

Potential of New Microbial Isolates for Biosurfactant Production using Combinations of Distillery Waste with other Industrial Wastes

Kirti V Dubey^{1*}, Pravin N Charde², Sudhir U Meshram², Santosh K Yadav³, Sanjeev Singh³ and Asha A Juwarkar³

¹Sevadal Mahila Mahavidyalaya, Sakkardara Square, Umrer Road, Nagpur-440009, India

²North Maharashtra University, Jalgaon, 425001 Maharashtra, India

³Ecosystem Division, National Environmental Engineering Research Institute (NEERI), Nehru Marg, Nagpur-440015, India

Abstract

In the present study, combinations of Distillery Waste (DW) with other industrial wastes viz. curd Whey Waste (WW), Fruit Processing Waste (FPW) and Sugar Industry Effluent (SIE) were evaluated to replace the use of water that was reported earlier for biosurfactant production from 1: 3 diluted distillery waste by using four new bacterial cultures BS-A, BS-J, BS-K and BS-P, isolated from soil collected from a distillery unit. These isolates have the potential to produce biosurfactant from these individual wastes and in their combinations. Highest biomass and biosurfactant yields with higher reduction in the Chemical Oxygen Demand (COD), total sugars, nitrogen and phosphate levels were obtained in 1:1:1 proportion of DW+WW+FPW followed by DW+WW+ SIE and individual wastes. The combinations of wastes improved the yields of biosurfactants by 18-41% and reduced COD of the combined wastes by 76-84.2%. Total sugars, nitrogen and phosphate levels reduced in the range of 79-86%, 58-71% and 45-59%, respectively. Among the four microbial isolates tested, BS-J and BS-P were the efficient biosurfactant producers and were identified as *Kocuria turfanesis* and *Pseudomonas aeruginosa* based on the 16S rDNA sequence and phylogenetic analyses. Benefits derived by using combined distillery waste with other wastes are improved production of biosurfactant as resource and saving precious water and the costly nutrients with concomitant reduction in pollution load of the wastes.

Keywords: Biosurfactant; Distillery wastewater; *Kocuria turfanesis* strain BS-J; *Pseudomonas aeruginosa* strain BS-P; Combined wastewater

Introduction

Biosurfactants are defined as a class of surface-active molecules synthesized by microorganisms. In the past few decades, biosurfactants have gained attention because of their biodegradability, low toxicity, ecological acceptance and ability to be produced from renewable wastes as substrates and can be applied in bioremediation and wastewater treatment [1,2]. Some potential applications of biosurfactants are crude oil recovery, hydrocarbon degradation in soils, and hexa-chloro cyclohexane degradation, heavy metal removal from contaminated soils and hydrocarbon biodegradation in aquatic environment [3-6]. Apart from being active at extreme temperatures, pH and salinity, biosurfactants can be produced from industrial wastes and from by-products [7]. This last feature makes cheap production of biosurfactants possible as it allows utilization of waste substrates so that application of biosurfactants in environmental remediation can be realized and environmental use is currently considered to be one of the larger markets for biosurfactants [6,8,9].

Interest in potential applications of biosurfactants in various industries has significantly increased recently, particularly because of their environmental friendly nature and sustainability. Nevertheless, economical large scale production for established and new applications of biosurfactants remains a challenge [10]. The main factor limiting commercialization of biosurfactants is associated with non-economical large-scale production. Development of cheaper processes and the use of low cost raw material are the main factors which accounts for the 10-30% of the overall cost [11]. To overcome the cost of fermentation medium and to compete with synthetic surfactants, inexpensive substrates and new efficient microorganisms have to be isolated for biosurfactant production. Furthermore, important prerequisites for the competitive production of biosurfactants include high biosurfactant yields, alternative low cost substrates, and cost-effective bioprocesses.

Agro-industrial wastes are considered as the promising substrate for biosurfactant production and can alleviate many processing industrial waste management problems [1]. If industrial and /or municipal wastewaters which contain organic load could be utilized as substrates for biosurfactant production, a double benefit could be obtained i.e. treating the waste and recovering valuable product in the form of biosurfactant with even a potential of generating a profit through the sale of biosurfactant [12]. Another approach involving the use of raw substrates with negligible or no-cost value appears simple; however, the main problem associated with this approach is the selection of suitable waste material with the right balance of nutrients that permits cell growth and product accumulation. Thus, the promising future of biosurfactants appears to be specifically dependent upon the use of abundant and low cost raw materials and the optimization of the operational cultivation conditions in order to achieve high yields.

The major classes of biosurfactants that have been studied until now using alternative low-cost substrates, like vegetable oils and agro-industrial wastes, are rhamnolipids produced by *Pseudomonas aeruginosa* and surfactin produced by *Bacillus subtilis* [13]. Therefore, use of other different types of no-cost industrial wastes as growth medium for new biosurfactant producing microbial isolates to

***Corresponding author:** Kirti V Dubey, Sevadal Mahila Mahavidyalaya, Sakkardara Square, Umrer Road, Nagpur-440009, India, Tel: +91 -0712-275037, 2751344; Fax: +91 -712 - 275037; E-mail: kirtivijay_dubey@yahoo.com

Received April 09, 2012; Accepted May 07, 2012; Published May 07, 2012

Citation: Dubey KV, Charde PN, Meshram SU, Yadav SK 3, Singh S, et al. (2012) Potential of New Microbial Isolates for Biosurfactant Production using Combinations of Distillery Waste with other Industrial Wastes. J Petrol Environ Biotechnol S1:002. doi:10.4172/2157-7463.S1-002

Copyright: © 2012 Dubey KV, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

minimize the cost of fermentation medium has to be explored. Earlier, we have reported various aspects of cost-effective production, recovery and application of biosurfactant produced from 1:3 diluted distillery waste [4,14,15]. Industrial wastes such as distillery waste and curd whey are the viable alternative sources for biosurfactant production and it has been demonstrated that distillery waste cannot be used as such in its original state as complete fermentation medium without dilution with water in 1:3 proportion due to the presence of large amounts of sulphate ions and potash in the waste, which inhibits the growth of biosurfactant producing microbial cultures [14]. Distillery waste has been reported as a viable medium for biosurfactant production, however, its dilution with precious water does not seem economical if an appropriate cost-effective process for biosurfactant production has to be developed using distillery waste as fermentation medium. Therefore, present study was carried out with an aim to save precious water that was required for the dilution of distillery waste by other industrial wastes before using it as no-cost medium for biosurfactant production. In the present study, a comparative account on scope of using different combinations of distillery waste with other industrial wastes as no-cost medium for production of biosurfactant by four different newly isolated microbial cultures is reported. This study forms an attractive and environmentally safe basis for developing new strategies for biosurfactant production from distillery waste by minimizing the pollution problem of distillery waste and other food industry wastes also (that were used for dilution of the distillery waste) as well as recovery of resource in the form of biosurfactants by newly isolated microbial cultures.

