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Biosurfactants- A Current Perspective on Production and Applications

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ABSTRACT

Biosurfactants are surface active compounds produced by a great diversity of microorganisms. They act by lowering the surface tension at the interfaces of solid, liquid and gases. The chemical surfactants that are practiced on a large commercial basis are toxic to the environment and non-biodegradable. These synthetic compounds may bio-accumulate and also their production, processes and by-products causes various environmental hazards. Due to the rising concern of environmental safety, biosurfactants have gained much importance. Biosurfactants are biodegradable, effective under extreme conditions, and are less toxic; which is advantageous in comparison to their chemical counterparts. In-depth studies have been carried out in the final few decades, bringing out their widespread application in different fields. Nevertheless, practical applications of biosurfactants are limited by their high production cost, and less information about their interactions with cells and the abiotic environment. In this paper, we have reviewed the diverse group of organisms that are capable of producing biosurfactants, use of alternative cheap substrates by these organisms to reduce the production cost and their various applications in the field of environmental remediation and petroleum industry.

INTRODUCTION

Surfactants are surface active agents, i.e. they are compounds that lower the surface tension between different phases such as gas, liquid and solid. Surfactants can be either natural or synthetic and contain various functional groups that impart them specific properties, making them suitable for different industrial and consumer uses. These compounds can be used as detergents, wetting agents, emulsifiers, foaming agents, dispersants, and constitute a major factor of household products as shampoo, personal care products, pharmaceuticals to paints (Das et al. 2013, Subhrasekhar et al. 2013). The worldwide market for surfactants has grown enormously over the past few decades. It has been estimated that there was a global production of 13 million tones of surfactants in 2008 and attained a volume of more than 33 billion US-dollars in 2014 (Reznik et al. 2010). Currently the anionic surfactant alkyl benzene sulfonate (LAS) is widely used for commercial purposes. Although the synthetic/chemical surfactants hold the biggest percentage in the overall surfactants market, some of them are known to be toxic to animals, ecosystems, and humans; and increase the dissemination of other environmental contaminants. For this reason some of the surfactants such as PFOS (Perfluorooctane-sulfonic acid) and PFOA (Perfluorooctanoic acid) have voluntary restrictions on their uses. These stringent regulations have made room for the role of biosurfactants. Biosurfactants are derived from microorganisms and have several advantages over the use of chemical/synthetic surfactants viz., biodegradability, low toxicity, biocompatibility and digestibility, cost effective production, ecological acceptability, and specificity (Kosaric 1992). A few biosurfactants have already been commercialized, mainly in the Far East Asia.

BIOSURFACTANTS- STRUCTURE AND TYPES

Biosurfactants are made up of a hydrophilic moiety, which may be an acid, peptide, cations, anions, mono-, di-, or polysachharides; and a hydrophobic moiety, which may be unsaturated, or saturated hydrocarbon chains, or fatty acids (Banat 2010). These compounds are structurally diverse and can be classified mainly by their chemical nature and microbial origin. They are broadly divided into two classes- low molecular weight molecules which include glycolipids, lipopeptides and flavolipids; high molecular weight molecules such as polysaccharides, proteins, lipopolysaccharides and lipoproteins. The low molecular surface active agents are known as biosurfactants and the high molecular weight agents are bioemulsifiers (Timmis 2010a, Timmis 2010b). Established on their chemical nature, biosurfactants can be further split into six classes: glycolipids, lipopolysachharides, lipoproteins-lipopeptides, fatty acids, neutral lipids and phospholipids, polymeric and particulate biosurfactants (Desai & Banat 1997). Different microbial groups such as yeasts, actinomycetes, filamentous fungi and bacteria have been reported so far as the producers of biosurfactants (Karthik et al. 2010, Grusha et al. 2014).

GLYCOLIPIDS

These are the most common biosurfactants. Structurally they are sugars in combination with long chain aliphatic acids or hydroxyaliphatic acids. The commonly known glycolipids are as follows.

Rhamnolipids: These are the best studied glycolipids. *Pseudomonas aeruginosa* is the most studied potent producer of rhamnolipids. However, *P. aeruginosa* is an opportunistic pathogen. Hence, extensive studies have been executed on non pathogenic strains. Among the non pathogenic strains, *Acinetobacter calcoaceticu*, *Enterobacter hormaechei*, *E. asburiae*, *Streptomyces coelicoflavus* and *S. matensis* have been reported (Rooney et al. 2009, Hoskova et al. 2013, Kalyani et al. 2014).

