# Utilization of Agro-industrial Wastes as Substrates for Biosurfactant Production.

**Neboh, H. A.**
World Bank African Centre of Excellence,
Institute of Petroleum Studies (IPS)
University of Port Harcourt, Rivers State,
Nigeria.

**Abu, G. O.**
Department of Microbiology,
University of Port Harcourt, Rivers State,
Nigeria.

**Uyigue, L.**
Department of Chemical Engineering,
University of Port Harcourt, Rivers State,
Nigeria.
**Corresponding author:** hopenwanagu@yahoo.com**.**

***Abstract***
*Advances in technology involving the use of natural resources as an alternative source of energy have led to the manufacture of biosurfactants with high value in the global market. Biosurfactants are amphiphilic biomolecules that partition at liquid/liquid, liquid/gas or liquid/solid interfaces. They have a wide-range of applications due to their unique properties like low toxicity, environmentally friendly nature, specificity and relative ease of preparation. Interest in biosurfactant production has increased because of the potential advantages they offer over their synthetic counterparts in various field of applications such as bioremediation, biodegradation, enhanced oil recovery, pharmaceutics and food processing. Biosurfactant production is considered as one of the key technologies for development in the 21st century. The key factor governing the success of biosurfactant production is the development of economical processes that use low-cost materials (wastes) to give high yields. The selection of such wastes with the proper balance of nutrients that will allow microbial growth and consequent biosurfactant production is critical. Agro - industrial wastes with a high content of readily metabolizable carbohydrates or lipids is ideal for use as substrate. This paper reviews the current knowledge and latest advances in the search for cost effective and sustainable agro-industrial substrates for biosurfactant production.*

***Keywords:*** *Biosurfactant, agro-industrial wastes, toxicity, microorganisms.*

## INTRODUCTION

Biosurfactants are a group of structurally diverse molecules produced by different microorganisms classified mainly by their chemical structure and microbial origin (Neboh and Abu, 2015). Biosurfactants are mainly produced by aerobic microorganisms in aqueous media with a carbon feedstock, such as carbohydrates, hydrocarbons, fats and oils. It is believed that biosurfactants are secreted into the culture medium to assist in the growth of the microorganisms by facilitating the translocation of insoluble substrates across cell membranes (Silva *et al,* 2014). Their interesting properties such as lower toxicity, higher degree of biodegradability, higher foaming capacity and optimal activity at extreme conditions of

temperatures, pH levels and salinity according to Neboh and Abu (2015), has attracted the attention of the scientific and industrial community. Industries are currently seeking to replace some or all chemical surfactants with sustainable biosurfactants (Amaral *et al*, 2006), but the high production cost is a major drawback (Silva *et al*, 2014). The choice of inexpensive raw materials is important to the overall economics of the process because they account for 50% of the final product cost. The best way to reduce substrate cost for biotechnology at present is to use wastes with the right balance of carbohydrates and lipids to support optimum growth of the organisms and biosurfactants production (Smyth *et al*, 2010a). This review is a compilation of literature for studies carried out in exploring production of biosurfactants using different substrates developed mainly from renewable agro-industrial wastes.

**Potential Agro-industrial Substrates for Biosurfactant Production.**

Production economy is the major bottleneck in biosurfactant production, the amount and type of raw material used for its production can contribute considerably to the cost. It is estimated that raw materials account for 10 - 30% of the total production costs in biosurfactant production (Kamalijeet and Sokhon, 2014). Thus to reduce costs, it is desirable to use low-cost raw materials like agro-industrial wastes. Agro-industrial wastes are sustainable source of organic bio-materials based on renewable substrates. Bioconversion of these waste materials is considered to be of prime importance for the near future because of its favorable economics, low capital and energy cost, reduction in environmental pollution and relative ease of operation (Makkar, 2011), availability of cheaper substrates in huge quantities and its ecofriendly nature. Producing biosurfactants from agro industrial waste is thus a feasible and favorable option (Moldes *et al*, 2007). These inexpensive agro- industrial waste substrates include cassava waste water, palm oil mill effluent, groundnut waste, soybean waste, olive mill waste, orange peel waste, potato peel waste and sugar waste. The use of such waste materials does not only produce biosurfactant but also reduces waste disposal (Makkar, 2011).

