

## Assessment of Nutritional Value of Single Cell Protein from Waste Activate Sludge

E.M.N. Chirwa\*, D. Manyisi and T.M. Lebitso

Water Utilisation Division, Department Of Chemical Engineering,  
University of Pretoria, Pretoria, 0002, South Africa. \* Tel. 27 (12) 420 5894, Fax 27  
(12) 362 5089, Email: [Evans.Chirwa@up.ac.za](mailto:Evans.Chirwa@up.ac.za)

With the fast growing population of Southern Africa, pressure is exerted on the feed industry to produce enough animal feed to meet the region's nutritional requirements. Due to the rising cost of meat, people in poor communities of Southern Africa - especially children - suffer from severe malnutrition. The national requirement of protein supplement for South Africa is 90,000 metric tonnes per year. On the other hand, the protein value of sludge produced in treatment plants in Gauteng province alone (1/8 of the countries population) is approximated at 95,000 metric tonnes per year, enough to replace the commercial supply for the whole country. Sludge from wastewater treatment plants treating domestic wastewater is shown to contain approximately 50% (as concentration) of amino acids from fishmeal - the common protein supplement for livestock. The protein content in sludge and fishmeal is validated in this study. A method for extracting toxic heavy metals and its impact on the concentration of amino acids is also investigated.

### 1. Introduction

South Africa is a developing country with a fast growing population. Therefore more animal feed is required to serve the escalating population. The current local animal feed production, however, is insufficient to cater for the demands. Therefore the country is dependent on importation of Fishmeal and other nutrients sources in order to meet the required needs. The cost of importation of these animal feeds is extremely high. The purpose of this project is to study the nutritive potential of Waste Activated Sludge (WAS) as a cheaper replacement of Fishmeal in animal feed.

The final tailings of the sewage sludge are dealt with by soil application as fertilizers or buried in the ground, landfill, combustion and ocean dumping (Hwang et al., 2008). These methods require huge capital investments more than any other part of waste water treatment (Vriens et al., 1989; Hwang et al., 2008). Soil application of sludge is also rendered environmental unfriendly (Kasselman, 2004). However, WAS from sewage treatment plants depicts enormous potential to be used for animal feed due to its wide range of important nutrients. This is supported by feeding experiments reported supplements conducted earlier by others such as Vriens et al. (1989).

The sludge contains comparable amount of mineral elements, vitamins, nucleic acids, and amino acid proteins, to whole egg, symba yeast sludge, soybean and fishmeal meals

(Vriens et al., 1989). The ratio of mineral elements in sludge to that in the feedstock was also reported to be significantly higher (Vriens et al., 1989). This indicates that the sludge has sufficient minerals required by animals. Sludge was also found to be a very good source of vitamins, particularly vitamin B12 (Vriens et al., 1989).

However, the level of heavy metal content is reported to be more than two orders of magnitude higher than the levels in conventional protein sources (Vriens et al., 1989; Yoshizaki and Tomida, 2000; Hwang et al., 2008). Therefore it is recommended that the feed sludge be pre-processed to reduce heavy metal content to acceptable level. It was reported that washing of WAS with acids can lower the toxic levels of heavy metals significantly (Yoshizaki and Tomida, 2000).

In this study, protein and heavy metal analysis is conducted on both the WAS from local sewage plants and Fishmeal to determine the comparability of the two protein sources.

## 2. Materials and Methods

### 2.1 Sample Collection and Mass Analysis

Three samples from the thickened sludge reactors were collected on a 1litre plastic bottle each. They were dried in an oven in two glass beakers of 1Litre volume each. They were then manually crushed using a pestle on a porcelain dish. Leaching processes were done in a 400 mL glass beaker. All the mass measurements were obtained from a Precisa 4000C instrument after filtering the samples as shown in Figure 1 (below) through a 40 µm pore filter using a Neuberger N022AN pump as the vacuum pump.

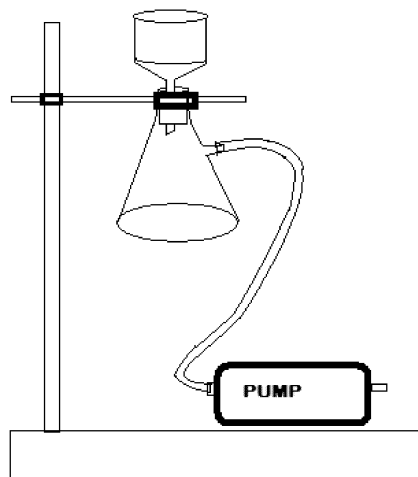


Figure 1. Experimental setup for filtration.