Trehalolipids: Trehalolipids are the best described surfactants produced by actinobacteria, such as *Gordonia*, *Nocardia*, *Arthrobacter*, *Corynebacterium*, *Micrococcus* and *Tsukamurella* (Franzetti et al. 2010, Christova et al. 2015, Kugler et al. 2015). Among the different species of *Rhodococcus* which have been found to produce trehalose lipids, *R. erythropolis* has been most commonly used as a producer (Desai & Banat 1997). In addition, different species of *Mycobacterium* such as *M. tuberculosis*, *M. bovis*, *M. phlei* and *M. flavescens* are another group of trehalose lipid producers (Kugler et al. 2015). However, toxicity associated with the pathogenic strains of *Mycobacteria* limits their application.

Sophorolipids: The first biosurfactant to become available in the market were sophorolipids. Reports on production of sophorolipids by several yeast species began in the early sixties, including *Candida apicola, C. bombicola*, *Rhodotorula bogoriensis* and *Torulopsis gropengiesseri* (Spencer et al. 1970, Jones 1967). Yeasts produce these biosurfactants as a mixture of several structural forms, for example *C. batistae* produces mainly the acid form, *C. apicola* produces a heterogenous mixture consisting of di-O-acetyl, mono-O-acetyl and non acetyl sophorolipidsin, the free acids and lactone forms, *Wickerhamiella domercqiae* produces more than six sophorolipids (Konishi et al. 2008, Van Bogaert et al. 2007, Chen et al. 2006).

LIPOPEPTIDES AND LIPOPROTEINS

A large number of cyclic lipopeptides, which includes decapeptide antibiotics and lipopeptide antibiotics have been proved to be powerful surface active agents (Gharaei-Fathabad 2011). *Bacillus* and *Pseudomonas* are widely recognized to produce lipopeptide surfactants. *Pseudomonas* sp. produces surfactants as viscosin amide and amphisin. Surfactin is one of the most effective lipopeptide biosurfactant produced by different *Bacillus subtilis* strains (Cooper et al. 1981). Lipopeptide biosurfactant produced by *Bacillus natto* was found to have strong surface activity (Cao et al. 2009). Halophilic bacteria *Kocuria marina* BS-15 was found to produce biosurfactant of the lipopeptide group (Sarafin et al. 2014). Also, actinomycetes such as *Actinomycetes nocardiopsis* (strain A17), *Breviacterium aureum* MSA13 (brevifactin), and *Nocardiopsis alba* MSA 10 have been reported to produce lipopeptide based biosurfactants (Chakraborty et al. 2015, Seghal et al. 2010, Gandhimathi et al. 2009).

FATTY ACIDS, PHOSPHOLIPIDS AND NEUTRAL LIPIDS

Greater amounts of fatty acids and phospholipids are produced by a broad diversity of bacteria and yeast during their growth on n-alkane enriched medium. Bile salts can act as biosurfactants and have been found to be produced by *Myroides* sp., including, *M. odoratus* and *M. odoramitimus* (Maneerat et al. 2005). Fatty acids, which also act as bio surfactants are produced by both actinobacteria as well as yeast species such as *Nocardia* sp., *Mycobacterium* sp., *Candida* sp., and *Cladosporium* sp. (Rehm & Reiff 1981). Phospholipid based biosurfactants were found to be produced by *Rhodococcus erythroides* (phosphatidy-lethanolamine), *Pseudomonas putida*, and *Pseudomonas aeruginosa* (Kretschmer et al. 1982, Janek et al. 2013, Robert et al. 1989).

POLYMERIC BIOSURFACTANTS

Emulsan is the best studied example of polymeric biosurfactant. *Acinetobacter calcoaceticus* RAG-1 has been reported to produce emulsan (Rosenberg et al. 1979). *Saccharomyces cerevisae* contains mannoprotein as a major cell wall component, which acts as a remarkable bioemulsifier (Cameron et al. 1988). Alasan, is a high molecular weight polymeric biosurfactant produced by *A. radioresistens* KA53 (Navonvenezia et al. 1995). These biosurfactants are also produced by different yeast species, including *Candida lipolytica*, *C. tropicalis*, *S. cerevisae* etc. (Cameron et al. 1988, Desai & Banat 1997). Recently, halophilic bacteria *Halomonas sp.* has been reported to produce 1,2-Ethanediamine, N,N,N',N'-tetramethyl, a polymeric biosurfactant at high quality level (Donio et al. 2013).

