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RESEARCH ARTICLE

STUDY ON EFFECT OF MONOVALENT AND DIVALENT SALTS ON THE PRODUCTION OF BIOSURFACTANT AND EMULSIFICATION INDEX.

C.C. Okore[1*], L.U. Nwaehiri[1], O.N. Mbanefo[1], T.E. Ogbulie[2], A.U. Ugenyi[2], I.B. Ogbuka[1], A.E. Ejele[3] and I.A.Okwujiako[2].

1. Environmental Biology Dept, Federal Polytechnic Nekede Owerri, Imo-State.
2. Biotechnology Dept, Federal University of Technology Owerri, Imo-State.
3. Chemistry Dept, Federal University of Technology Owerri, Imo-State.

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Abstract

Biosurfactants are produced by micro organisms and are used in industrial and environmental applications as a result of their resistivity to harsh conditions. Their production can be affected by extreme environmental conditions like pH, salinity, temperature, aeration. In view of this we studied the effect of monovalent salt (KCl) and divalent salt ($MgCl_2$) on the biosurfactant producing activity and emulsification index of five bacterial strains (*Staphylococcus aureus*, *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas aeruginosa* and *Proteus* sp.). The KCl and $MgCl_2$ increased the biosurfactant production of all bacterial strains. The KCl affected emulsification index (E24) of all the strains except *Proteus* sp. that recorded the highest E24 of 41.6% with addition of 0.4 g KCl. The $MgCl_2$ increased the E24 of *Bacillus* sp. (52.4%; 0.1 g); *Corynebacterium* sp. (50%; 0.1 g); *Pseudomonas aeruginosa* (56%; 0.2 g); *Proteus* sp. (57.7%; 0.4 g). These bacterial isolates can be used in industries and environmental applications as a result of their tolerance to salt.

Introduction:-

Surface active agents which are produced by different groups of micro organisms are known as biosurfactant. Biosurfactants are environmentally friendly, biodegradable, less toxic and non harzardous. They have better foaming properties and higher selectivity. Biosurfactants are active at extreme temperatures, pH, salinity and can be produced from industrial waste and by-products (Kosaric, 2001). This is important for industrial and biotechnological applications. Biosurfactants are very stable and effective in the culture medium that is used for their synthesis (Pacwa-Płociniczak *et al.*, 2011). The industrial needs for biosurfactants are constantly increasing. Biosurfactants are widely used in different industries, such as cosmetics, food and beverages, pharmaceuticals, agriculture, cleaners, and in petroleum industry. In the petroleum industries, biosurfactants are used, in bioremediation of hydrocarbon polluted soils and waters, in microbial enhanced oil recovery, to reduce the heavy oil viscosity, clean up oil storage tanks, increase flow through pipelines and stabilize fuel water emulsion (Sharma and Pant, 2000, Makkar and Cameotra, 1997). During *in situ* application, bacteria for microbially enhanced oil recovery (MEOR) must be able to grow under extreme condition encountered in oil reservoir such as high temperature, pressure, salinity and low oxygen level. Emulsification of the hydrocarbons in water is a prerequisite that paves the way for biodegradation of environmental pollutants by many bacteria. The efficiency of biologically enhanced oil recovery

Corresponding Author:-C.C. Okore.
Address**:-**Environmental Biology Dept, Federal Polytechnic Nekede Owerri, Imo-State.

has been proven in field studies in the United States, Czech Republic, Romania, Hungary, Poland and Holland with significant increases in oil recovery observed in all cases (Karanth *et al*., 1999). The industrial and environmental applications of the biosurfactant depend on their stability at extreme conditions of temperature, salinity, and pH (Joshi *et al*., 2008). Biosurfactant production rate by bacteria, like any other chemical reaction is affected by a number of factors which either increase or decrease its productivity rate, such include pH, salinity and temperature (Joshi *et al*., 2008, Maneerat *et al*., 2005). Desai and Banat, (1997), ABU-Ruwaida *et al*., (1991) also affirm the fact that environmental factors and growth condition such as pH, temperature, agitation, salinity, oxygen availability, affect cellular activity. Therefore the search for biosurfactant producing bacteria that can withstand harsh environmental conditions and rigorous industrial processes without affecting them led to this study on effects of monovalent and divalent salts (KCl and $MgCl_2$) on the production of biosurfactants and emulsification index.