### 2.2 Sample Pre-treatment

Three samples of thickened WAS, each 1 litre volume, were collected from the Zeekoegat sewage works. The samples were dried in an oven at 105°C for a period of

three days. The dry sample was crushed into fine powder of a maize meal grade. The samples were weighed before and after treatment to determine the percent solids. The total sample solids recovered (that was usable in the experiments) was 103.9 grams.

### **2.3 Acid Leaching**

The heavy metals found in higher quantities are zinc, copper, cadmium, manganese and palladium. These metals are highly toxic to animals and humans when taken in large quantities. Hence they have to be reduced. In this study 1N of Hydrochloric acid was used to leach heavy metals from the sample. 50 g of dry, pre-treated, sludge was mixed with water to make it a pulp. The pulp was then mixed with 150ml of 1N hydrochloric acid. The mixture was stirred for 1 hour then allowed to separate. The solids were retained while the liquid was filtered to recover further solids. The solids were then dried while the filtrate was neutralized.

### **2.4 Preparation for Amino Acid Analysis**

The neutralization of the acidic filtrate (wastes) was done using sodium hydroxide pellets (Sigma-Aldrich) with red litmus paper used as neutralization control. The samples sent for elemental analysis were stored in medium-sized plastic containers while those sent for amino acid analysis were stored in small plastic containers.

During leaching and neutralization steps, the mixtures were stirred using a Pyrex glass rod. A South African premium grade Fishmeal was used for comparison purposes.

### **2.5 Amino Acid Analysis**

The amino acid analysis method used is pre-column derivatization with reversed-phase chromatography earlier reported by Bidlingmeyer et al (1984). The method consists of two sequential reaction steps (Hydrolysis of proteins and peptides and derivatization of amino acids with PITC) before the analysis of the samples in a chromatographical system.

### **2.6 Metals Analysis**

Samples were prepared as pressed powder briquettes and introduced to the ARL 9400XP+ XRF spectrometer. Analysis was executed using the UniQuant software. The software analyzed all elements in the periodic table between Na and U, but only elements found above the detection limits were reported. The values were normalised, as no LOI was done to determine crystal water and oxidation state changes. All metal elements were reported as oxides.

## **3. Results and Discussion**

### **3.1 Heavy Metal Content**

Heavy metals found in sewage sludge include zinc, copper, manganese, lead, cadmium and few others (Vriens et al., 1989). Some of these have undesirable health effects such as carcinogenesis and toxicity to animal tissue. The drop in mass of the retained sample in the acid leaching process is indicative that heavy were indeed extracted from the sample. The results of the samples assay are summarized in Table 3 below.

As can be clearly seen, the extraction ratio of the metals was very low. This could be attributed to a number of factors. Firstly, the time allowed for the acid to react with the elements in the sludge sample was insufficient. This may have seen the mixed sample

being isolated before the reaction goes to completion. The second factor that could have contributed to low extraction is the difference in densities of the metals in the powdered sample. Although extreme care was exercised to ensure that the powder is thoroughly mixed, heavier metals may have concentrated in one portion of the sample with others being found in the other. This can affect the distribution of the samples to the storage containers. The last factor could have been the higher content of acid insoluble metals. This would have meant that most of the samples were not dissolved in hydrochloric acid. It is also reported that in practice extraction in solid samples is lower than extraction in liquid samples (Kasselmann, 2004).