**2.1 Cassava waste waters**

This is a starch rich substrate with huge application obtained during the preparation of cassava flour and is an attractive alternative substrate in fermentation processes (Saharan *et al*, 2011). Major nutrients present in cassava waste are sugars and mineral salts which are quite attractive substrates for biotechnological processes. Nitschke and Pastore (2003) studied biosurfactant production from 5 cassava flour waste water, they reported the best to be the group with no solids that showed a surface tension of 26.59mN/m and reciprocal of CMC of over 100. Nitschke *et al* (2004) studied biosurfactant production on cassava effluent as a *substrate* using two *Bacillus subtilis* strains. Both *B. subtilis* ATCC 21332 and *B. subtilis* LB5a, exhibited good surface activity and produced similar yields of surfactin. Cassava waste water was also used for surfactin production by *B. subtilis* (Nitschke and Pastore, 2006). Siddhartha *et al* (2009) used cassava waste water as a substrate for the simultaneous production of rhamnolipids and polyhydroxyalkanoates by *Pseudomonas aeruginosa.*

**2.2 Palm Oil Mill Effluent (POME)**

POME is high strength organic waste slurry that contains high levels of fat, oil and grease (Jeremiah *et al*, 2014). It has a lot of nutritional values which microorganisms make use of as a source of energy for their growth and in turn produce useful metabolites such as biosurfactants (Neboh and Abu, 2015). Saimmai *et al* (2012a) produced biosurfactant from palm oil contaminated sites. Chanika *et al* (2013) produced biosurfactant from POME using *Nevskia ramose* NA3, the produced biosurfactant was found to reduce the surface tension of water from 72mN/m to 27mN/m. Surfactin, a biosurfactant from *Bacillus* was also produced by Mohd *et al* (2013). Kanokkrat *et al* (2013) isolated bacteria from POME for biosurfactant production and all the isolates reduced surface tension of water from 72mN/m to 40mN/m.

**2.3 Groundnut waste/Peanut oil cake**

Groundnut oil refinery residue is a high protein content solid residue rich in arginine but low in lysine (Swetha and Dhanya, 2009). It is also a rich source of carbohydrate and lipids. Sobrinho *et al* (2008) produced biosurfactant from *Candida sphaerica* using 5.0% groundnut oil refinery residue plus 2.5% corn steep liquor as substrates. The biosurfactant had high surface tension reducing activity (26 mN/m), a low CMC value (0.08%) and a yield of 4.5 $gl^{-1}$. Coimbra *et al* (2009) also reported biosurfactant production by six *Candida* strains grown in insoluble (n-hexadecane) and soluble substrates (soybean oil, ground-nut oil refinery residue, corn steep liquor and glucose). These biosurfactants were able to remove 90% of the hydrophobic contaminants from sand. Thavasi *et al* (2008a) used peanut oil cake for biosurfactant production, they confirmed that *Bacillus megaterium*, *Azotobacter chroococcum* and *Corynebacterium kutscheri* had the capability of using these substrates for biosurfactant production with better yields achieved with peanut oil cake. Recently the authors have reported biosurfactant production by *Lactobacillus delbrueckii* using peanut oil cake as the carbon source. The biosurfactant produced (5.35 mg/ml) was capable of promoting biodegradation to a large extent (Thavasi *et al*, 2011).

**2.4 Soybean waste**

Soy molasses, a by-product of soybean oil processing contains high fermentable carbohydrate (30% w/v) and is about 60% of solid carbohydrate which makes it well suited for economical production of biosurfactants (Makkar *et al*, 2011). Soy molasses were used to produce sophorolipids by *Candida bombicola* with yields of 55 g/l (Solaiman *et al*, 2007). Rufino *et al* (2008) applied sequential factorial design to optimize biosurfactant production by *Candida lipolytica* using soybean oil refinery residue as substrate. In this study they evaluated the impact of three cultivation factors, amounts of refinery residue, glutamic acid and yeast extract. The biosurfactant product showed high surface activity and emulsifying ability and was very stable at wide range of pH (2-12), temperatures (0-120°C) and salinity (2-10% NaCl).

**2.5 Olive Oil Mill Effluent (OOME)**

Olive Oil Mill Effluent (OOME) is black liquor containing a water-soluble fraction of ripe olives and water that is used in the process of olive oil extraction. This waste contains 80-96% water, 3.5-15% organics and 0.5-2% mineral salts (Maria *et al*, 2012). Mercade *et al* (1993) were the first group to show the production of rhamnolipids by *P. aeruginosa* 47T2 when grown on Olive Oil Mill Effluent (OOME) as the sole carbon source. Camargo *et al* (2003) studied the production of a glycolipid with emulsifier properties during cultivation of *Penicillium citrinum* on mineral medium with 1% olive oil as carbon source. The growth associated emulsifier production reached maximal activity at 60 h of cultivation with the production yield (Yp/s) of 0.54.

