Research Paper

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Biosurfactant Production by New Microbial Isolates From Soil Co- Contaminated With Lube Oil and Distillery Spent Wash

Science

KEYWORDS : Biosurfactant; Distillery waste; Emulsification index, Surface tension

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ABSTRACT

Present work presents utilization of industrial wastes such as distillery spent wash, curd whey, fruit processing waste and sugar industry effluent for cost-effective production of biosurfactant by four new microbial isolates designated as BS-A, BS-J, BS-K and BS-P isolated from soil sample contaminated with lube oil and distillery spent wash) collected from a distillery unit. These isolates have the potential to produce biosurfactant from mineral salt medium and also from individual wastes viz. distillery waste, sugar industry effluent, fruit processing waste and curd whey waste. Results have shown that highest biomass and biosurfactant yields were obtained in curd whey followed by distillery waste, fruit processing waste and sugar industry effluent by all the four isolates. The surface tension of the fermented wastes reduced from an initial range of 56-60 mN/m to 27-39 mN/m. The fermented wastes showed good emulsification property and the emulsification index (E24) obtained was in the range of 51-54%. Biosurfactant yields obtained from individual wastes were in the range of 0.0043-1.1631 g/l. Reductions in pollutional load of the wastes were observed as total nitrogen, phosphate, and chemical oxygen demand (COD) levels reduced significantly during biosurfactant production by these isolates. This study has shown that these newly isolated biosurfactant producers can find their application in cost-effective production of biosurfactant from different types of industrial wastes without supplementing with costly nutrients.

Introduction

Surfactants and emulsifiers are indispensable components of daily life. They are widely used in the pharmaceutical, cosmetic, petroleum and food industries. Most of the different types of surfactants that are already being used in industry are synthesized chemically and are of petroleum origin. Most of them are toxic to environment, not easily biodegradable and their manufacturing processes and by-products can be environmentally hazardous. Increasing environmental awareness and strict legislations has made environmental compatibility of surfactants an important factor in their application for various industries (Maier and Soberon-Chavez, 2000).

In past few decades, biosurfactant have gained attention because of biodegradability, low toxicity, ecological acceptance and ability to be produced from renewable substrates (Ishigami 1997, Makkar and Cameotra 2002, Maneerat 2005, Mukherjee et al. 2006). Several structurally diverse varieties of surface active molecules are being produced by a wide spectrum of microorganisms (bacteria, fungi and yeast). Biosurfactants have been tested to impart several environmental solutions such as bioremediation and dispersion of pesticides, enhanced oil recovery, and transfer of crude oil through pipelines, and therefore, it is thought to be the potential candidate to replace synthetic surfactants in the future, especially in the food and health care industries, industrial cleaning of oil coated surfaces and in agricultural chemicals (Banat et al., 2000; Makkar and Cameotra 2002, Karanth et al., 1999). In the view of these multifaceted benefits of the microbial surfactants in comparison to synthetic surfactants, it has become an essential prerequisite to develop a cost-effective process technology for biosurfactant production so that application of biosurfactant in environmental remediation can be realized. It has been demonstrated that fermentation medium can represent almost 30% of the cost for a microbial fermentation (Rodrigues et al., 2006). This is feasible by developing a fermentation process which explores use of different types of no-cost industrial wastes as growth medium for microbial production of surfactants combating at the same time their polluting effects that balance the overall costs. If industrial and /or municipal waste waters which contain organic pollutants could be utilized as substrates for biosurfactant production , a double benefit would be obtained: The polluted waters would be treated and valuable product in the form of biosurfactant would result. This approach reduces the cost for wastewater treatment with even a potential of generating a profit through the sale of biosurfactant. Earlier, we have reported various issues pertaining to cost-effective production of biosurfactant from 1:3 diluted distillery waste, developed a new technique of adsorption-desorption process for recovery of di-rhamnolipid biosurfactant from fermented distillery waste and its application in the removal of heavy metals from contaminated soil (Dubey and Juwarkar, 2001, Dubey et al., 2005, Juwarkar et al., 2007 and 2008). We have reported that industrial wastes such as distillery waste and curd whey are the viable alternative sources for biosurfactant production and 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 owing to the presence of large amount of sulphate ions in the waste, which inhibits the growth of biosurfactant producing microbial cultures (Dubey and Juwarkar, 2001).