PARTICULATE BIOSURFACTANTS

A strain of *Acinetobacter* sp. HO1-N cells has been reported to produce accumulations of extracellular membrane vesicles. These vesicles have a diameter of 20-50nm, buoyant density of 1.158 g/cm^2, and consist of protein, phospholipids and lipopolysachharides. They are capable of forming microemulsions from hydrocarbons and aid in the uptake of alkane by the cells (Kappeli & Finnerty 1979).

PRODUCTION

In order to commercialize biosurfactants, economical strategies must be devised to make them cheap. Biosurfactants can be produced from various substrates, which account for 50% of the total production cost. Ideally, in order to make biosurfactant production economical, the process must use low cost substrates accompanied with a high yield of a product obtained. Other strategies to make the production of biosurfactant economical include the optimization of fermentative conditions, developing low cost efficient downstream processing and development of overproducing strains (Banat et al. 2010). In this review, we have focused on the different low cost substrates that are currently used for biosurfactant production.

Vegetable oil and oil wastes: Oil production occurs mostly in the food industries and accounts for nearly 2.5-3 million tons, generating huge quantities of wastes, tallow, lard, marine oil, soap sticks and free fatty acid during extraction of oil from seeds. Waste disposal creates problem and therefore has attracted the attention of researchers to the utilization of wastes in microbial transformation. In one study the effect of different vegetable oils as soybean, olive, castor, sunflower and coconut fat on the production of biosurfactant by *Serratia marcescens* strains was tested and best result was obtained with sunflower oil (Ferraz et al. 2002). Nitschke et al. (2005) reported that *Pseudomonas aeruginosa* LBI yielded 11.7 g/L of rhamnolipid using soybean soapstick. While in another study, *Pseudomonas* sp. was found to produce rhamnolipid successfully using olive oil mill effluent as the sole carbon source (Mercade et al. 1993). Many plant-derived oils, which are not suited for human use, are available at cheap monetary values and therefore have been practiced in different research works for biosurfactant production. Jatropha oil, which is non-edible, has been used for sophorolipid production by *Starmerella bombicola* NBRC 10243 in high yields (122.6g/L) as well as rhamnolipids by *Pseudomonas aeruginosa* ATCC 10145 in a concentration of 4.55 g/L which was comparable to that of the most common oils (Imura et al. 2013). Further, restaurant oil wastes have been employed in a few studies for biosurfactant production. Virgibacillus salaries showed better production of biosurfactants with waste cooking oil than other vegetable oils like jatropha oil, jojoba oil, canola oil and castor oil (Elazzazy et al. 2015).

Animal fat: Meat processing industries which include food and leather industries, produce large amounts of animal fat, lard and tallow. However the demand for animal fat is relatively low and thus utilization as well as disposal becomes a major problem. Thus, this can be used as an inexpensive substrate for biosurfactant production. Sophorolipid was produced from the yeast *Candida bombicola* in a medium containing animal fat and glucose as a carbon source (Deshpande & Daniels 1995). Low cost media containing animal fat has been used to measure the production of glycolipid biosurfactant by *Candida lipolytica* (Santos et al. 2013).

Agro-industrial wastes: Several agro-industrial wastes which include merchandise such as bran, hull of soy, corn, rice, sugar cane molasses, beet molasses, cassava flour and its wastewater, corn steep liquor, etc., are low cost renewable substrates and can be used for microbial biosurfactant production at industrial level. Orange peel was found to be the best substrate for biosurfactant production by *Bacillus licheniformis* (KC710973) with a yield of 1.796 g/L (Kumar et al. 2016). Potato peel was successfully used as a carbon source for biosurfactant production by *Bacillus pumilus* DSVP18 and the biosurfactant thus produced had better properties like stability over a wide range of temperatures, pH and salt stress (Sharma et al. 2015). Similarly, alkaliphilic bacterium *Klebsiella* sp. showed high yields of biosurfactant production with corn powder and also the biosurfactant had unique stability under adverse conditions (Jain et al. 2013). Cassava wastewater was found to be a good substrate when *Bacillus subtilis* LB5a was used in a pilot scale production of biosurfactant (2.4 g/L) (Barros et al. 2008). In another study, it was reported that a culture medium containing corn steep liquor (10% (v/v)) and molasses (10% (w/v)) resulted in biosurfactant production (3.2 g/L) by *Pseudomonas aeruginosa* strain as a mixture of eight different rhamnolipid congeners, with mono-rhamnolipid Rha-C_{10}-C_{10} being the most abundant (Gudina et al. 2015).