Materials and Methods

Collection and processing of industrial waste waters for biosurfactant production

Fresh waste waters viz. distillery waste, curd whey, fruit processing waste and sugar industry effluent were collected for biosurfactant production from the respective industries as indicated in Table 1 and were immediately transferred into a deep freezer working at 2°C (Remi Instruments, Vasai, India). Among these wastes, curd whey required processing to remove casein exhaustively from the whey by following earlier described method [14]. Briefly, curd whey was neutralized with 5N NaOH followed by steaming for 10 min. Casein settled in the bottom in the form of sodium caseinate and the supernatant was removed

as partially de-proteinised whey which was further filtered through membrane filter (0.45µm) before sterilization at 15lb/square inch pressure. Distillery waste, fruit processing waste and sugar industry effluent had low pH in the range of 4.8-6.8 and were neutralized to pH-7.0 with 5N NaOH. Neutralized wastes were separately sterilized at 15lb/square inch pressure and were then used in further study.

Screening of biosurfactant producing microorganisms

Biosurfactant producing microorganisms were isolated from soil contaminated with lube oil and distillery spent wash by using selective enrichment procedure and plating serially diluted enriched culture on sterile nutrient agar followed by incubation at 37°C for isolated colonies [14]. Soil used for isolation purpose was collected from an area just below the spent wash pumping device of a distillery unit. Isolates so obtained were individually screened for biosurfactant production from each of the above processed waste on the basis of stability of foam, emulsification index, surface tension reduction, and biosurfactant yield [14].

Biosurfactant production potential of new microbial isolates in distillery waste and other different wastes

Distillery waste (diluted with tap water in 1:3 ratio), whey waste, sugar industry effluent and fruit processing waste, 100 ml of each in four sets were taken in 250 ml Erlenmeyer flasks, and were sterilized at 121°C for 20 min. For inoculum build-up, one set of each of these sterile waste were then inoculated separately with the microbial isolates designated as BS-A, BS-J, BS-K, and BS-P under aseptic conditions and were then kept in a gyro rotatory incubator cum shaker working at 150 rpm for 24 h. One ml of each of this inoculum was then inoculated into an individual sterile waste in triplicates and the flasks were incubated in a gyro rotatory shaker working at 150 rpm for 120 h. Before and after incubation, parameters such as biomass developed in fermented waste in terms of c.f.u./ml, COD, surface tension reduction and biosurfactant yield (g/l) were determined.

Biosurfactant production potential of new microbial isolates in combination of distillery waste with other industrial wastes

No-cost fermentation medium having combinations of distillery waste with other industrial wastes were tested for biosurfactant production by new microbial isolates to nullify the use of precious water that was previously required for diluting distillery waste so that

Types of wastes	Sources of collection of wastes	Parameters					
		pH	COD (mg/l)	BOD (mg/l)	Total sugars (g/l)	Total Nitrogen (mg/l)	Total Phosphorus (mg/l)
Distillery wastes (DW)	Purti Sakhar Karkhana limited, Bela, Tal. Umred, Nagpur, India.	4.8±0.02	98013±9.43	37000±245	12.4±0.04	708±1.63	235±2.45
Whey waste (WW)	Amruta Dairy, Sakkardara square, Umred Road, Nagpur, India.	4.3±0.02	56000±73	28000±20	6.8±0.02	987±2.45	352±4.90
Fruit processing waste (FPW)	Noga factory, MIDC Hingna, Nagpur, India.	5.4±0.02	2133±42	1090±7	2.0±0.02	784±3.27	122±5.72
Sugar industry effluent (SIE)	Purti Sugar factory limited, Bela, Tal. Umred, Nagpur, India.	6.8±0.05	1050±7	959±2	1.5±0.04	643±6.53	135±1.63

Mean + Standard deviation; n=4

Table 1: Characteristics of different types of industrial wastewaters collected from different sources for biosurfactant production.

it can be used as substrate for biosurfactant production and secondly, combinations of wastes can lead to improved nutritional status of distillery waste which can be beneficial for improved biosurfactant production. Following two different sets of combinations of wastes were tested: i. Distillery waste + curd whey + sugar industry effluent (DW+WW+SIE) in 1:1:1 ratio & ii. Distillery waste + curd whey + fruit processing waste (DW+WW+FPW) in 1:1:1 ratio.

Four sets of four 250 ml Erlenmeyer flasks each containing 100 ml of combined distillery waste of above mentioned combinations were sterilized at 121°C for 20 min. One set of four flasks with each combination were inoculated with the microbial isolates designated as BS-A, BS-J, BS-K, and BS-P under aseptic condition. These inoculated flasks were then kept in a gyro rotatory incubator cum shaker for 120 h and after incubation same parameters as mentioned above were analysed to screen suitable combination of distillery waste with other industrial wastes and also the efficient microbial isolate for biosurfactant production in combined waste system.

Analytical methods

Measurement of the growth of biosurfactant producing isolates in no-cost industrial wastes: Growth of the biosurfactant producing isolates were monitored in terms of c.f.u./ml of fermented waste by performing serial dilution and pour plate technique using nutrient agar as the growth medium [14].

Surface tension measurements

Individual waste waters and the combined wastes before and after incubation time were centrifuged at 12,000 rpm and 4°C for 20 min in a C-24 Remi cold centrifuge (Remi Instruments, Vasai, India) to remove the microbial cells. The cell free supernatants so obtained were used for surface tension measurement(s) using a du Nouy ring-type tensiometer (Kruss, GmbH, Hamburg, Germany). The surface tension measurements were carried out at room temperature after dipping the platinum ring in the solution for a while in order to attain equilibrium condition. For calibration of the instrument, the surface tension of distilled water was first measured. The measurement was repeated at least three times, and an average value was used to express the surface tension of the sample [16].

Estimation of biosurfactant yield

Bacterial cells were removed by centrifugation of 120 h old fermented waste (12,000 rpm and 4°C, for 15 min). Cultural supernatant was acidified with 6 N HCl to obtain the pH of 2.0 and kept overnight in a refrigerator at 4°C. The precipitated biosurfactant was extracted three times with two volumes of di-ethyl ether. Pooled solvent extracts were concentrated using an evaporator (EYELA SB-651, Tokyo, Japan) under reduced pressure the yield of biosurfactant was gravimetrically estimated [14].

Physico-chemical characterization of no-cost fermented wastes and combined waste before and after biosurfactant recovery

Physico-chemical characterization of each of the no-cost waste and as well as combined waste was performed before and after biosurfactant recovery, with respect to parameters mentioned in Table 1 according to protocols of standard methods [17]. Total nitrogen was estimated by using semi-micro Kjeldahl method, total phosphate content was analysed by vanadomolybdo phosphoric acid spectrophotometric method and COD was estimated by closed reflux titrimetric method

by using COD digestion unit, Hicon Engineering Company Private Limited, Greater Noida, India. Total sugars were estimated by phenol-sulphuric acid method [18].

Identification of the efficient biosurfactant producing isolates

The two microbial isolates BS-J and BS-P having potential to produce high biosurfactant yields were identified on the basis of morphological, cultural and biochemical methods [19]. Results of these identification tests were compared with those in Bergey's Manual of Systemic Bacteriology [20].