In this study, we report a comparative account on using different industrial wastes as no-cost medium for production of biosurfactant by four different new microbial isolates. This work was carried out to with an aim to replace the use of costly nutrient medium for biosurfactant production. Moreover, utilization of wastes for biosurfactant production will minimize the pollution problem of distillery waste and other different industrial wastes used in the present study.

This paper describes an attractive and environmentally safe alternative to reduce the pollution problem of distillery waste and other food industry wastes such as curd whey, fruit processing waste and sugar industry effluent with simultaneous recovery of resource in the form of biosurfactants by newly isolated microbial cultures.

Materials and Methods

Collection of industrial waste water for biosurfactant production: For biosurfactant production, distillery spent wash and different types of other waste waters, such as sugar industry effluent, curd whey (lactic acid whey), and fruit processing waste were collected from respective industries (**Table- 1)**. Fresh waste waters were immediately transferred into a deep freezer working at 2° C (Remi Instruments, Vasai, India). Among these wastes, curd whey required following below given steps of processing to remove casein exhaustively from the whey. Curd whey was firstly neutralized with 5N NaOH followed by steaming for 10 minutes. Casein in the form of sodium caseinate so formed settled at the bottom and the supernatant was removed as partially deproteinised whey and it was further filtered through membrane filter and then used in further studies.

Physico-chemical characterization of wastes: Physico-chemical characterization of these wastes was performed before incubation (i.e. as control) and after recovery of biosurfactant as per the standard methods. Total sugars were estimated by phe-

nol-sulphuric acid method of Dubois *et al*., 1956. Total nitrogen was estimated by using semi-micro Kjeldhal method and total phosphate content was analyzed by vanadomolybdo phosphoric acid colorimetric method. COD was estimated by closed reflux titrimetric method (APHA, AWWA, WPCF, 1989).

Isolation of biosurfactant producing microorganism from different sources and screening of an efficient isolate: Biosurfactant producing microorganisms isolates were screened from different sources on the basis of stability of foam, emulsification index, surface tension measurement, quantitative assessment of biosurfactant yield, pH and biomass yield. Soil sample contaminated with lube oil and distillery spent wash present at spent wash pumping device of the a distillery unit was added aseptically to 100 ml mineral salts medium which consisted of (g/l) $NaNO_3$, 2.0; K_2HPO_4 , 1.0; KH_2PO_4, 0.5; $MgSO_4$. $7H_2O$, 0.5; KCl, 0.1 and $FeSO_4.7H_2O$, 0.01 amended with 1.0 % (v/v) waste frying vegetable oil as sole carbon source. The flask was incubated at 37 °C for 8 days at 150 rpm on an orbital shaker (Remi Instruments, Vasai, India) and was visually examined for heavy growth and excessive stable foam formation. The cultures were then enriched in increasing concentrations of waste frying vegetable oil (1.0-5%) by following culture enrichment technique (Finnerty & Singer 1984), and were grown on nutrient agar at 30° C for 24 h. Different types of bacterial colonies obtained on the plates were then purified. Each isolate was initially assessed qualitatively and quantitatively to screen for the efficient isolates which has the capacity to produce biosurfactant in each of the individual waste.

Parameters analyzed for qualitative assessment of biosurfactant production by the isolates were:

i) Foaming : Foam produced by hand shaking of the fermented culture broth for several minutes was observed for its stability for a period of two hours.(Abouseoud *et al. 2007)*.

ii) Emulsification index (E24): E24 of culture samples was determined by adding 5ml of cell free culture broth to 5ml of kerosene, mixing with a vortex for 2 minutes and leaving to stand for 24 hours. The E24 index is given as percentage of height of emulsified layer (mm) divided by total height of the liquid column (Cooper & Goldenberg, 1987).

iii) Surface tension measurement: The surface tension measurements of the cell free supernatant was determined by du Nouy ring detachment method . The values reported are the mean of three measurements. All measurements were made on cell-free broth obtained by centrifuging the culture broths at 8000 rpm for 20 minutes (Dubey & Juwarkar, 2001).