Table 1. Quantities of heavy metals found in samples

<b>Element (as Oxide)</b>	<b>Average Standard error</b>	<b>Dry Sludge (g/100g)</b>	<b>Fish meal (g/100g)</b>	<b>Leached Sludge*</b> (g/100g)
<b>TiO<sub>2</sub></b>	0.03	0.85	0.06	1.12
<b>Al<sub>2</sub>O<sub>3</sub></b>	0.10	3.79	3.98	4.02
<b>Fe<sub>2</sub>O<sub>3</sub></b>	0.22	9.77	0.69	8.97
<b>MnO</b>	0.00	0.16	0.02	0.08
<b>MgO</b>	0.10	1.80	0.36	0.90
<b>CaO</b>	0.29	4.88	22.60	2.34
<b>Cr<sub>2</sub>O<sub>3</sub></b>	0.00	0.13	0.00	0.14
<b>NiO</b>	0.00	0.04	0.00	0.02
<b>ZrO<sub>2</sub></b>	0.00	0.03	0.00	0.04
<b>BaO</b>	0.00	0.11	0.00	0.12
<b>CuO</b>	0.01	0.16	0.01	0.19
<b>ZnO</b>	0.05	3.14	0.07	1.88
<b>PbO</b>	0.00	0.03	0.01	0.03

\* Acid leached sludge

### 3.2 Amino Acid Distribution

The results of the amino acid analysis of dry sludge, fish meal and acid-leached sludge is indicated in Figures 2, 3 and 4 below. Looking at Figure 2, it can be clearly seen that all essential amino acids are present in WAS from sewage. This clearly indicates that sludge from sewage has the required nutritional value. Further analysis of the results indicates that the ratio of lysine to methionine, an important nutritional factor (Vriens et al., 1989), is nearly equal to 2, which compares well with that of Fishmeal (which is nearly 2.1).

Looking at Figures 2 and 4, it is noticed that the loss in amino acids during leaching occurred. It can also be noticed that the degree of amino acid loss is nearly the same for all essential amino acids. Therefore the degree of this loss appears to be having no prominent effect in the nutritional factors.

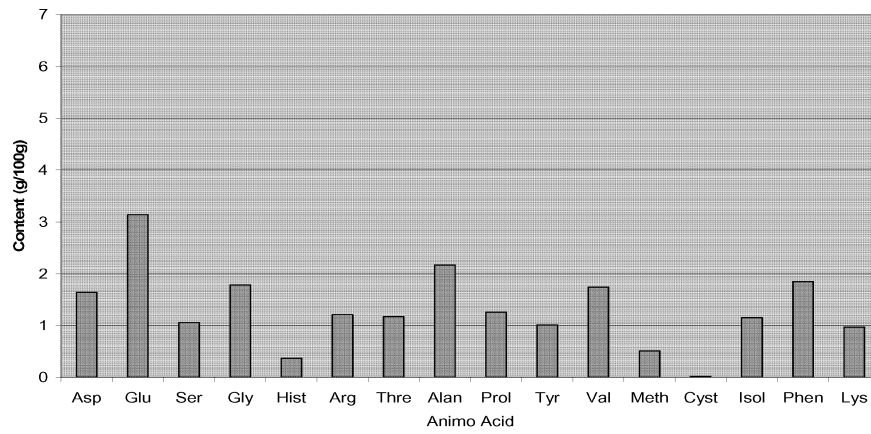


Figure 2: Quantity of Amino Acid in Dry Sludge.

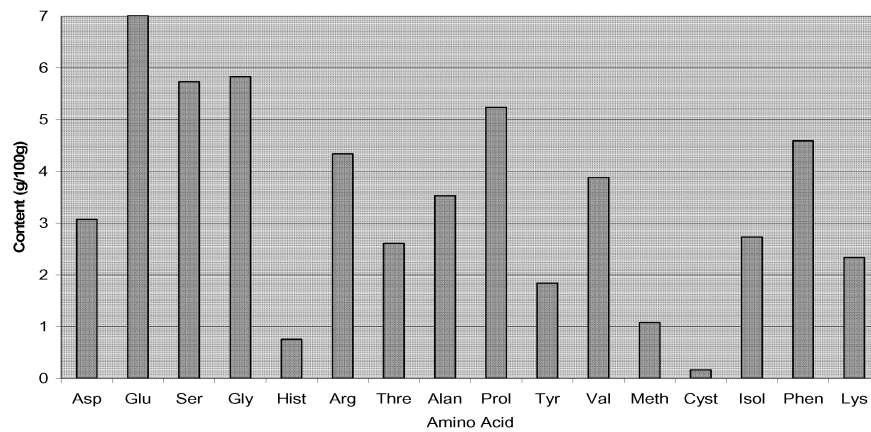


Figure 3: Quantity of Amino acids in Fish meal.