Methodology:-

Production of biosurfactants:-

The test tubes were washed with hypo and detergent, peptone water and test tubes were sterilized in the auto clave for 15 minutes. Test tubes were labelled according to the isolates. In this study five (5) bacteria genera (*Bacillus* sp., *Corynebacterium* sp. *Proteus* sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*) positive for biosurfactant production were used. A loopful of each of the bacterium culture in agar slants was picked and inoculated into five different test tubes containing 4.5 ml of peptone water and allowed to stand for 24 h at 37^{o}C. At the end of the incubation, different salt quantity (0.0 g control, 0.1 g, 0.2 g, 0.3 g, 0.4 g and 0.5 g) was added to each of the broth culture and incubated for 7 days. At the end of the incubation, each of the broth culture of test organisms containing the salt solutions were screened for biosurfactant production using emulsification index method to determine the various effects or degrees to which the test organisms produce biosurfactants. Also, the biosurfactants produced by each of the test organisms was extracted and compared with the control samples (0.0 g) without salt. The result obtained in this study is recorded and represents the effects of salts on biosurfactant production of the test organism.

Emulsification index:-

The emulsification capacity of the biosurfactant on kerosene 2 ml was studied using 2 ml of the cell free supernatant obtained after vortexing the 7 days sample culture at high speed for 2 min and allowed to stand for 24 h. The emulsification index (E24) was calculated as the percentage of height of the emulsified layer (mm) divided by the total height of the liquid/aqueous column (mm) (Maneerat, 2005, Okore *et al*., 2013, Okore *et al*., 2017).

Extraction of Biosurfactant:-

The extraction was performed by acid precipitation followed by liquid-liquid extractions. After 7 days of incubation sterile Petri plates were taken and the weight of the plate measured before and after. The cell free supernatant or crude biosurfactant (1 ml), got after centrifugation at 5,000 rpm for 15 min, was poured on the plates and acidified with equal volume of acid (1 ml, 2 M H_2SO_4) to attain a pH of 2.0 and extracted with an equal volume of solvent, chloroform: methanol (2:1). The resultant aliquot was shaken well for mixing and placed on the hot air oven for drying at 100^{o}C for 30 min. The white coloured sediment obtained was the crude biosurfactant and weighed (Morikawa *et al.,* 2000). The dry weight of the biosurfactants was calculated by the following formula:

Dry weight of biosurfactant = Weight of the plates after drying-weight of the empty plates.

Result:-

The result of the weight of biosurfactant produced by the different bacteria strains enhanced with different quantities of potassium chloride is presented in Table 1. Table 2 is the result for the emulsification index produced by the different bacteria strains enhanced with different quantities of potassium chloride. Table 3 is the result for the weight of biosurfactant produced by the different bacteria strain enhanced with different quantities of magnesium chloride while Table 4 is the result for the emulsification index produced by the different bacteria strain enhanced with different quantities of magnesium chloride.

Table 1:- Effect of potassium chloride salt on biosurfactant production.

Isolate	Weight of Petri dish before extraction (g)	Weight of Petri dish after extraction (g)	Weight of biosurfactant (g)	Quantity of KCl in bacterial suspension(g)
Staphylococcus aureus	46.7	47.2	0.5	0.1
	35.7	36.7	1.0	0.2
	42.0	42.3	0.3	0.3
	36.7	36.9	0.2	0.4
	36.0	36.5	0.5	0.5
	38.0	38.3	0.3	0
Bacillus sp.	32.3	33.0	0.7	0.1
	33.5	34.0	0.5	0.2
	38.0	38.5	0.5	0.3
	45.5	46.0	0.5	0.4
	40.5	40.7	0.2	0.5
	43.2	43.3	0.1	0
Corynebacterium sp.	40.1	40.7	0.6	0.1
	41.8	42.3	0.5	0.2
	43.0	44.5	1.5	0.3
	46.2	46.5	0.3	0.4
	35.5	36.7	1.2	0.5
	42.0	42.2	0.2	0
Pseudomonas aeruginosa	35.0	35.3	0.3	0.1
	31.9	32.9	1.0	0.2
	30.3	30.7	0.4	0.3
	36.0	36.3	0.3	0.4
	36.8	37.0	0.2	0.5
	32.0	32.1	0.1	0
Proteus sp.	35.0	37.5	2.5	0.1
	43.5	43.6	0.1	0.2
	34.3	34.5	0.2	0.3
	35.9	36.2	0.3	0.4
	31.5	31.7	0.2	0.5
	43.3	43.4	0.1	0

Table 2:- Effect of potassium chloride salt on Emulsification index.