16S rRNA gene amplification and sequencing of biosurfactant producing isolates

The 16S rRNA gene amplification and sequencing of biosurfactant producing microbial isolates BS-J and BS-P were performed by the following procedure: DNA was extracted by using ZR fungal/bacterial genomic DNA extraction kit (Zymo Research Corporation 17062 Murphy Ave. Irvine, CA 92614, U.S.A.) according to the manufactures instructions and used for PCR amplification of 16S rRNA gene. The 16S rRNA gene was amplified by PCR using universal bacterial primers 6-27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). The amplified product was subjected to electrophoresis on 1% agarose gel and a band corresponding to 1.5 Kb was cut and was eluted using QIA quick PCR purification Kit (Qiagen, Hilden, Germany). This purified amplicon was then sequenced using Big Dye Terminator cycle sequencing kit and an ABI PRISM model 3130xl analyser automatic DNA sequencer (Applied Biosystems, USA) with 3 different internal sequencing primers (27f, 535r and 1492r). The sequences obtained were aligned, edited manually and the aligned sequence was used for BLAST search.

Alignment of 16S rRNA sequence and construction of phylogenetic tree of biosurfactant producing isolates

The sequence of 16S rRNA gene was aligned with closely related sequences using CLUSTAL_X Windows interface and edited manually [21]. Neighbour-joining analysis was done with Kimura 2-parameter model, using TREECON; version 1.3b [22,23]. The stability among the groupings of phylogenetic tree was assessed by taking 1000 replicates. For J-strain *Kytococcus sedentarius* (X87755) was used as an out-group. For BS-P strain, *Halomonas elongate* (M93355) was used as an out-group. Strain J formed a clade with *Kocuria turfanesis* Ho-9042T. Strain BS-P formed a clade with *Pseudomonas aeruginosa* LMG 1242T. Based on nucleotide homology and phylogenetic analysis, the biosurfactant producing BS-J and BS-P strains were identified as *Kocuria turfanesis* and *Pseudomonas aeruginosa* at IMTECH, Chandigarh, India, and MTCC allotted numbers are 10635 and 10636, respectively.

Statistical analysis

Statistical analysis was performed using the Sigma Plot 11 software program (Systat Software Inc., San Jose, USA). The data were analyzed by analysis of variance (ANOVA). All values are given the mean of four replicates \pm standard deviation.

Results and Discussion

Collection of distillery and other liquid wastes for biosurfactant production

Industrial wastes such as distillery waste and curd whey are the viable alternative sources for biosurfactant production and it has been demonstrated that distillery waste cannot be used as such in its original

state as complete fermentation medium without dilution with water in 1:3 proportion due to the presence of large amounts of sulphate ions and potash in the waste, which inhibits the growth of biosurfactant producing microbial cultures [14]. Therefore, present study was carried out with an aim to replace the use of precious water by some appropriate industrial waste, so that distillery waste can be used as no-cost medium for biosurfactant production after combination with industrial wastes instead of water. To evaluate a suitable combination of distillery waste with other industrial wastes for biosurfactant production, distillery waste and other different types of waste waters, viz. sugar industry effluent, curd whey (lactic acid whey), and fruit processing waste were collected from different industries during winter season in the month of December because of plenty availability of milk, variety of fruits and sugar cane which are utilized for industrial production of curd, jam and jellies and cane sugar, respectively (Table 1).

Physico-chemical characteristics of distillery and other liquid wastes used for biosurfactant production

Results presented in Table 1 show that distillery waste had high COD, sugar and nitrogen levels as compared to curd whey (lactic acid whey), followed by fruit processing waste and sugar industry effluent. Although, these wastes had sufficient organic load required for biosurfactant producers to grow, however, suitability of these individual wastes for biosurfactant production was assessed by studying the growth profile of biosurfactant producing isolates and their biosurfactant production potential in each waste. Results have shown that wastes from fruit processing industry and sugar factory had comparatively lower COD, sugar and nitrogen levels and hence were less suitable for biosurfactant production. These two wastes then can be used to combine distillery waste in a specific ratio and such a reconstitution can avoid the use of precious water required for biosurfactant production from distillery waste as no-cost fermentation medium. Moreover, dilution of distillery waste with sugar industry effluent will be more desirable as the waste generated from distillery and sugar factory are situated near to each other can be utilized as nutrient medium for biosurfactant production as a resource without input of transportation cost for supplying sugar industry effluent to biosurfactant production site at distillery unit. Other wastes such as curd whey can be transported to the biosurfactant production site without using any special cryo-preservation techniques because continued fermentation of curd whey by indigenous lactic acid bacteria of curd will lead to more production of lactic acid which is a preferred substrate for biosurfactant production since the biosurfactant producers used in this study are efficient utilize lactic acid for growth [20]. Curd whey is generated during the preparation of Chakka which is an intermediate product obtained by draining of curd (a type of fermented milk from lactic acid bacteria) for preparation of "Shrikhand", a well known popular Indian dessert. During preparation of 1 kg Chakka, 9 litre of lactic acid whey is generated [24]. During winter season different types of fruits such as apples, oranges, pineapple, and pomegranate are available in plenty and are used in preparation of mixed fruit jam from these fruits leading to generation of fruit processing waste, which can be used for biosurfactant production. Logistics for availability of these wastes throughout the year has to worked out before putting up a pilot plant at distillery unit for production of biosurfactants from no-cost industrial wastes.

Isolation and screening of efficient microbial isolates used for biosurfactant production from different industrial wastes

In order to form a basis for using no-cost wastes as potential

alternative fermentative medium for biosurfactant production, present study focused on the use of industrial wastes such as distillery waste, curd whey, sugar industry waste and fruit processing waste as nutrient medium for biosurfactant production by new microbial isolates. For isolation and screening of biosurfactant producing microorganisms, the phenomenon of reduction of surface tension of the culture medium and emulsification index was selected as described earlier [14]. Based on the results of these screening criteria, four isolates designated as BS-A, BS-J, BS-K and BS-P, were screened from 23 different types of microbial cultures obtained from lube oil and distillery spent wash contaminated soil which was collected from spent wash pumping device of a distillery unit (data not shown). These four isolates designated as BS-A, BS-J, BS-K and BS-P were used for further studies on biosurfactant production from distillery waste and other industrial wastes and in their combinations.