Dairy and distillery wastes: Dairy industries produce a huge amount of waste in the form of whey such as curd whey, whey waste, cheese whey and lactic whey. Lactic whey waste was found to be a comparatively better substrate than synthetic media for rhamnolipid production on a commercial scale (Mukherjee et al. 2006). Dubey et al. (2012a) reported that curd whey was a good substrate for biosurfactant production by *Pseudomonas aeruginosa* strain PP2 and *Kocuria turfanesis* strain-J. The biosurfactant produced were highly potent to emulsify pesticides under adverse environmental conditions (wide range of temperature, low pH and saline conditions). Two probiotic bacteria *Lactococcus lactis* 53 and *Streptococcus thermophilus* A strains showed 1.2-1.5 times increase in the mass of biosurfactant production per gram dry cell weight in media formulation using supplemented cheese whey medium as well as molasses compared to that of synthetic media MRS and M17 broths (Rodrigues et al. 2006).

On the other hand, distillery waste, also known as stillage, is a byproduct of biological process (fermentation of molasses by yeast), and is found to contain lysed yeast cells. Distillery wastes contain all the essential nutrients needed to hold the development of microorganisms. Biosurfactant production from an oily sludge isolate *Pseudomonas aeruginosa* strain BS2 was found to give the best result with whey waste followed by diluting distillery waste rather than with synthetic medium (Dubey & Juwarkar 2001). Likewise, in some other study, dilution of distillery waste with whey waste and sugar industry effluent gave satisfactory biomass and biosurfactant yield produced by the bacterial isolates. Individual wastes also gave satisfactory results for biosurfactant production by two new bacterial isolates identified as *Kocuria turfanesis* strain BS-J and *Pseudomonas aeruginosa* strain BS-P (Dubey et al. 2012b).

APPLICATIONS

Biosurfactants have widespread applications in different fields of environmental remediation, food processing, biomedical sciences, petroleum and cosmetic industry etc. The biggest market for biosurfactants has been in the petroleum industry. In this review article, we have concentrated on the various novel applications of biosurfactants in the major areas of bioremediation, and petroleum industry (Banat et al. 2000).

Biosurfactants in bioremediation: The purpose of bioremediation is to provide with cost effective, contaminant specific and environmentally friendly techniques to eliminate individual or mixed contaminants from the environment. Biosurfactants are effective remediation and dispersing agents and have been used for different purposes of bioremediation.

Marine Bioremediation: Large amounts of toxic wastes are discharged every year in different contaminated sites worldwide, and their common sink being the coastal marine regions. Oil spills are a major concern of pollution in the marine environment. Oils may contain different proportions of hydrocarbon. Biosurfactants play a role in the metabolism of hydrocarbon substrates by microorganisms. The majority of biosurfactants make the hydrocarbon substrates more amenable to degradation, but do not have the ability to degrade hydrocarbons by themselves. Still, there are exceptions, polyaromatic hydrocarbon anthracene was metabolized and converted to biosurfactant by the marine form of *Bacillus circulans* (Das et al. 2008). The addition of biosurfactants to the environment of non-producer, hydrocarbon degrading microbes would fulfill the purpose of bioremediation. Among all the other microorganisms, bacteria and their consortia have been shown to be efficient in getting rid of hydrocarbons from the brine. Other organisms, including yeast, algae and protozoa are a major area of on-going research (Morales & Paniagua 2014, Kim 2014). A remediation agent JE1058BS contained a biosurfactant prepared from the culture broth of *Gordonia* sp. strain JE-1058 had low toxicity and stimulated the biodegradation of weathered crude oil through the activities of indigenous marine bacteria (Saeki et al. 2009). Chhatre et al. (1996) created a consortium consisting of four bacterial isolates which efficiently degraded 70% of the crude oil in 72 hrs. One of the members of this consortium produced the biosurfactant rhamnolipid that emulsified the crude oil effectively for successful degradation by the other bacterial isolates. A bioemulsificant exopolysachharide (EPS_{2003}) increased five times the oil degradation capacity of hydrocarbon degrading bacteria in an experimentally created seawater microcosm, supplemented with crude oil and EPS_{2003} (Cappello et al. 2012).

