# Comparative Studies on Synthetic and Agricultural Product on Lysine Production by *Alcaligenes aquatilis*

**ABSTRACT-** Production of lysine by *Alcaligenes aquatilis* from agricultural products (banana and soyabean) was compared to glucose and ammonium sulphate as carbon and nitrogen source. Ammonium sulphate was constant as nitrogen source when the two carbon sources were investigated and glucose constant as carbon source when the nitrogen sources where investigated. The production of lysine was examined quantitatively by acidic ninhydrin method. The results showed that banana and soyabean gave the maximum lysine yield (1.158mg/mL and 1.279mg/mL) for the fermentation period of 96h.

## INTRODUCTION

L-lysine, 2, 6 diaminohexanoic acid ($C_6H_{14}N_2O_2$) is a basic amino acid having two amino groups, one, on α- position and other at ε- position (Tome and Bos, 2007; Rao *et al*., 2011; Malothu *et al*., 2012). L-Lysine is generally deficient in the food supply of man and meat producing animals (Pellett and Ghosh, 2004). Since animal feeds such as grain and defatted oil seeds contain only a small quantity of lysine, poultry, cattle and other livestock are unable to synthesize these amino acids. It must be added in feed to provide a balanced diet (Nasab *et al*., 2007). Agricultural by-products may be used as low-cost carbohydrate sources for microbial production of high value added products such as amino acids (Buzzini and Martini, 1999). Microbial fermentation provides 100% L-amino acids whereas by chemical method 50% D and 50% L- amino acids are obtained (Khan *et al*., 2006). Anastassiadis (2007) revealed that fermentation process is more economical, optical active and the sterospecificity (the L-isomer) make it more advantageous compared with synthetic processes. The present report demonstrated a comparative studies between synthetic and agricultural products on lysine production which show that that banana and soyabean gave the maximum lysine yield (1.158mg/mL and 1.279mg/mL) for the fermentation period of 96h.

## MATERIALS AND METHODS

### Microorganism

*Alcaligenes aquatilis* was isolated from soil in Unizik Awka, Nigeria. It was maintained on starch casein agar slants at 4$^{o}$C. The medium for seed culture consisted of peptone, 10.0g;

yeast extract, 10.0g; NaCl, 5.0g; distilled water, 1L; pH adjusted to 7.2 with 1N NaOH. The medium was sterilized at 121$^{o}$C. Two loopful of a 24h culture of the isolate on nutrient agar was inoculated into 2ml of the sterile seed medium in a test tube and incubated on a Searchtech HY-2A orbital shaker at 160rpm and 30°C for 48h.

**Fermentation**

The basal medium for fermentation experiments was composed of $KH_2PO_4$, 0.5g; $K_2HPO_4$, 0.5g; $MgSO_4.7H_2O$, 0.001g; $MnSO_4.H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $CaCO_3$, 0.02g; $(NH_4)_2SO_4$, 10g; glucose, 20g; water, 1 litre while the pH was adjusted to 7.2, was used for lysine production. After sterilization the flask was cooled to room temperature and 1mL (ca $1.8 \times 10^7$) of a 24h seed inoculum of the isolate was inoculated into the fermentation medium. The experiment was performed in duplicate, with uninoculated flask serving as control. The flask was incubated on a rotary shaker (160 rpm) at 30$^{o}$C for 96h. Bacterial growth and lysine production were determined from the broth culture.

**Comparative difference between Glucose and Banana**

Two carbon sources [glucose and banana (*Musa acuminata*)] were studied for their effects on lysine accumulation by the isolate. Fermentation was carried out in a medium consisting of $KH_2PO_4$, 0.05g; $K_2HPO_4$, 0.05g; $MgSO_4.7H_2O$, 0.1g; $MnSO_4.4H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $CaCO_3$, 2.0g; carbon source, 20g; $(NH_4)_2SO_4$, 10g; distilled water, 1L, pH 7.2. A 100ml Erlenmeyer flask containing 20ml of the fermentation medium was inoculated with 2ml of seed inoculum (two loopful) and the flask incubated on an orbital shaker (160rpm) at 30°C. Duplicate flasks were used and uninoculated flasks served as control. After 96h fermentation, lysine production was determined as previously described. Thus, sorghum gave the maximum lysine accumulation.

**Comparative difference between ammonium sulphate and soyabean**

Two nitrogen sources [$NH_4SO_4$ and soyabean (*Glycine max*)] were examined for their effects on lysine production by the isolate. Fermentation was carried out in a medium consisting of $KH_2PO_4$, 0.05g; $K_2HPO_4$, 0.05g; $MgSO_4.7H_2O$, 0.1g; $MnSO_4.4H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $CaCO_3$, 2.0g; glucose, 20g; nitrogen source, 10g; distilled water, 1L, pH 7.2. A 100ml Erlenmeyer flask containing 20ml of the fermentation medium was inoculated with 2ml of the inoculums (two loopful) and the flask incubated on an orbital shaker (160rpm) at

30°C. Duplicate flasks were used and uninoculated flasks served as control. After 96h fermentation, lysine production was determined as previously described.

