, Pruthi P and Pruthi V. Structural Characterization and Antimicrobial Activity of a Biosurfactant Obtained From *Bacillus pumilus* DSVP18 Grown on Potato Peels. Jundishapur J Microbiol 2015; 8(9): e21257. DOI: 10.5812/jjm.21257

[33]. Sivasubramani A and Selvaraj R. Isolation, Screening and Production of Biosurfactant by *Pseudomonas aeruginosa* SD4 Using Various Hydrocarbon Sources. International Journal of Science and Research. 2017; 6(2):2007-2012.

[34]. Sneha KS, Padmapriya B and Rajeswari T. Isolation and Screening of Biosurfactants Produced by *Pseudomonas aeruginosa* from Oil Spilled Soils. International Journal of Pharmaceutical & Biological Archives 2012; 3(2): 321-325.

[35]. Velioglu Z and Urek RO. Optimization of cultural conditions for biosurfactant production by *Pleurotus djamor* in solid state fermentation. Journal of Bioscience and Bioengineering 2015; 120(5): 526–531.

[36]. Wei YH, Lai CC and Chang JS. Using Taguchi Experimental Design Methods to Optimize Trace Element Composition for Enhanced Surfactin Production by *Bacillus subtilis* ATCC 21332. Process Biochem 2007; 42: 40–45.

[37]. Zhi L, Xing-zhong Y, Hua Z, Guang-ming L, Zhi-feng L, Xiao-ling MA and Ya-ying Z. Optimizing rhamnolipid production by *Pseudomonas aeruginosa* ATCC 9027 grown on waste frying oil using response surface method and batch fed fermentation. Journal of Central South University 2013; 20: 1015–1021.

[38]. Zhu Y, Gan J, Zhang G, Yao B, Zhu W, Meng Q. Reuse of waste frying oil for production of rhamnolipids using *Pseudomonas aeruginosa* zju.u1M. J Zhejiang Univ Sci A. 2007; 8:1514-1520.

Table 1. Utilization of potato peel at different time interval with the effective biosurfactant production

Time (hrs)	Total carbohydrate utilized (g/l)	Reducing sugars utilized (g/l)	Non-reducing sugar utilized (g/l)	Cell Biomass* (g/l)	BS production (g/l)	Emulsification Index (%)
0	33.14 ± 0.1	17.36 ± 0.1	18.23 ± 0.1	ND	ND	ND
12	31.47 ± 0.1	14.52 ± 0.2	17.58 ± 0.2	0.57 ± 0.2	0.19 ± 0.1	17.44± 0.1
24	29.61 ± 0.2	12.02 ± 0.1	16.41 ± 0.1	0.68 ± 0.1	1.07 ± 0.2	28.63± 0.2
36	28.32 ± 0.2	10.43 ± 0.1	15.83 ± 0.1	0.93 ± 0.1	1.58 ± 0.1	31.14± 0.1
48	27.83 ± 0.1	9.67 ± 0.3	14.62 ± 0.1	1.12 ± 0.2	1.71 ± 0.1	34.71± 0.3
60	26.01 ± 0.1	8.21 ± 0.2	14.13 ± 0.1	1.37 ± 0.1	2.16 ± 0.1	37.56± 0.1
72	25.84 ± 0.3	6.11 ± 0.1	13.18 ± 0.2	1.69 ± 0.1	2.84 ± 0.2	43.52± 0.3
84	24.11 ± 0.1	5.64 ± 0.2	12.47 ± 0.1	2.11 ± 0.3	3.45 ± 0.1	50.21± 0.3
96	23.75 ± 0.2	5.12 ± 0.1	12.01 ± 0.1	2.23 ± 0.1	3.78 ± 0.4	60.07± 0.2

The values are represented in Mean ± standard deviation, * - Dry cell weight, ND – Not determined.

Table 2. Growth Kinetics of the Biosurfactant production using the agro industrial waste as substrates

Substrate	BS yield (g/l)	YP/S (g/g)	YP/X (g/g)
Homogenized Potato peel extract	3.78 ± 0.4	0.213 ± 0.004	1.28 ± 0.02
Curd whey	3.67 ± 0.2	0.192 ± 0.003	1.12 ± 0.004

BS yield - biosurfactant yield, YP/S - biosurfactant yield on the substrate utilization, YP/X - biosurfactant production to dry cell biomass.