Biosurfactant production potential of microbial isolates in different industrial wastes

The success of biosurfactant production depends on the development of cheaper processes and the use of low-cost raw materials, which account for 10-30% of the overall cost [25]. A great variety of agro-industrial wastes have been studied as potential substrates for biosurfactant production. A by-product of the sugar cane industry, fruit processing industry, whey wastes represents an alternative medium for the biosurfactant production process as these have no-cost as compared to other known substrate sources, and they possess valuable nutrients required for the fermentation process. These wastes can therefore, be used to dilute the distillery waste as proposed in the present study. By doing so, there will be improvements in the nutrient content of the distillery waste and this approach can provide a better solution in improving the strategies of biosurfactant production from distillery waste as substrate instead of using precious water for diluting distillery waste which was reported earlier [14,15,26]. Results presented in Table 2 show the biosurfactant production potential of the new microbial isolates from different types of waste waters as fermentative medium used in the present study. Results have shown that the isolates having accession No. as BS-A, BS-J, BS-K, and BS-P showed variations in regard to biosurfactant production capacities in different types of waste waters tested. This is evident by production of stable foam formation in the fermented distillery and curd whey waste by all the four isolates that lasted up to 2 h of standing. However, in sugar industry effluent and fruit processing waste, isolate BS-P could only produce stable foam formation, whereas in case of isolates BS-A, BS-J and BS-K, foam collapsed within 60 min of standing. All the four isolates reduced the surface tension of the fermented distillery waste and curd whey wastes from an initial range of 56 ± 1.3 - 58 ± 1.0 mN/m to 27 - 29 mN/m indicating the production of effective biosurfactant and also showed good emulsification property as the emulsification index E24 was in the range of 40 ± 0.8 - 55 ± 1.5 % was obtained. Biosurfactant yield produced by isolates in the four different wastes was in the range of 0.004 ± 0.0003 - 1.631 ± 0.0045 g/l. Results have shown that all the four isolates produced highest yields of biomass and biosurfactant in curd whey followed by distillery waste, fruit processing waste and sugar industry effluent. This is owing to the fact that curd whey is a very rich source of organic carbon, nitrogen and minerals like calcium, phosphorus, potassium, sodium, copper and iron. It is also a good source of vitamins of B complex group viz. riboflavin and pantothenic acid that are readily available for the growth of biosurfactant producing isolates [27]. Low yields of biomass and biosurfactant in sugar industry effluent and fruit processing waste is owing to the low COD of 1052 ± 2

Parameters	Industrial waste	Control	Microbial isolates			
			BS-A	BS-J	BS-K	BS-P
Foaming	Distillery waste	-ve	+ve	+ve	+ve	+ve
	Curd Whey	-ve	+ve	+ve	+ve	+ve
	Sugar industry effluent	-ve	-ve	-ve	-ve	+ve
	Fruit processing waste	-ve	-ve	-ve	-ve	+ve
Emulsification index (%)	Distillery waste	-	43±0.8	47±0.8	40±0.8	54±0.8
	Curd Whey	-	50±2.4	51±2.2	49±1.6	55±1.5
	Sugar industry effluent	-	20±3.3	23±0.8	22±2.4	30±2.8
	Fruit processing waste	-	35±0.8	37±2.4	36±2.4	40±2.2
Surface tension (mN/m)	Distillery waste	58±1.0	27±0.0	27±0.0	29±1.3	27±0.0
	Curd Whey	56±1.3	27±0.0	27±0.0	27±0.0	27±0.0
	Sugar industry effluent	62±5.7	39±0.8	37±0.8	38±0.8	35±0.8
	Fruit processing waste	60±1.0	29±0.8	28±0.0	29±0.8	27±0.0
pH	Distillery waste	7.0±0.0	8.7±0.0	8.7±0.0	8.9±0.0	8.9±0.0
	Curd Whey	7.0±0.0	8.4±0.0	8.6±0.0	8.9±0.0	9.0±0.0
	Sugar industry effluent	7.0±0.0	6.5±0.0	5.9±0.1	5.7±0.0	6.4±0.1
	Fruit Processing waste	7.0±0.0	7.8±0.1	7.5±0.3	7.9±0.0	7.5±0.0
Biomass yield (c.f.u./ml)	Distillery waste	12±1x10 ²	57±4x10 ⁸	38±2x10 ⁸	55±3x10 ⁷	66±4x10 ⁸
	Curd Whey	12±2x10 ²	85±3x10 ⁸	83±3x10 ⁸	79±2x10 ⁷	98±1x10 ⁹
	Sugar industry effluent	12±2x10 ²	26±3x10 ⁴	33±2x10 ⁴	72±4x10 ⁴	82±2x10 ⁴
	Fruit Processing waste	12±4x10 ²	66±2x10 ⁴	53±5x10 ⁴	70±3x10 ⁴	96±1x10 ⁵
COD (mg/L)	Distillery waste	30880 ±24	18110 ±33	20899 ±25	24520 ±41	20000 ±39
	Curd Whey	37250 ±456	19320 ±38	19340 ±45	20091 ±58	21008 ±65
	Sugar industry effluent	1052±2	678±5	549±2	556±4	659±5
	Fruit Processing waste	2108±4	780±10	890±4	798±8	653±3
Biosurfactant yield (g/l)	Distillery waste	0.001± 0.0002	0.648± 0.0017	0.575± 0.0014	0.696± 0.0051	1.421± 0.0037
	Curd Whey	0.001± 0.0002	0.786± 0.0004	0.590± 0.0004	0.876± 0.0022	1.631± 0.0045
	Sugar industry effluent	0.001± 0.0002	0.006± 0.0004	0.005± 0.0006	0.004± 0.0003	0.008± 0.0001
	Fruit Processing waste	0.001± 0.0002	0.008± 0.0004	0.006± 0.0002	0.006± 0.0005	0.010± 0.0006

Mean ± Standard deviation; n=4

Table 2: Variations in biosurfactant production potential and other related parameters of the new microbial isolates from distillery waste, and other industrial waste as fermentation medium (After 120 h of incubation).

mg/l and 2108 ± 4 mg/l, respectively and low levels of nutrients such as sugars, nitrogen, and phosphate in the wastes. Reductions in COD and total nitrogen, sugars, and phosphate contents were observed in each of the waste which indicates decrease in pollution load of the waste during biosurfactant production.

Biosurfactant production by the microbial isolates in the combination of distillery waste with other industrial wastes

This is a first report on use of combination of distillery waste with other industrial wastes such as curd whey, sugar industry effluent and fruit processing waste for biosurfactant production by new microbial isolates. Our research focused on using distillery spent wash, a type distillery waste because it is one of the most complex, troublesome and strongest organic industrial effluents, having a chemical oxygen demand (COD) value of 80,000-100,000 mg/l and 40,000-50,000 mg/l biological oxygen demand (BOD) and currently, about 40.72 million m³ of spent wash is generated annually from distilleries alone in India. Increased demand for alcohol for applications in pharmaceuticals, food, perfumery industries and recently as an alternate fuel has increased the amounts generated of this waste. The ever increasing amount of distillery spent wash and its disposal problems has stimulated the need for developing new technologies to reduce the pollutant load of the waste partially to some extent and economically use it for producing biosurfactants which are of tremendous industrial importance. The world's total annual production of alcohol from sugarcane molasses is more than 13 million m³ and the spent wash generated is approximately 12-15 times by volume of product alcohol [28]. Due to high concentration of organic load, distillery spent wash is a potential source of renewable energy. It contains high amount of nutrients such as nitrogen, phosphorous, potassium, sulphur and a large amount of micronutrients necessary for microbial growth and proliferation.

In the present study following two different sets of combinations of distillery waste tested were i. Distillery waste combined with whey and sugar industry effluent (DW+WW +SIE) in 1:1:1 ratio and ii. Distillery waste combined with whey and fruit processing waste (DW+WW+FPW) in 1:1:1 ratio. The main aim of combining distillery waste with other industrial wastes was firstly, to nullify/replace the use of precious water that was previously required for diluting distillery waste by combining it with other different types of wastes used in the present study before using it as substrate for biosurfactant production and secondly, combinations of wastes can lead to improved nutritional status of distillery waste which can be beneficial for improved biosurfactant production.