**2.6 Orange peel**

Citrus fruits are one of the most important value added fruit crop in international market and is mostly used for orange juice production which generates large quantities of waste (Adalgisa *et al*, 2005). George and Jayachandran (2008) reported the use of orange fruit peeling as sole carbon source for rhamnolipid production using *P. aeruginosa* MTCC 2297. The substrate was able to give a yield of 9.18g/l and surface tension of 31.3mN/m. Kumar *et al* (2016) reported orange peel as the best substrate for biosurfactant production with a yield of 1.796g/l and emulsification activity of 75.17% against diesel.

**2.7 Potato peel**

Processing of potatoes results in starch rich waste water, potatoes peels, un- consumable potatoes, which are rich substrates for microbial growth. It is estimated that only 59% of the potato crop are processed

into consumable products and most of what remains represent a starchy rich wastes which is difficult to dispose (Makkar, 2011). Fox and Bala (2000) evaluated potato substrate as a carbon source for biosurfactant production using *B. subtilis* ATCC 21332. They compared growth, surface activity and carbohydrate utilization of *B. subtilis* ATCC 21332 on an established potato medium, simulated liquid and solid potato waste media and a commercially prepared potato starch in a mineral salts medium. The results obtained indicated the utilization of potato substrate and production of surfactant as indicated by high surface tension reduction. Das and Mukherjee (2007) reported the efficiency of two *Bacillus subtilis* strains for the production of biosurfactants in two fermentation systems using powdered potato peels as substrate. Wang *et al* (2008a) also applied a *Bacillus subtilis* strain B6-1, for production of biosurfactant using soybean and sweet potato residues in solid-state fermentation.

**2.8 Sugar waste**

Molasses are a co-product of sugar production, both from sugar cane and sugar beet industry resulting from the final step of sugar crystallization after which further sugar crystallization becomes uneconomical (Maneerat, 2005a). Molasses are mainly composed of sugars (sucrose; 48-56%), non-sugar organic matter (9-12%), proteins, inorganic components and vitamins. The total fermentable sugar is in the range of 50-55% by weight. Maneerat (2005b) reported specific production rate of rhamnolipid when using 2%, 4%, 6%, 8% and 10% of molasses with biomass yield of 0.003, 0.009, 0.053, 0.041 and 0.213 respectively. Abdel-Mawgoud *et al* (2008) carried out an optimization study of environmental and nutritional production conditions for surfactin production by *Bacillus subtilis* using 16% molasses, 5 g/l $NaNO_3$ and the trace elements to give a surfactin yield of 1.12 g/l.

**3.0 Other factors affecting bio surfactant production**

Many factors affect the production of biosurfactant aside from nutritional factors. Physicochemical and environmental factors are extremely important in the yield and characteristics of the biosurfactant produced. In order to obtain large quantities of biosurfactant, it is necessary to optimize the process conditions because the production of biosurfactant is affected by such factors;

**3.1 Oxygen availability**

Oxygenation is one of the crucial parameters for aerobic organisms; many intracellular enzymatic activities are regulated by oxygen. Fontes *et al* (2010) investigated the influence of aeration and agitation speed on biosurfactant production by *Yarrowia*. The results from the batch fermentation showed that as the agitation speed increases from 160rpm to 250rpm, biosurfactant production increased as determined through the three different methods used to measure biosurfactant activity. In the batch fermentation of *Pseudomonas aeruginosa* EM1 when the agitation was increased from 50 to 250 rpm, the rhamnolipid production increased to 80% (Wei *et al*. 2005). Amaral *et al* (2006) also mentioned that biosurfactant production increased with increase in agitation speed with optimum production at 250rpm. Kronemberger *et al* (2008) has also shown that a rhamnolipid production depends on specific oxygen uptake rate. The agitation speed affects the mass transfer efficiency of both oxygen molecules and medium components.

**3.2 Temperature**

Various microbial processes are temperature dependant and get affected by a little change. Bhardwaj (2013b) reported biosurfactant productions in a temperature range of 25 - 30$^{o}$C. A lipopeptide biosurfactant produced by *Serratia marcescens* was able to retain its properties at 100 $^{o}$C (Anyanwu *et al*, 2011). In culture of *Candida antarctica*, temperature causes variations in the biosurfactant production while the highest mannosylerythritol production was observed at 25 $^{o}$C for the production of both growing and resting cells. *Yarrowia lipolytica* was reported to grow best at a favourable temperature of 27$^{o}$C (Bhardwaj, 2013b). The optimum temperature for the *Bacillus* strains isolated from the marine sediments of Tamil Nadu coastal area was 37°C (Gnanamani *et al*, 2010). Saharan *et al* (2011) reported that the amount of sophorolipids obtained in culture medium at temperatures between 25 - 30 $^{o}$C was similar. Nevertheless the fermentation performed at 25 $^{o}$C recorded a lower biomass growth and a higher glucose

consumption rate in comparison to the fermentation performed at 30 $^{o}$C. It was also observed that the growth of *Candida bombicola* reaches a maximum at a temperature of 30 $^{o}$C while 27 $^{o}$C is the best temperature for the production of sorphorolipids.