Figure 1. Influence of the Homogenized potato peel extract (HPPE) on Biosurfactant activity

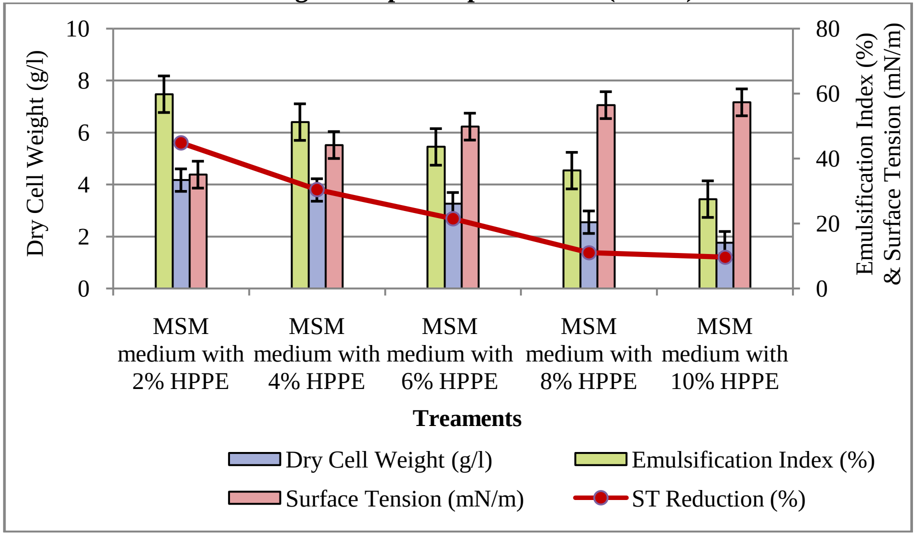

Figure 2. Utilization of the Homogenized potato peel extract as substrate influencing the Biosurfactant activity at different time interval

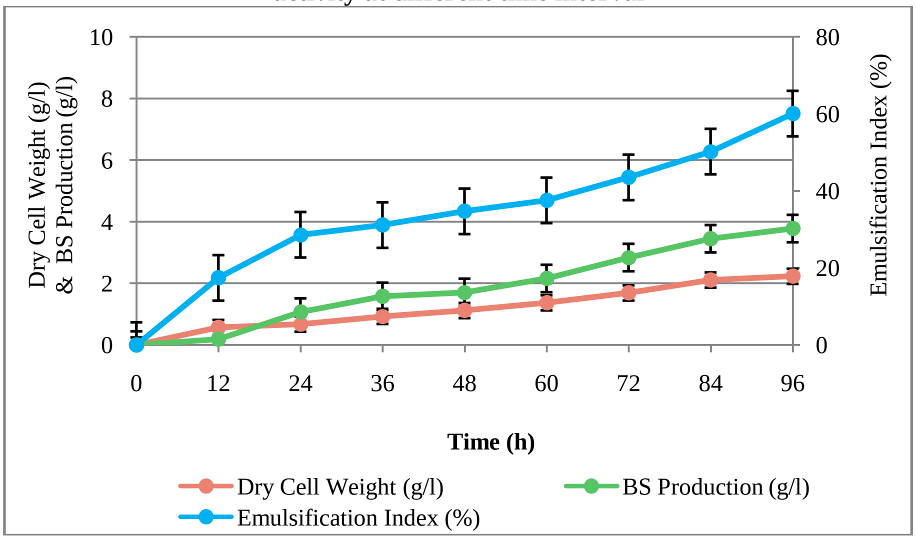

Figure 3. Influence of Curd whey as substrate on Biosurfactant activity

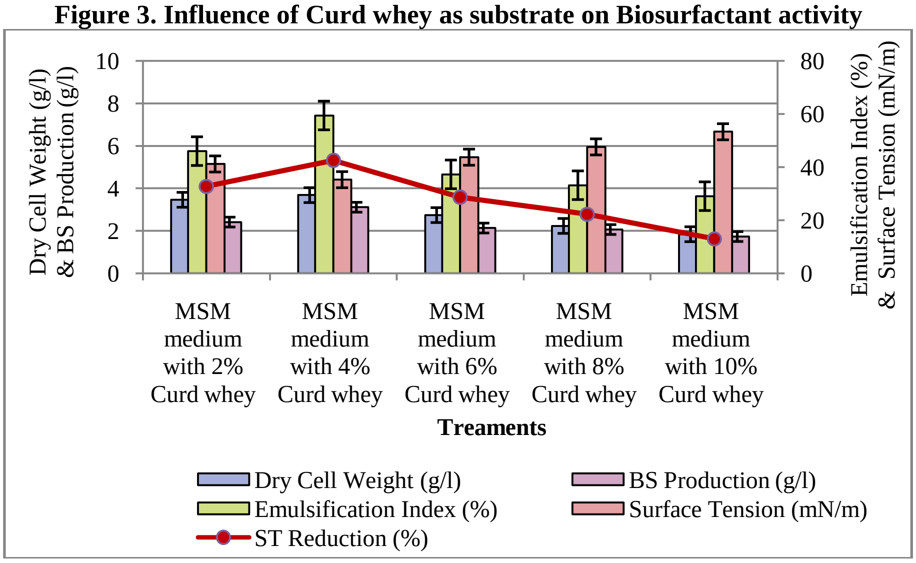

Figure 4. Phosphorous source in the Curd whey medium influencing the biosurfactant activity