Isolate	Quantity of KCl in bacterial suspension (g)	Emulsification index E24(%)
Staphylococcus aureus	0	50.0
	0.1	9.0
	0.2	4.5
	0.3	10.0
	0.4	9.0
	0.5	13.0
Bacillus sp.	0	68.0
	0.1	10.5
	0.2	6.8
	0.3	9.5
	0.4	22.7
	0.5	6.6
Corynebacterium sp.	0	25.9
	0.1	7.6
	0.2	4.0
	0.3	4.0
	0.4	3.3

	0.5	10.0
Pseudomonas aeruginosa	0	4.0
	0.1	4.0
	0.2	4.0
	0.3	4.7
	0.4	3.3
	0.5	16.6
Proteus sp.	0	10
	0.1	25.0
	0.2	28.0
	0.3	21.4
	0.4	41.6
	0.5	17.2

Table 3:- Effect of magnesium chloride salt on biosurfactant production.

Isolate	Weight of Petri dish before extraction	Weight of Petri dish after extraction	Weight of biosurfactant	Quantity of $MgCl_2$ in bacterial suspension (g)
Staphylococcus aureus	35.5	36.6	1.1	0.1
	24.0	24.2	0.2	0.2
	24.3	25.2	0.9	0.3
	34.7	35.3	0.6	0.4
	39.5	41.5	2.0	0.5
	24.8	25.2	0.4	0
Bacillus sp.	32.8	33.0	0.2	0.1
	51.0	51.3	0.3	0.2
	32.0	32.5	0.5	0.3
	30.65	31.5	0.85	0.4
	20.60	22.2	1.6	0.5
	20.0	20.8	0.8	0
Corynebacterium sp.	20.0	20.5	0.5	0.1
	20.0	20.4	0.4	0.2
	20.0	20.3	0.3	0.3
	40.9	41.3	0.4	0.4
	43.0	43.8	0.8	0.5
	42.1	42.5	0.4	0
Pseudomonas aeruginosa	33.0	33.4	0.4	0.1
	31.8	32.2	0.4	0.2
	28.5	28.8	0.3	0.3
	33.0	33.5	0.5	0.4
	50.0	50.3	0.3	0.5
	31.5	31.8	0.3	0
Proteus sp.	35.3	36.0	0.7	0.1
	51.0	51.5	0.5	0.2
	51.0	51.5	0.5	0.3
	40.0	40.3	0.3	0.4
	27.5	27.3	0.2	0.5
	31.5	31.9	0.4	0

Table 4:- Effect of magnesium chloride salt on Emulsification index.

Isolate	Quantity of $MgCl_2$ in bacterial suspension (g)	Emulsification index E24 (%)
Staphylococcus aureus	0	12
	0.1	3.8

	0.2	6.25
	0.3	3.7
	0.4	3.3
	0.5	4
Bacillus sp.	0	45
	0.1	52.4
	0.2	47.6
	0.3	25
	0.4	10
	0.5	10
Corynebacterium sp.	0	12
	0.1	50
	0.2	40
	0.3	10
	0.4	8
	0.5	44.4
Pseudomonas aeruginosa	0	33
	0.1	7.1
	0.2	56
	0.3	4.3
	0.4	4
	0.5	4
Proteus sp.	0	10.3
	0.1	44
	0.2	44
	0.3	37.9
	0.4	57.7
	0.5	34.4

Discussion:-

In this study the quantity of biosurfactant production increased with the addition of both monovalent salt KCl and divalent salt $MgCl_2$ (Table 1 and Table 3). The quantity of biosurfactant produced by *Staphylococcus aureus* (Table 1) was highest 1.0 g with the addition of 0.2 g KCl to the broth medium; 0.2 g biosurfactant was produced with the addition of 0.5 g $MgCl_2$ (Table 3). The highest quantity of biosurfactant produced by *Bacillus* sp., is 0.7 g (Table 1) when 0.1 g KCl was used; also 1.6 g was produced with the addition of 0.5 g $MgCl_2$ (Table 3). *Corynebacterium* sp. produced the highest quantity of biosurfactant 1.5 g (Table 1) when 0.3 g KCl was added to the medium; and 0.8 g produced with the addition of 0.5 g $MgCl_2$ to the medium (Table 3). The biosurfactant produced by *Pseudomonas aeruginosa* was highest 1.0 g (Table 1) with the addition of 0.2 g KCl; and 0.5 g produced when 0.4 g $MgCl_2$ was added to the medium (Table 3). *Proteus* sp. produced its highest quantity of biosurfactant 2.5 g (Table 1) with the addition of 0.1 g KCl and 0.7 g biosurfactant produced with the addition of 0.1 g $MgCl_2$.