To make biosurfactant production process economical, some researchers followed an approach of using mixed substrates as by utilizing the capability of *Candida bombicola* to produce sophorolipid biosurfactant when grown in medium composed of two different carbon sources and a nitrogen source. One of the carbon sources was a readily available sugar to maximize biomass production and the second was sunflower oil and they were able to achieve 120 g/l sophorolipid in 8 days under the best operational conditions [29]. Strain *Pseudomonas aeruginosa* 47T2 produced 2.7 g/l of rhamnolipid with a production yield of 0.34 g/g with waste frying cooking oil (sunflower and olive oil) as substrates [30]. In an effort to economize biosurfactant production, Rau et al., (2001) used oleic acid or rapeseed oil respectively, as additional carbon sources in addition to glucose in an optimized feed-batch and continuous cultivations and with these substrates more than 300 g/l sophorolipid with increased productivities of 57 g/l/d (feed-batch) and 76 g/l/d (continuous mode), respectively, were produced

under optimized cultivation conditions [31]. Apart from studies where lipidic vegetable oils alone or mixed with other vegetable oils or other water insoluble carbon sources that are highly power intensive substrate used for biosurfactant production, researchers are now looking at more economic process of using wastes having water soluble nutrients especially carbon source so that the biosurfactant production process becomes less power intensive. By keeping this in view, in the present study, no lipidic waste was used, instead a combination of distillery waste with curd whey and sugar industry effluent was used, which resulted into a good combination of nutrient source containing total sugars (5000 mg/l), nitrogen (700 mg/l) and phosphates (200 mg/l) for biosurfactant producing organisms.

Biosurfactant production by different isolates in distillery waste combined with curd whey and sugar industry effluent in 1:1:1 ratio:

Results presented in Table 3 highlights that biomass yield increased in the range of $90 \pm 5 \times 10^7$ c.f.u./ml to $68 \pm 4 \times 10^9$ c.f.u./ml from an initial inoculum size of $12 \pm 1 \times 10^2$ c.f.u./ml indicating nearly more than four folds increase in the number of each isolate that was used for biosurfactant production within 120 h of fermentation. In the previously reported study, where distillery waste was diluted with water in 1:3 ratio resulted in maximum cell counts of *Pseudomonas aeruginosa* strain BS2 i.e. 54×10^8 c.f.u./ml from an initial inoculum size of 1×10^5 c.f.u./ml which is comparatively lower than that obtained on using combination of distillery waste with curd whey and sugar industry effluent [14]. Combination of distillery waste with curd and sugar industry effluent has also resulted in highest biosurfactant yield of 1.86 ± 0.01 g/l in case of isolate BS-P as compared to other isolates which is 23.5% higher than the yield produced in distillery waste diluted with tap water. As compared to our earlier reported yield of biosurfactant (0.91 g/l) produced by *Pseudomonas aeruginosa* strain BS2 in 1:3 diluted distillery waste, there is 50% increase in the yield of biosurfactant production by an isolate BS-P which is a new strain used in the present study. On using combination of distillery with whey and sugar industry effluent, isolates BS-A, BS-J, and BS-K also showed the improvements in the biosurfactant yield in the range of 18.2-26.9%. It was observed that combination of distillery waste with whey and sugar industry effluent has improved the biomass and biosurfactant yields produced by all the four microbial isolates. There was increase in the pH of the combined waste from 7.0 to a range of 8.7 to 8.9 indicating that there was not much variation in the rise in the pH of the combined waste during biosurfactant production by different isolates. The COD of the combined waste decreased in the range of 76.0 ± 1.31 - $82.4 \pm 0.90\%$ and it was found that the biosurfactant high yielding strains were also efficient in reducing the COD of the waste. During biosurfactant production, these isolates depleted the level of total sugars in the combined waste in the range of 79.2 ± 0.87 - $86.2 \pm 0.91\%$ and nitrogen and phosphate levels depleted in the range of 58.42 ± 0.24 - 71.28 ± 0.88 and 48.0 ± 0.24 - $59.5 \pm 0.37\%$, respectively. It was noticed that among the four isolates tested, isolate BS-A resulted in maximum reduction in levels of COD, total sugars, nitrogen and phosphate during biosurfactant production in the combined waste. However, it could result in lower biosurfactant yield. This result suggest that such a strain can be explored to have twin benefit simultaneously i.e. for waste treatment option and it can also work well for biosurfactant production from industrial wastes, however, after improving the strain after genetic manipulation for high yields. This study has shown that the new microbial isolates viz. BS-J and BS-P are comparatively promising strains, better than the previously reported culture *Pseudomonas aeruginosa* strain BS2 for

Parameters	Control	BS-A	BS-J	BS-K	BS-P
pH	7.0±0.0	8.7±0.0	8.7±0.0	8.9±0.1	8.9±0.0
Biomass yield (c. f. u. /ml)	12±1x10 ²	94±4x10 ⁸	89±6x10 ⁸	90±5x10 ⁷	68±4x10 ⁹
Surface tension (mN/m)	61±5.2	27±0.0	27±0.0	28±0.0	27±0.0
Emulsification index (%)	-	47±0.8	52±0.8	34±0.8	58±1.4
Biosurfactant yield (g/l)	0.00±0.00	0.79±0.01 (18.2%)*	0.79±0.00 (26.9%)*	0.90±0.01 (22.3%)*	1.86±0.01 (23.5%)*
% reduction in COD	0.0±0.00	82.4±0.90	80.5±0.41	76.0±1.31	80.5±0.78
Total sugars reduction (%)	0.0±0.00	86.2±0.91	82.6±1.10	79.2±0.87	81.4±0.37
Total nitrogen reduction (%)	0.00±0.00	71.3±0.88	62.3±0.87	58.4±0.24	62.8±0.66
Total phosphate reduction (%)	0.0±0.00	59.5±0.37	57.0±0.49	48.0±0.24	56.5±0.37

Mean ± Standard deviation; n=4

*values presented in the parentheses indicates the percent rise in the yield of biosurfactant production by the respective isolates by replacing 1:3 diluted distillery waste with combination of distillery waste, curd whey and sugar industry effluent in 1:1:1 ratio

Table 3: Biosurfactant production by different isolates in distillery waste combined with curd whey and sugar industry effluent in 1:1:1 ratio (After 120 h of incubation).

biosurfactant production from distillery waste as they have an potential to grow well and produce biosurfactant with higher production capacities in the distillery waste alone as well as in combination of distillery waste with whey and sugar industry effluent. There are reports on utilization of commercial sugar, sugarcane juice and cane molasses, sugarcane juice alcohol stillage, glycerol, mannitol, and soybean oil for production of biosurfactant by *Bacillus subtilis* ATCC 6633 indicating the feasibility to produce biosurfactants resulting in lower surface tension and higher emulsification indexes from a renewable and low-cost carbon source. Daverey and Pakshirajan, (2009) used combination of sugarcane molasses and three different oils (soybean, sunflower or olive oil) as a low cost media for the production of sophorolipids (SLs) from the yeast *Candida bombicola* that resulted biosurfactant yield of approximately 24 g/l in mixed media in comparison to media with single constituents and yield was comparable to the costly conventional synthetic medium containing yeast extract, urea, soybean oil and glucose [32]. Mixture of whey concentrate and rapeseed oil as substrate has resulted high yields of sophorolipids production [33]. There are also reports on use of molasses in combination with cheese whey for fermentative production of biosurfactant by *Lactococcus lactis* 53 and *Streptococcus thermophilus* which resulted 1.2-1.5 times increase in the mass of produced biosurfactant per gram cell dry weight with 75% cost reduction indicating that supplemented cheese whey and molasses media can be used as a relatively inexpensive and economical alternative to synthetic media for biosurfactant production by these probiotic bacteria [25]. These studies suggest that conventional medium containing glucose has been very well replaced with the present low-cost fermentative medium. However, it was also observed from the available information that all these low-cost nutrient media required some addition of costly substrates like glucose, yeast extract, oleic acid for biosurfactant production.