Quantitative assessment of biosurfactant yield: Biosurfactant in the form of brown paste was recovered by diethyl ether extraction method (Ramana and Karanth 1989) and was quantified by using analytical balance (Shimadzu AUW220D, Japan).

pH and Biomass measurements: The pH of the cell free culture broth was measured with a digital pH-meter MK VI (Systronics, Naroda, Ahmedabad). Biomass development of biosurfactant producing different isolates in individual and combined wastes was monitored in terms of c.f.u./ml of fermented wastes by serial dilution and pour plate technique using nutrient agar as the growth medium (Dubey & Juwarkar, 2001).

Studies on biosurfactant production and changes in the physico-chemical characteristics of distillery waste and other different individual waste alone before and after biosurfactant production by different isolates: Distillery waste (diluted with tap water in 1:3 ratio), whey waste, sugar industry effluent and fruit processing waste, 100 ml each taken in 250 ml Erlenmeyer flasks, were sterilized at 121° C and 15 lb/inch^2 pressure for 20 minutes and then were then inoculated with the microbial isolates given accession No. as BS-A, BS-J, BS-K, and BS-P under aseptic conditions. These inoculated flasks were then kept in a gyrorotatory incubator cum shaker for 120 hours and after incubation parameters such as biomass yield in terms of c.f.u./ml, COD reduction (%), Total sugars reduction (%), surface tension reduction and biosurfactant yield (g/l) were determined before and after biosurfactant recovery from wastes. Physico-chemical characterization of these wastes was performed before incubation (i.e. as control) and after recovery of biosurfactant as per the standard methods (APHA, AWWA, WPCF, 1989). Total sugars were estimated by phenol-sulphuric acid method of Dubois *et al*., 1956. Total nitrogen was estimated by using semi-micro Kjeldhal method and total phosphate content was analyzed by vanadomolybdo phosphoric acid colorimetric method.

Results and Discussion

Collection of distillery and other liquid wastes for biosurfactant production and their physico-chemical characteristics:

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 (Rodrigues et al., 2006; Makkar et al., 2002). Therefore, studies were carried out on exploring the potential use of alternative no-cost fermentative medium formulations for biosurfactant production by utilising industrial wastes. Different types of industrial wastes were collected from different sources for biosurfactant production (**Table 1**).

Table. 1:- Types of industrial waste waters collected from-different sources for biosurfactant production

S. No.	Types of waste	Sources of collection
1.	Distillery waste	Purti Sakhar Karkhana limited, Bela, Tal. Umred, Nagpur
2.	Sugar industry effluent	Purti Sakhar Karkhana limited, Bela, Tal. Umred, Nagpur
3.	Curd Whey waste	Amruta Dairy, Sakkardara square, Umred road, Nagpur
4.	Fruit processing waste	Noga factory, MIDC Hingna, Nagpur.

Results of characterization of different wastes presented in **Table 2** show that distillery waste had high COD, BOD, sugar and nitrogen levels as compared to curd whey (lactic acid whey), followed by fruit processing waste and sugar industry effluent which indicates it can be as good nutrient source for micro organisms to grow and produce biosurfactant. However, suitability of these individual wastes for biosurfactant production will be assessed by studying the growth profile of biosurfactant producing isolates and their biosurfactant production potential in each of these collected wastes.

Table: 2. Characteristics of different industrial wastes used for biosurfactant production

Parameters	Types of wastes			
	Distillery wastes (DW)	Whey waste (WW)	Fruit processing waste (FPW)	Sugar industry effluent (SIE)
pH	4.8	4.3	5.4	6.8
COD (mg/l)	98,000	56,000	2100	1050
BOD(mg/l)	37,000	28,000	1090	959
Total sugars (g/l)	12.4	6.8	2.03	1.54
Total N (mg/l)	710.0	987.0	784.0	643.0
Total P (mg/l)	235.0	352.0	122.0	135.0