The result on Table 2 showed that the monovalent salt KCl affected the E24 recorded by the *Staphylococcus aureus*. The bacterial suspension with salt 0 g recorded the highest E24 (50%). This value dropped as the quantity of KCl increased. The salt KCl also affected the E24 shown recorded by *Bacillus* sp. (Table 2). The control 0 g recorded the highest value for E24 as 68% and increased quantities dropped this value. The E24 recorded by *Corynebacterium* sp. with the 0 g of KCl was 25.9%. This value dropped with the addition of KCl which has a negative effect on the E24 (Table 2). The bacteria *Pseudomonas aeruginosa* (Table 2) produced a highest E24 of 16% with the addition of 0.5 g of KCl. The salt is needed by the organism for growth and breakdown of hydrocarbon. The *Proteus* sp. (Table 2) also recorded the highest E24 value with the addition of 0.4 g of KCl to 41.6%. The salt KCl is tolerant to the *Proteus* sp. used in this study.

The effect of the divalent salt $MgCl_2$ on emulsification index in the study recorded higher values with more of the bacteria studied than with the addition of the monovalent salt KCl to the bacterial broth medium. *Bacillus* sp. (Table 4) recorded 52.4% E24 with the addition of 0.1 g of the salt $MgCl_2$ to the broth medium. This value decreased to

47.6% with the addition of 0.2 g $MgCl_2$. *Corynebacterium* sp. recorded a high E24 of 50% (Table 4) with the addition of 0.1 g $MgCl_2$ and dropped to E24 of 44.4% with the addition of 0.5 g $MgCl_2$. *Pseudomonas aeruginosa* recorded (Table 4) E24 of 56% with the addition of 0.2 g $MgCl_2$ to the growth medium. *Proteus* sp. tolerated salt content (Table 4). The E24 value recorded with the addition of 0.4 g $MgCl_2$ is 57.7%. The divalent salt was not tolerated by *Staphylococcus aureus* only (Table 4), the addition of $MgCl_2$ affected negatively the E24. The E24 recorded without the addition of the $MgCl_2$ is 12% but this value dropped to 3.3% with the addition of the salt.

This study showed that *Pseudomonas aeruginosa* and *Proteus* sp. tolerated the monovalent salt KCl (Table 2) added to the growth medium as this increased the E24 while *Staphylococcus aureus, Bacillus* sp. and *Corynebacterium* sp. were affected by the addition of the monovalent salt (Table 2). The study using divalent salt (Table 4) $MgCl_2$ gave contrasting result to the monovalent salt KCl used. The *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas aeruginosa* and *Proteus* sp. tolerated the $MgCl_2$ recording high E24 values. *Staphylococcus aureus* (Table 4) was intolerant to the addition of $MgCl_2$ in the culture medium.

Maneerat and Phetrong, (2007) studied the effect of monovalent salt NaCl and divalent salt $MgCl_2$ on emulsification index of *Bacillus* sp. They found out that $MgCl_2$ ranging from 0 to 0.1 M had no effect on emulsifying index. The monovalent salt they used ranging from 0 to 9 M had no effect on E24 but the higher molarity from 12 M to 21 M affected the E24 drastically as there was no E24 activity recorded. The result of this current study confirms this finding. Karanth *et al.,* (1999) in their study found out that biosurfactant produced from *Pseudomonas strains* MEOR 171 and MEOR 172 were not affected by temperature, pH, calcium, magnesium concentration in the ranges found in many oil reservoirs.

Conclusion And Recommendation:-

In conclusion only *Staphylococcus aureus* was intolerant to both KCl and $MgCl_2$ used in the study (Table 2 and Table 4) while the other bacterial isolates can be used in the industries and environmental applications due to their tolerance to salt.

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