Biosurfactant production by different isolates in distillery waste combined with curd whey and fruit processing waste in 1:1:1 ratio:

Another combination of distillery waste used in the present study for biosurfactant production by the new microbial isolates was with curd whey and fruit processing waste in 1:1:1 proportion. Results

presented in Table 4 indicates that high biomass yield of $87 \pm 6 \times 10^9$ c.f.u./ml was obtained at 120 h of incubation in case of isolate BS-P which correspondingly yielded highest biosurfactant yield of 1.976 g/l indicating that combination of distillery with whey and fruit processing waste is suitable combination of waste for biosurfactant production by isolate BS-P. It was also found that this yield is 28.1% higher than that obtained on using 1:3 diluted distillery wastes for biosurfactant production. However, it was observed that in comparison to 1:3 diluted distillery waste, and among the four isolates tested, isolate BS-J preferred this combination for biosurfactant production which was evident from higher (40.5%) improvements in the biosurfactant yield as compared to that produced by isolate BS-P (28.1%). Isolates BS-A and BS-K could however, resulted 25.9 and 24.6% improvements in the biosurfactant yield on using combination of distillery with whey and fruit processing waste. Improvements in the biosurfactant production on using combined distillery waste was also evident from the improvement in the emulsification indices of the corresponding cell free fermented waste (48.1 ± 0.36 to $67.0 \pm 0.45\%$). It was also observed that the pH of the combined waste varied from 8.1 to 8.8 during biosurfactant production by different isolates. This is due to the alkaline nature of the biosurfactant produced by the different isolates in combined waste. The COD of the combined waste before fermentation was 38890 mg/l which reduced during biosurfactant production in the range of 80.4 ± 0.30 - $84.2 \pm 0.05\%$. Total sugars, nitrogen and phosphate levels also reduced from an initial levels of 5.3 mg/l, 742 mg/l and 220 mg/l, to a range of 79.4 ± 0.15 - $86.4 \pm 0.02\%$, 59.6 ± 0.08 - $70.4 \pm 0.07\%$ and 45.5 ± 0.02 - $59.1 \pm 0.04\%$, respectively. In combination of distillery waste with curd whey and fruit processing waste also, isolate BS-A could result in highest reduction in the COD, total sugars, nitrogen and phosphate levels during biosurfactant production. On comparing the results of the two combinations of distillery waste studied, it is found that combination of the distillery waste with whey and fruit processing waste in 1:1:1 combination is a better for growth of biosurfactant producing isolates and also for biosurfactant production. George and Jayachandran (2008) reported the use of orange fruit peeling as sole carbon source for rhamnolipid production using *P. aeruginosa* MTCC 2297 [34]. Another substrate which has found use for production of biosurfactants is the byproduct of cashew industry which is important

Parameters	Control	BS-A	BS-J	BS-K	BS-P
pH	7.0±0.0	8.8±0.0	8.6±0.0	8.1±0.0	8.7±0.0
Biomass yield (c. f. u. /ml)	12±1x10 ²	45±3x10 ⁹	56±2x10 ⁹	55±2x10 ⁸	87±6x10 ⁹
Surface tension (mN/m)	59±0.0	27±0.0	27±0.0	28±0.0	27±0.0
Emulsification index (%)	-	48.1±0.36	52.0±0.30	49.0±0.20	67.0±0.45
Biosurfactant yield (g/l)	0.001±0.0002	0.875±0.0025 (25.9%)*	0.967±0.0035 (40.5%)*	0.923±0.0025 (24.6%)*	1.976±0.0000 (28.1%)*
% reduction in COD	0.0±0.00	84.2±0.05	81.6±0.12	80.4±0.30	82.5±0.20
Total sugars reduction (%)	0.0±0.00	86.4±0.02	82.3±0.03	79.4±0.15	83.5±0.03
Total nitrogen reduction (%)	0.0±0.00	70.4±0.07	63.6±0.04	59.6±0.08	68.9±0.11
Total phosphate reduction (%)	0.0±0.00	59.1±0.04	55.5±0.04	45.5±0.02	55.9±0.10

Mean ± Standard deviation; n=4

*values presented in the parentheses indicates the percent rise in the yield of biosurfactant production by the respective isolates by replacing 1:3 diluted distillery waste with combination of distillery waste, curd whey and fruit processing waste in 1:1:1 ratio

Table 4: Biosurfactant production by different isolates in distillery waste combined with curd whey and fruit processing waste in 1:1:1 ratio after 120 h of incubation.

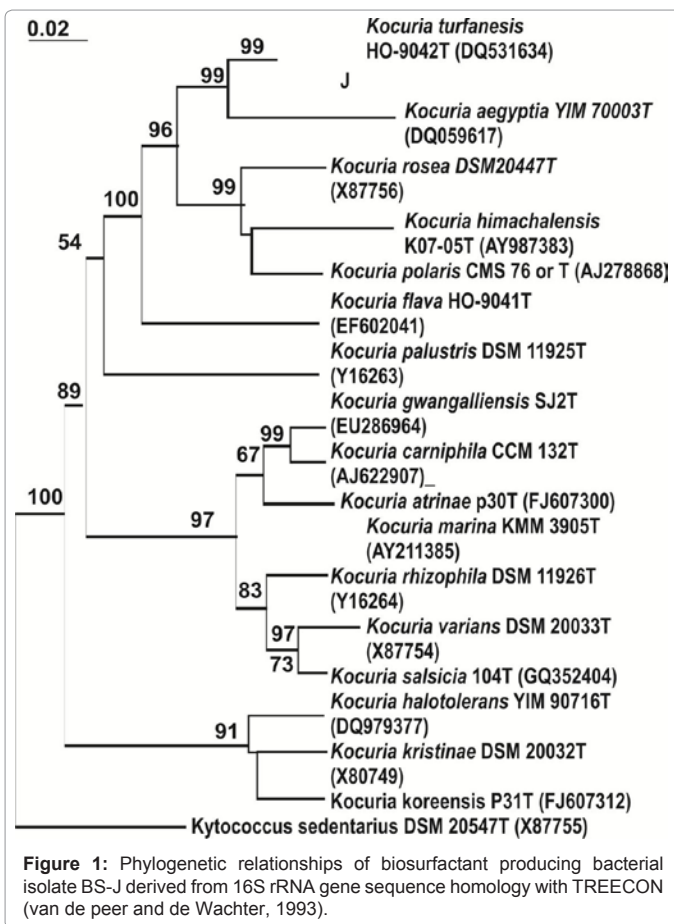
Tests	Isolate BS-J	Isolate BS-P
Morphological tests:		
Colony morphology		
Configuration	Circular	Circular
Margin	Regular	Regular
Elevation	Flat	Flat
Surface	Smooth	Smooth
Pigment	Orange	Light Green
Opacity	Opaque	Translucent
Cell Morphology		
Gram's reaction	+	-
Shape	Coccus	Rods
Size (µm)	0.5-1 µm	2-3 µm
Arrangement	Bunches	Scattered
Spore (s)	+	-
Position	central	-
Shape	round	-
Sporangia bulging	Bulged	-
Motility	-	+
Physiological tests:		
Growth at temperatures		
4° C	+	-
10° C	+	-
20° C	+	+
30° C	+	+
37° C	+	+
42° C	+	+
55° C	-	-
Growth at pH		
pH 4.0	-	-
pH 5.0	-	-