Soil bioremediation: Soil pollution is a major environmental concern and results from the accumulation of a wide range of chemical compounds generated either by natural or industrial processes. Biosurfactants can increase the bioavailability of hydrophobic organic compounds. Barkay et al. (1999) reported that alasan produced by *Acinetobacter radioresitens* enhanced the solubilization of polycyclic aromatic hydrocarbons (50-500 µg/mL). They also reported that alasan influenced mineralization of phenanthrene and fluoranthene by *Sphingomonas paucimobilis* EPA505. Aqueous solutions of biosurfactants can also be used in soil washing techniques to increase the bioavailability of low solubility contaminants. SPB1 lipopeptide biosurfactant produced by *Bacillus subtilis* was able to remove 87% of oil in a diesel contaminated soil. The study also reported the optimum washing parameters using the Taguchi experimental design (Mnif et al. 2014). Biosurfactant produced by *Rhodococccus ruber* IEGM 231 showed high potential in removing polyaromatic hydrocarbons from soil. The study was carried out in soil columns spiked with model mixtures of major petroleum constituents, and the produced biosurfactant showed 2.5 times greater washing activity than synthetic surfactant Tween-60 in non-aqueous phase liquid (containing PAH dissolved in alkanes C_{10}-C_{19}) spiked soil. The biosurfactant also maintained its activity at a high (5% w/w) contamination level (Ivshina et al. 2016). Kang et al. (2009) reported that incorporation of sophorolipid in soil increased the biodegradation of 2-methylnapthalene, hexadecane and pristine. Also, it caused effective biodegradation of crude oil in soil.

Heavy metal bioremediation: Heavy metals such as cadmium, copper, lead, mercury, nickel and zinc are hazardous and are included in the priority list of EPA. Biosurfactants are able to form complexes with heavy metals resulting in heavy metal removal. Surfactin from *Bacillus subtilis* removed 15% copper and 6% zinc; rhamnolipid from *Pseudomonas aeruginosa*, was able to remove 65% copper and 18% zinc; sophorolipid from *Torulopsis bombicola* removed 25% copper and 60% zinc in a single washing. Further speciation of the heavy metals were determined and it was found that rhamnolipid and surfactin could remove organically bound copper, while sophorolipid removed carbonate and oxide bound-zinc (Mulligan et al. 2001). In another study carried out with biosurfactants produced from four microbial species: *Pseudomonas putida* T1 (8), *Bacillus subtilis* 3K, *Acinetobacter* sp., and *Actinobacillus* sp., reported the ability of biosurfactants to remove heavy metals from sediments of sludge. Biosurfactant from *Acinetobacter* sp. showed the highest removal of copper (14.04%), and biosurfactant from *Pseudomonas putida* T1(8) showed the highest removal of copper (2.01%) and zinc (6.5%) (Hidayati et al. 2014).