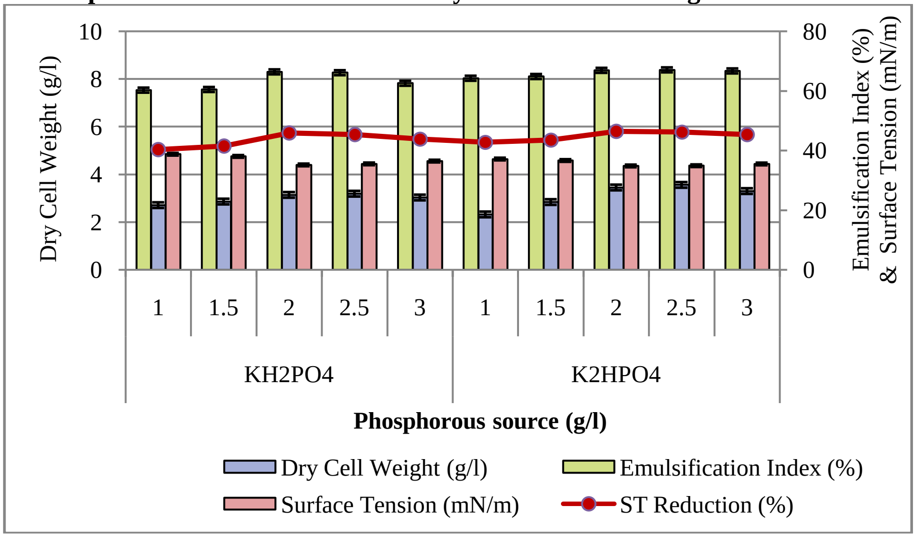

Figure 5. C/P ratio in the Curd whey medium influencing the biosurfactant activity

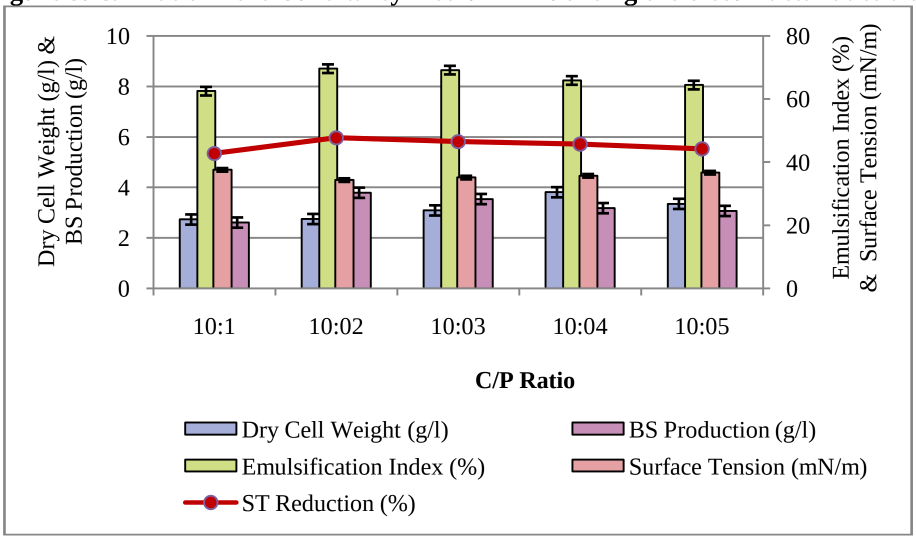

Figure 6. Various Metal ions in the Curd whey medium influencing the biosurfactant activity