pH 6.0	+	+
pH 7.0	+	+
pH 8.0	+	+
pH 9.0	+	+
pH 10.0	+	+
pH 11.5	+	+
Growth on NaCl (%)		
2.0	+	+
4.0	+	+
6.0	+	+
8.0	+	+
10.0	+	+
Growth under anaerobic condition		
	-	-
Biochemical tests:		
Growth on MacConkey	-	+ (NLF)
Indole test	-	-
Methyl red test	-	-
Voges Proskauer test	-	-
Citrate utilization	-	+
H ₂ S production	-	+
Gas production from glucose	-	-
Casein hydrolysis	-	+
Esculin hydrolysis	-	+
Gelatin hydrolysis	-	+
Starch hydrolysis	(+)	-
Urea hydrolysis	+	+
Nitrate reduction	+	-
Catalase test	+	+
Oxidase test	-	+

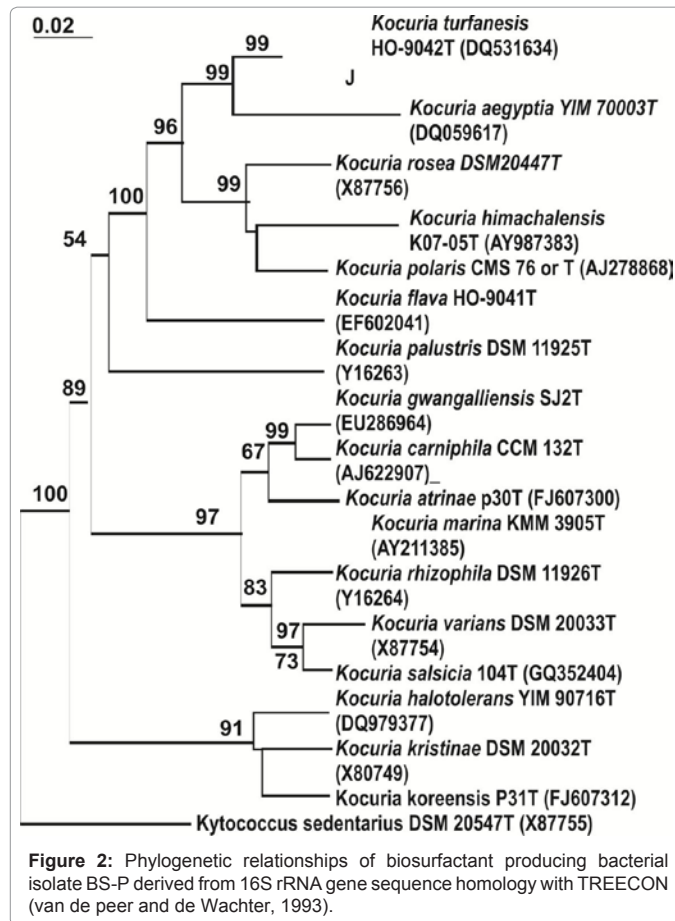
Ornithine decarboxylase	-	-
Lysin decarboxylase	-	-
Tween 20 hydrolysis	-	(+)
Tween 40 hydrolysis	-	(+)
Tween 60 hydrolysis	-	-
Tween 80 hydrolysis	-	-
Acid production sugars		
Dextrose	+	+
Lactose	-	-
Sucrose	-	-

+: Positive; -: Negative; (+): Weak positive; NLF: Lactose Fermenting

Table 5: Morphological, physiological and biochemical characteristics of efficient biosurfactant producing isolates.



in Brazil. Cashew apples are rich in reducing sugar, vitamins and minerals salts and are cheap (US \$ 0.50/kg) which makes them an interesting and inexpensive culture medium [35]. Study on the ability of *Pseudomonas aeruginosa* to produce biosurfactants using Cashew Apple Juice (CAJ) and mineral media supplemented with peptone and nutrient broth has shown that there was effective reduction in surface tension of the medium which indicated that CAJ could be used as medium for growth and biosurfactant production [36]. In various combination of supplementation tested, utilization of Mineral



Medium Containing Clarified Cashew Apple Juice (MMCAJC) for biosurfactant production by *Bacillus subtilis* LAMI008 strain, favoured highest reduction in surface tension specifically when cultivation on MM-CAJC, supplemented with yeast extract was used as fermentation medium. This facilitated biosurfactant (surfactin) production of 3.5 g/l which exhibited good surface and emulsifying activity [37]. This has lead to ascertain the fact that it was feasible to produce surfactin from CCAJ, a renewable and low-cost carbon source. However, in the present study, comparatively a contrast phenomenon was observed showing that a combination of distillery waste with other different wastes studied did not require any supplementation of costly substrates as components of mineral medium for biosurfactant production by the new microbial isolates. However, more studies are required to overcome the problems associated with batch variability and ways to standardize the pre-treatment requirement of these substrates for more productive output. Further studies are required to work out the best possible combination of distillery waste with curd whey and fruit processing waste. Detailed physico-chemical characterization of biosurfactants produced by new microbial isolates viz. *Kocuria turfanensis* strain BS-J and *Pseudomonas aeruginosa* strain BS-P in the combined distillery waste is being carried out to ascertain novelty of the compound.

Identification of efficient biosurfactant producing microbial isolates

Based on morphological, biochemical, physiological characteristics presented in Table 5, the efficient biosurfactant producing isolates BS-J and BS-P were identified as *Kocuria turfanensis* and *Pseudomonas*

aeruginosa, respectively. Both of these biosurfactant producing isolates are the new strains as revealed by 16S rRNA sequence pattern and phylogenetic tree analysis. For isolates BS-J and BS-P *Kytococcus sedentarius* (X87755) and *Halomonas elongate* (M93355) were used as an out-groups, respectively. Strain-J formed a clade with *Kocuria turfanesis* Ho-9042T (Figure 1). Strain BS-P formed a clade with *Pseudomonas aeruginosa* LMG 1242T (Figure 2). Based on nucleotide homology and phylogenetic analysis carried out at IMTECH, Chandigarh, India, the biosurfactant producing BS-J and BS-P strains were identified as *Kocuria turfanesis* and *Pseudomonas aeruginosa*, respectively and isolates BS-J and BS-P were catalogued as MTCC 10635 and MTCC 10636, respectively.