BIOSURFACTANTS IN PETROLEUM INDUSTRIES

Microbial enhanced oil recovery: It is a tertiary oil recovery technique, which uses microbes and/or their metabolites to recover remaining oil from reservoir after the primary and secondary recovery procedures. Biosurfactants produced by microorganisms' aid in microbial enhanced oil recovery (MEOR). Widespread research has been carried out on a laboratory scale on sand pack columns and field trials have also been conducted in this area. Amani et al. (2010) carried out an experiment with three indigenous strains of bacteria *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. All the bacterial strains produced biosurfactants which was efficient in emulsifying crude oil. Further the biosurfactants showed a good stability at extreme environmental conditions (pH 4, 25 g/L of salinity and a temperature of 120°C) similar to those found in oil reservoirs. Laboratory scale oil displacement experiment was carried out with kerosene and 25% of residual oil was recovered by the biosurfactant from *Bacillus subtilis*. Fermentative production of the biosurfactant from *Bacillus* strains was carried out and efficiency of crude biosurfactant preparation varied from 30.22-34.19% of the water flood residual oil saturation in sand pack column (Joshi & Desai 2013). There have been numerous reports on the ability of biosurfactants produced by *Bacillus* strains for MEOR (Pathak & Keharia 2014, Al-Wahaibi et al. 2014, Borah and Yadav 2016). Other studies which reported the efficiency of biosurfactants in enhanced oil recovery includes, rhamnolipids produced by *Pseudomonas aeruginosa* recovered 27% of the original oil after water flooding in sand packed column (Amani et al. 2013); biosurfactant produced by *Rhodococcus* sp. strain TA6 recovered 70% of residual oil from oil saturated sand packs (Shavandi et al. 2011).

Oil storage tank cleaning: Biosurfactants can be applied to take out the oily sludge deposits that are produced during oil transportation to refineries in large containers (tankers, barges, trucks) as well as during oil production and processing. In a field trial conducted at the Kuwait Oil Company using two tons of rhamnolipid biosurfactant produced by a culture broth, sludge was efficiently lifted and mobilized from the bottom of the tank and was solubilized within the emulsion. Approximately 91% of the hydrocarbons were recovered from the sludge (Banat et al. 1991). Since then extensive researches have been carried out and led to the development of the BioRecoil process patented in 2004 by Idrabel Italia and Jeneil Biosurfactant Company (Sen 2010).

Oil transportation and pipelining: After extraction from the fields, crude oil often needs to be transported over long distances to reach the refineries. One of the limiting factors of pipelining is high oil viscosity that slows down the oil flow. In a field trial, it was found that emulsan reduced the viscocity of Boscan crude oil from 200000 cP to 70 cP, which was then pumped at a rate of 380 miles over 64 hours. It was also calculated that under optimum conditions the transportation rate could be raised to 26000 miles (Sen 2010). In another study, emulsan produced by *Acinetobacter calcoeceticus* PTCC 1318 at 25°C, 30 mg/L, with a water-oil ratio of 1:2 showed 98% emulsification of crude oil and also demonstrated tube cleaning with removal percentages of 100% at room temperature, depending on washing conditions (Amani & Kariminezhad 2016). On the other hand, waxy crude oil transportation suffers from the problem of paraffin precipitation that may reduce or even block the internal diameters of pipelines and may also cause a change in oil composition. Biosurfactants produced by *Gordonia amicalis* LH3 had potential in paraffin control (Hao et al. 2008). *Pseudomonas* and *Bacillus* and a mixed consortium were capable of degrading n-paraffin used in the treatment of two paraffinic oils (Lazar et al. 1999).

CONCLUSION

Extensive research work has been done on biosurfactants and they have hit a vital phase in their commercial development. Due to their widespread application in different fields, biodegradability and non toxic nature, biosurfactants have reached the top of the agenda of many companies. During the last few decades, many studies have been carried out on the production of different types of biosurfactants from novel organisms. A diverse group of microorganisms, including bacteria, yeasts, filamentous fungi, actinomycetes has been shown to produce biosurfactants. It has potential applications in the arena of oil industry, and environmental remediation. In case of bioremediation, relatively less is known about the biosurfactant production potential of microorganisms in situ. The vast potential of biosurfactants in the area of petroleum industry is played up by the large number of related patents. Extremophilic and hyper-extremophilic biosurfactant producers should be used in this field to allow use in the adverse conditions of oil reservoirs. In addition, molecular techniques and gene expression monitoring should be applied in order to supervise and control activities and processes in situ, and in substantial time. It is necessary to get information about the structure of biosurfactants and their interaction with contaminants and cells, to increase their applicability in this area. In spite of various advantages, the high cost of production is a major limiting factor to the commercial success of the biosurfactants. A lot of work has been done on the use of cheap alternative substrates for biosurfactant production, which can reduce the production cost alone by 50%. In order to compete with the market for toxic chemical surfactants, and to obtain widespread commercialization of biosurfactants, would require strain improvement by genetic engineering; improving the economics of the production process by optimizing production parameters and developing low cost downstream processing.

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