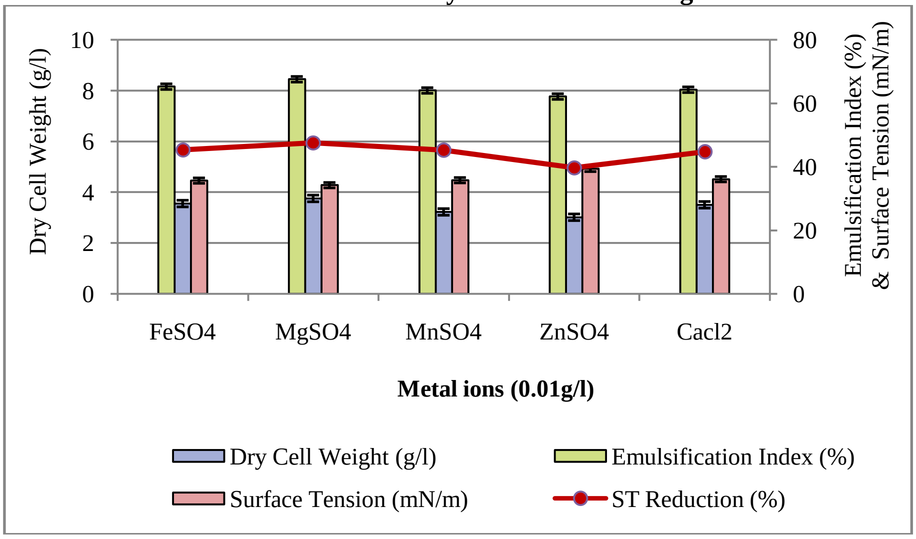

Figure 7. Synergistic effect of Metal ions in the Curd whey medium influencing the biosurfactant activity

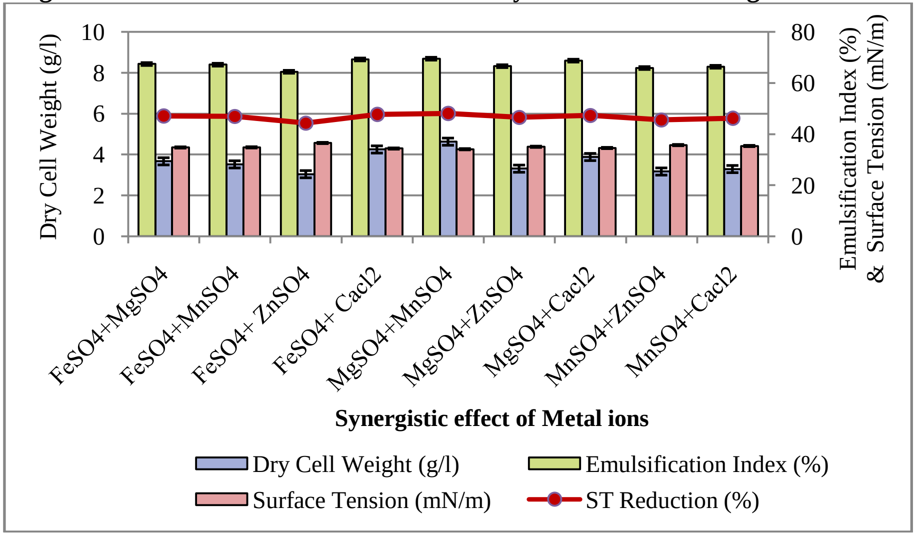

Figure 8. Concentration of Metal ions in the Curd whey medium influencing the biosurfactant activity

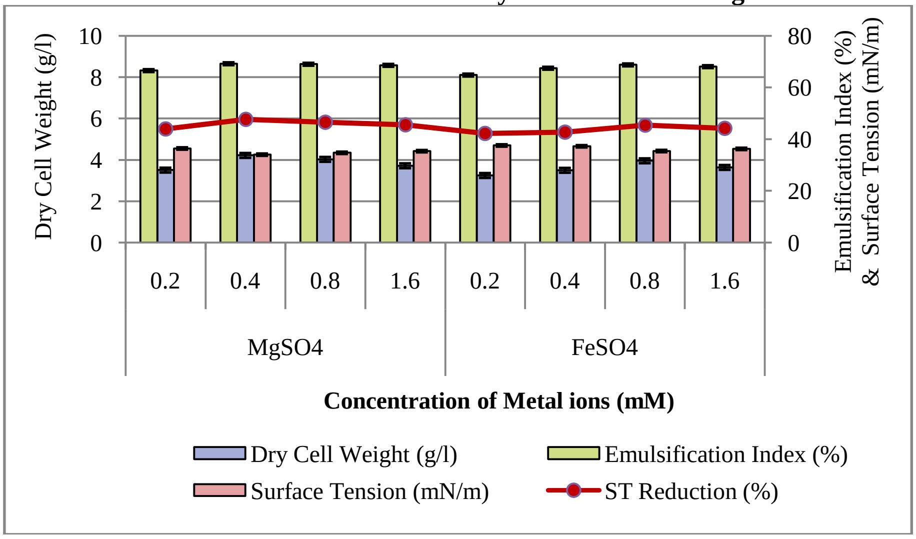

Figure 9. Combinned effect of C/P and metal ion in the Curd whey medium influencing the biosurfactant activity