**3.3 PH**

The acidity of the production medium was the parameter studied in the synthesis of glycolipids by *Candida antarctica* and *Candida apicola*. When pH is maintained at 5.5, the production of glycolipids reached a maximum. The synthesis of the biosurfactant decreased without the pH control indicating the importance of maintaining it throughout the fermentation process (Bednarski *et al*, 2005). *Yarrowia lipolytica* as reported by Sarubbo *et al*, (2006), produced maximum biosurfactant at a pH of 5.0. Optimum pH for the *Bacillus* strains isolated from the marine sediments of Tamil Nadu coastal area was $7.2 \pm 0.2$ (Gnanamani *et al*, 2010). *Candida lipolytica* at pH of 5.0 and *Candida batistae*, at pH of 6.0 produced maximum biosurfactant (Sarubbo *et al*, 2006; Bhardwaj, 2013b). Amaral *et al* (2006) reported the production of Yansan, with a stable pH of between 3-9 from *Y. lipolytica*.

**3.4 Salinity**

A lipopeptide biosurfactant produced by *Serratia marcescens* was able to retain its properties at high NaCl concentrations up to 12% (Anyanwu *et al*, 2011). Salt tolerant strains of *Yarrowia lipolytica* have been isolated from hypersaline and marine locations implicating that this yeast may be playing a significant role in saline environment (Kim *et al*, 2007; Zinjarde *et al*, 2008). Souza *et al* (2012) in their study investigated the application of *Y. lipolytica* in the bioremediation of oil in seawater. The physiology of this yeast was investigated under saline conditions of the sea water in the presence of diesel oil. They came up with the result that the genus *Yarrowia* presented a high halophilic capacity because of its tolerance to sea water.

**3.5 Incubation time**

This plays a significant role in the production of biosurfactants. The effect of incubation time can be seen by monitoring the values of emulsification activity, surface tension and biomass concentration after a regular time interval e.g 5.86g/l of Rhamnolipid was produced at 72h (Soniyamby *et al*, 2011). *Pseudomonas fluorescence* after 36 h of incubation starts producing biosurfactant and reaches its maximum concentration after about 56 h (Abouseoud *et al*, 2007). The product yield increased to 70% when aeration is supplied to the *Pseudomonas aeruginosa* LBI in a batch feed culture (Benincasa *et al*, 2002). Rufino *et al* (2011) produced a biosurfactant with antimicrobial properties from *Candida lipolytica* UCP in 72 h fermentation at 28ºC in an orbital shaker at 150 rpm. The biosurfactant produced was able to reduce the surface tension from 50 mN/m to 25 mN/m.

**Optimization of Bio surfactant Production.**

Classical method of medium optimization involves changing one variable at a time, while keeping the others at fixed levels. However, this method is time consuming and does not guarantee the optimal metabolite production. A statistical optimization strategy; Response Surface Methodology (RSM) has been developed for the optimization of the process. RSM explores the relationship between several explanatory variables and one or more response variables. Sen and Swaminathan (2004) used this method to determine the optimum media, inoculums and environmental conditions for the enhanced production of surfactin by *Bacillus subtilis***.** RSM has also been applied to enhance biosurfactant production by *Pseudomonas aeruginosa* AT10 (Rodrigues *et al*, 2006d). Using the methods like experimental factorial design and response surface analysis, it is possible to conclude optimal operating circumstances to obtain a higher cellular growth, thus a higher cell-bound biosurfactant production yield. Optimization through factorial design and response surface analysis is a general practice in industrial biotechnology and numerous research workers have applied this technique for optimization of cultural conditions (Saharan *et al*, 2012).

## 5.0 CONCLUSION

In this review we have provided an overview of the use of alternative substrates (agrowastes) as an attractive strategy for biosurfactant production. The commercial realization of the biosurfactants which is restricted by the high production costs can be supported by optimized production conditions provided by utilization of the cheaper renewable substrates and optimization of cultural conditions. The true significance of these processes will be justified only when these studies can be scaled up to commercially viable processes.

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