## RESULTS AND DISCUSSION

Lysine producing bacteria need ample supply of a suitable carbon and nitrogen source. Trifonova *et al.* (1993) and Javed *et al*. (2011) used agricultural product as substrate for production of lysine by *Brevibacterium* spp. and citric acid by *Aspergillus niger,* respectively. The ability of these bacteria to utilize these agricultural products is supported by the work of Umerie *et al.* (2000), who used agricultural by-products as carbon and nitrogen sources for lysine production. Krishnamurth (1980), Nicoloni *et al. (*1987) and Nigam (2000) used agricultural by-products for single cell protein production. For this purpose, the concentrations were the same. As shown in Figures 1 and 2, maximum lysine of 1.158mg/ml was accumulated when sorghum was used while 1.279mg/ml was obtained when soyabean was used. This is in consistent with the report of Adnan *et al.* (2011), who studied the selection of substrates for L-lysine production by *Brevibacterium linens* DSN 20158 and observed maximum L-lysine production (2.213g/kg) with soyabean meal. Umerie *et al.*(2000) and Ekwealor and Orafu (2003), similarly, observed that defatted soyabean meal stimulated the highest amount of lysine in *Bacillus laterosporus* and *Bacillus* species, respectively. Also, Ikpeme *et al.* (2006) reported that soyabean and peanut meal were more effective in promoting antibiotic production in mutant strains of *Bacillus pumilus* Bpu 32. The result is also in agreement with the work of Pham *et al*. (1992), who reported the use of carbohydrate sugar cane juice, molasses, banana, cassava and coconut water as source of carbon for methionine production.

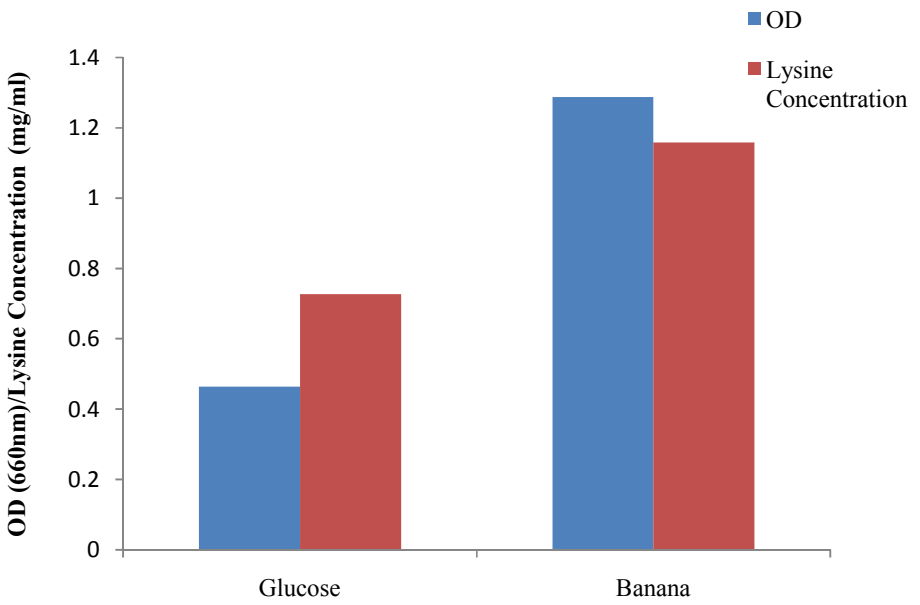


**Fig. 1: Effect of Carbon Sources on Lysine Production *Alcaligenes aquatilis***

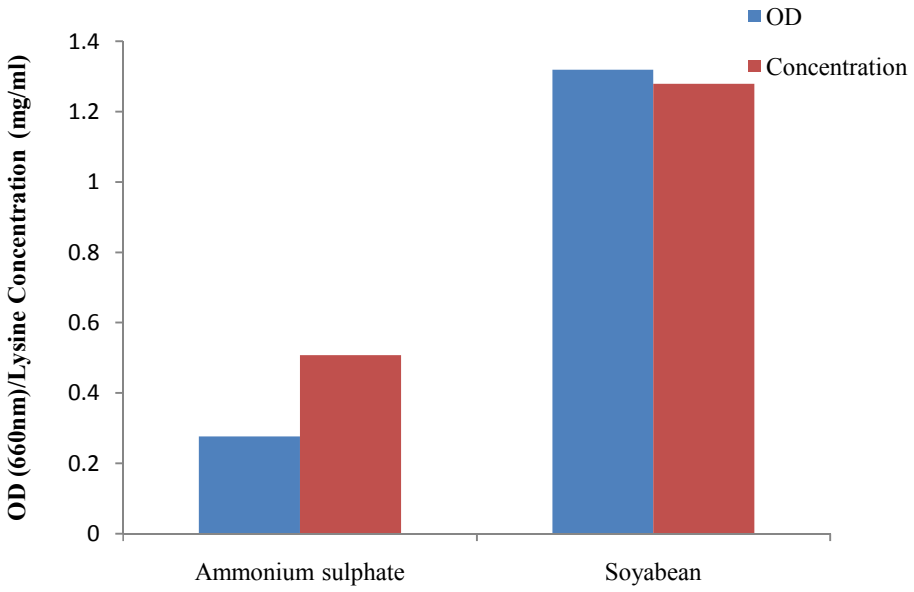


**Fig. 2: Effect of Nitrogen Sources on Lysine Production *Alcaligenes aquatilis***

## CONCLUSION

It was found that the fermentative method has the important advantage of yielding the optically active L-form of lysine directly. It was also established that agricultural products can be used for lysine production by fermentation and if well developed will reduce the importation of this product into the country and make it more readily available.

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