Variations in Biosurfactant production potential of microbial isolates in different wastes: Results presented in **Table 3** shows variations in the biosurfactant production potential of the different microbial isolates used in the present study in terms of qualitative and quantitative assessment parameters, respectively. Results have shown that all four of the isolates having accession No. as BS-A, BS-J, BS-K, and BS-P showed isolates have different capacities of biosurfactant production. All

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the isolates tested could result in stable foam formation in case of the fermented distillery and curd whey waste, which lasted up to two hours of standing. However, isolates viz. BS-A, BS-J and BS-K, could not produce stable foam formation in sugar industry effluent and fruit processing waste and in contrary isolate BS-P could do so. Reduction of the surface tension of the fermented wastes observed was from a range of 59-64 dynes/cm to 27-39 dynes/cm. The fermented broth showed good emulsification property and the emulsification index E24 was in the range of 51-54%. Biosurfactant yield produced by isolates was in the range of 0.0043-1.631 g/l. Results have also shown that all the four isolates yielded highest yields of biomass and biosurfactant in curd whey followed by distillery waste, fruit processing waste and sugar industry effluent. A reduction in COD was observed in case of individual waste which indicated decrease in pollutional load of the waste during biosurfactant production. Low yields of biomass and biosurfactant in sugar industry effluent and fruit processing waste is owing to the low COD and nutrient status of these wastes.

Table-3. Qualitative and quantitative assessment of biosurfactant production potential of different isolates in different wastes (After 120 hours of incubation).

Parameters	Industrial waste	Control	Microbial isolates			
			BS-A	BS-J	BS-K	BS-P
Qualitative parameters Foaming	DW	-ve	+ve	+ve	+ve	+ve
	WW	-ve	+ve	+ve	+ve	+ve
	SIE	-ve	-ve	-ve	-ve	+ve
	FPW	-ve	-ve	-ve	-ve	+ve
Emulsification index	DW	-	43	47	40	54
	WW	-	50	51	49	54
	SIE	-	20	23	22	30
	FPW	-	35	37	36	40
Surface tension (dynes/cm)	DW	59	27	27	29	27
	WW	56	27	27	27	27
	SIE	64	39	37	38	35
	FPW	60	29	28	29	27
Quantitative Parameters pH	DW	7.0	8.66	8.7	8.86	8.93
	WW	7.0	8.42	8.57	8.89	8.97
	SIE	7.0	6.5	5.9	5.7	6.4
	FPW	7.0	7.8	7.5	7.9	7.5
Biomass yield (c.f.u./ml)	DW	12x102	57x108	38x108	55x107	66x108
	WW	12x102	86x108	83x108	79x107	98x108
	SIE	12x102	26x104	33x104	72x104	82x104
	DW	12x102	66x104	53x104	71x104	96x105
COD (mg/L)	WW	30880	18110	20899	24520	20000
	SIE	37000	19320	19340	20091	21008
	FPW	1052	678	549	556	659
	DW	2108	780	890	798	653
Biosurfactant yield (g/l)	DW	0.0014	0.648	0.575	0.196	1.421
	WW	0.0011	0.7862	0.5897	0.876	1.631
	SIE	0.0011	0.0063	0.0046	0.0043	0.0082
	FPW	0.0012	0.0078	0.0056	0.0064	0.0098

DW-Distillery waste, WW- Curd whey waste, SIE-Sugar industry effluent, FPW-Fruit processing waste

Conclusion:

Four newly isolated bacterial cultures designated as BS-A, BS-J, BS-K and BS-P from soil sample (contaminated with lube oil and distillery spent wash) collected from a distillery unit were evaluated for cost-effective production of biosurfactant from distillery spent wash, curd whey, fruit processing waste and sugar industry effluent. These isolates have the potential to produce biosurfactant from mineral salt medium and also from the individual wastes viz. distillery waste, sugar industry effluent, fruit processing waste and curd whey waste. Results have shown that the yields of biomass and biosurfactant were higher in whey waste followed by distillery waste, fruit processing waste and sugar industry effluent. It was found that during biosurfactant production there was significant reduction in the chemical oxygen demand of the wastes by different isolates. This study has shown that these newly isolated biosurfactant producers can find their application in cost-effective production of biosurfactant from industrial wastes without supplementing the waste with costly nutrients.

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