Conclusions

Combination of distillery waste with other industrial wastes was assessed in order to constitute more nutritionally rich no-cost complete medium for biosurfactant production by replacing the use of precious water which was earlier reported for diluting distillery waste in 1:3 proportion. Individual wastes alone have resulted in high yields of biosurfactants by new efficient microbial isolates viz. *Kocuria turfanesis* strain BS-J and *Pseudomonas aeruginosa* strain BS-P. Biosurfactant yield further improved by using combination of distillery waste with other wastes viz. whey, fruit processing waste/sugar industry effluent in 1:1:1 proportion. This indicates a positive impact of waste combination on biosurfactant production capacities of the new microbial isolates and replacement of precious water with other wastes required for diluting distillery waste for biosurfactant production.

Acknowledgement

Authors acknowledge University Grants Commission (UGC), New Delhi, India for financial support to carry out the work presented in this paper.

References

- Makkar RS, Cameotra SS, Banat IM (2011) Advances in utilization of renewable substrates for biosurfactant production. AMB Express 1: 5.
- Cerqueira VS, Hollenbach EB, Maboni F, Vainstein MH, Camargo FA, et al. (2011) Biodegradation potential of oily sludge by pure and mixed bacterial cultures. Bioresour Technol 102: 11003-11010.
- Brown LR (2010) Microbial enhanced oil recovery (MEOR). Curr Opin Microbiol 13: 316-320.
- Juwarkar AA, Nair A, Dubey KV, Singh SK, Devotta S (2007) Biosurfactant technology for remediation of cadmium and lead contaminated soils. Chemosphere 68: 1996-2002.
- Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, et al. (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87: 427-444.
- Nitschke M, Costa SGVAO, Contiero J (2011) Rhamnolipids and PHAs: Recent reports on *Pseudomonas* -derived molecules of increasing industrial interests. Process Biochem 46: 621-630.
- Pacwa-Plociniczak M, Plaza GA, Piotrowska-Seget Z, Cameotra SS (2011) Environmental applications of biosurfactants: recent advances. Int J Mol Sci 12: 633-654.
- Das K, Mukherjee AK (2007) 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 Biochemistry 42: 1191-1199.
- Reis RS, Pereira AG, Neves BC, Freire DM (2011) Gene regulation of rhamnolipid production in *Pseudomonas aeruginosa* - a review. Bioresour Technol 102: 6377-6384.
- Bognolo G (1999) Biosurfactants as emulsifying agents for hydrocarbons. Colloids Surf A Physicochem Eng Asp 152: 41-52.
- Cameotra SS, Makkar RS (1998) Synthesis of biosurfactants in extreme conditions. Appl Microbiol Biotechnol 50: 520-529.
- Kosaric N (1992) Biosurfactants in industry. Pure and Applied Chemistry 64: 1731-1737.
- Nitschke M, Costa SG, Contiero J (2010) Structure and application of a rhamnolipid surfactant produced in soybean oil waste. Appl Biochem Biotechnol 160: 2066-2074.
- Dubey K, Juwarkar A (2001) Distillery and curd whey wastes as viable alternative sources for biosurfactant production. World J Microbiol Biotechnol 17: 61-69.
- Dubey KV, Juwarkar AA, Singh SK (2005) Adsorption-desorption process using wood-based activated carbon for recovery of biosurfactant from fermented distillery wastewater. Biotechnol Prog 21: 860-867.
- Cooper DG, Zajic JE (1980) Surface-Active Compounds from Microorganisms. Adv Appl Microbiol 26: 229-253.
- APHA, AWWA, WPCF (1989) Standard Methods for the Examination of Water and Wastewater, (Seventeenth ed) New York, USA.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350-356.
- Cappuccino JG, Sherman N (2010) Microbiology: A laboratory manual. Addison-Wesley Longman, Inc., Harlow, England, 199-204.
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams T (1994) Bergey's Manual of Determinative Bacteriology (9th edn) Williams & Wilkins, Baltimore.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-4882.
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120.
- Van de Peer Y, De Wachter R (1997) Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput Appl Biosci 13: 227-230.
- Bandhoopadhy AK, Mathur BN (1987) Indian milk products: a compendium. Dairy India 211-218.
- Rodrigues L, Moldes A, Teixeira J, Oliveira R (2006) Kinetic study of fermentative biosurfactant production by *Lactobacillus* strains. Biochem Eng J 28: 109-116.
- Dubey K, Juwarkar A (2004) Determination of genetic basis for biosurfactant production in distillery and curd whey wastes utilizing *Pseudomonas aeruginosa* strain BS2. Indian J Biotechnol 3: 74-81.
- Nickerson TK (1974) Lactose: Fundamentals of Dairy Chemistry. (Edited) Westport, Connecticut A VI, 273-324.
- Mohana S, Acharya BK, Madamwar D (2009) Distillery spent wash treatment technologies and potential applications. J Hazard Mater 163: 12-25.
- Casas JA, Garcia-Ochoa F (1999) Sophorolipid production by *Candida bombicola*: medium composition and culture methods. J Biosci Bioeng 88: 488-494.
- Haba E, Espuny MJ, Busquets M, Manresa A (2000) Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. J Appl Microbiol 88: 379-387.
- Rau U, Hammen S, Heckmann R, Wray V, Lang S (2001) Sophorolipids: a source for novel compounds. Ind Crops Prod 13: 85-92.
- Daverey A, Pakshirajan K (2009) Production, characterization, and properties of sophorolipids from the yeast *Candida bombicola* using a low-cost fermentative medium. Appl Biochem Biotechnol 158: 663-674.
- Daniel HJ, Otto RT, Reuss M, Sylidat C (1998) Sophorolipid Production with

- High Yields on Whey Concentrate and Rapeseed Oil without Consumption of Lactose. *Biotechnol Lett* 20: 805–807.
34. George S, Jayachandran K (2009) Analysis of rhamnolipid biosurfactants produced through submerged fermentation using orange fruit peelings as sole carbon source. *Appl Biochem Biotechnol* 158: 694–705.
35. Rabelo MC, Fontes CP, Rodrigues S (2009) Enzyme synthesis of oligosaccharides using cashew apple juice as substrate. *Bioresour Technol* 100: 5574–5580.
36. Rocha MV, Souza MC, Benedicto SC, Bezerra MS, Macedo GR, et al. (2007) Production of biosurfactant by *Pseudomonas aeruginosa* grown on cashew apple juice. *Appl Biochem Biotechnol* 137-140: 185–194.
37. Ponte Rocha MV, Gomes Barreto RV, Melo VM, Barros Gonçalves LR (2009) Evaluation of cashew apple juice for surfactin production by *Bacillus subtilis* LAMI008. *Appl Biochem Biotechnol* 155: 366–378.

This article was originally published in a special issue, **Microbial Biosurfactants/Marine Microbial Biosurfactants** handled by Editor(s). Dr. Thavasi Renga Thavasi, Polytechnic Institute of New York University, USA