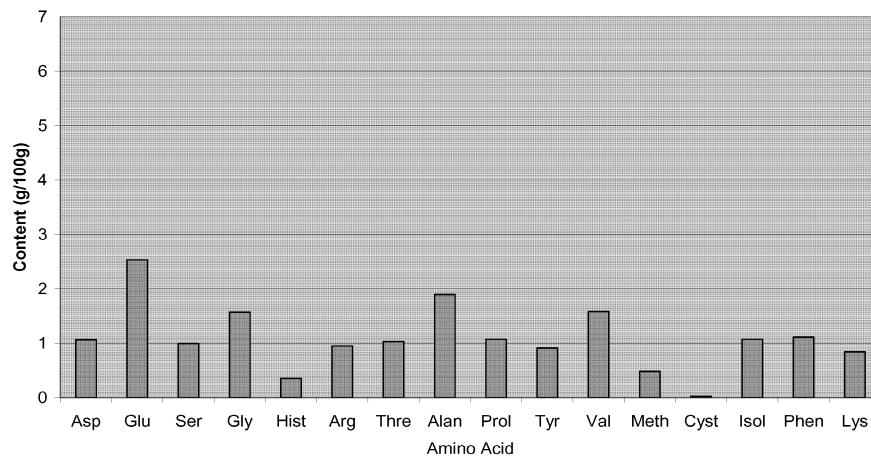


Figure 4: Quantity of Amino Acids in Dry Acid-Leached Sludge

However, when looking at Figures 2 and 3, it becomes apparent that the content of amino acids in fish meal is significantly higher than that in sewage activated sludge. The same can be seen for acid-leached dry sludge, as compared to fish meal, when looking at Figures 3 and 4. In actual fact, the ratios of amino acids in fish meal to dry sludge and acid-leached sludge are nearly uniform at 2 and 3 respectively.

The quantity of heavy metals in the sludge was extremely high. This could cause health problems in animals and accumulation in animal tissue making the meat unsuitable for human consumption. And very little heavy metals were removed by leaching with 1N hydrochloric acid. Better methods for removing the heavy metals are thus required in order to apply this technology to animals targeted for meat and dairy.

The acid leaching process was also seen to have washed out a certain quantity of amino acids (data not shown). Greater quantities of phenylalanine and aspartic acid, being at 35 and 40 percent respectively, were lost. The quantity of cysteine, however, was not washed out. Overall, the effect of the amino acid washed out is insignificant as the ratio is smoothed for all proteins.

When considering the nutritive value of the acid-leached sludge, it becomes evident that WAS from sewage is a rich source of proteins. The comparison of proteins in WAS and Fishmeal, though, sideline the use of WAS as the replacement animal feed for Fishmeal.

#### 4. Conclusion

If applied to animals the use of leached dried sludge is strongly recommended. At the current leaching rate, three volumes of leached sludge could need to be used to replace one volume of Fishmeal in order to maintain the amino acid proportion comparable to Fishmeal.

#### 5. Acknowledgements

We thank the University of Pretoria for the support through the Research Development Programme (UP-RDP Fund). We also thank the Departments of Biochemistry and Geography for assistance with analytical work.

#### References

- Yoshikazi, S. and Tomida, T. (2000). Principle and process of Heavy Metal Removal from Sewage Sludge. *Environ. Sci. Technol.* 34, (8), 1572-1575.
- Vriens, L., Nihoul, R. and Verachtert, H. 1989. Activated Sludge as Animal Feed: A Review. *Biolog. Wastes* 27, 161-207.
- Bidlingmeyer, B.A., Cohen, S.A. and Tarvin, T.L. (1984). Rapid analysis of Amino Acids using Pre-column Derivatization. *J. Chromatogr.* 336, 93-104.
- Chou, C.L., Haya, K., Burridge, L and Moffatt, J.D. (2002). Aquaculture-related trace metals in sediments and lobsters and relevance to environmental monitoring program ratings for near-field effects. *Mar. Pollut. Bull.* 44, 1259-1268.
- Kasselmann, G. (2004). An evaluation of predictive environmental test procedures for sewage sludge. Pretoria: University of Pretoria.
- Hwang, J., Zhang, L., Seo, S., Lee, Y. and Jahng, D. (2008). Protein recovery from excess sludge for its use as animal feed. *Bioresour. Technol.* 99, 8949–8954.