Eco. Env. & Cons. 29 (January Suppl. Issue) : 2023; pp. (S214-S216)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i01s.033

Valuable Material Recovery-protein from Faecal Sludge Collected during Sars-cov-2 Period from Bengaluru-rural

Hansa K.[1], M.C. Sampath Kumar[2] and Priya V.[3]

[1]Environmental Engineering, Department of Civil Engineering, BMS College of Engineering, Bull Temple Road, Bengaluru, Karnataka, India
[2,3]Department of Civil Engineering, BMS College of Engineering, Bull Temple Road, Bengaluru, Karnataka, India

(Received 2 June, 2022; Accepted 28 July, 2022)

ABSTRACT

This study describes the extraction of protein from faecal sludge as a valuable commodity or recovery of valuable. Recovery of valuable materials from waste would give rise to business opportunities which will pave way for feasible and sustainable sanitation. Sludge samples collected from Faecal Sludge Treatment Plant were used to extract proteins from faecal sludge. The Protein was extracted using simple alkali treatment followed by quantification of the protein by Lowry Assay using Bovine Serum Albumin (BSA) as standard.

Key words : *Faecal sludge, Protein extraction, Protein quantification, Upcycling, Resource Recovery, Recycle and Reuse*

Introduction

All over the world, the humankind generates approximately 290 billion Kg of faeces (TIME, 2015). The vast majority of this generated faecal matter remains untreated or is illegally dumped which ends up polluting water bodies. This has the potential to cause serious health problems as well as have a negative impact on the environment and economy and hence is a matter of concern. The solution to this problem is to look into material recovery options for faecal matter.

Faeces is composed of water, protein, undigested fats, polysaccharides, bacterial biomass, ash, and undigested food residues (Rose *et al*., 2015) and is a significant source for resource recovery. Given the current Covid-19 pandemic, it is necessary to highlight the variable composition of the faecal sludge to include the causative virus as a whole or/and viral RNA; outer coat proteins; any consequential traces thereof. The dynamics of the presence of virus, viral molecules have to be considered within the boundaries of this project. Based on viral detection carried out by Xiaoming Wang *et al*. (2020) in 69 stool samples, 20 samples resulted in a positive test for presence of virus as a whole or/and viral RNA; outer coat proteins; consequential traces. This is indicative of the virus finding itself a place in the microbiome of the infected human body ranging from the respiratory system where it displays extreme pathogenicity to the digestive system where the pathogenicity has caused relatively mild discomfort in the form of diarrheal stool which was one of the primary indicative symptoms of infection (Meng Guo *et al*., 2021). To further add to the newly emerging body of work co-relating human stool to the vi-

([1]PG Student, [2]Professor, [3]Assistant Professor)

rus, Chan *et al.* (2020) have studied the lifespan of the virus present in the stool of infected individuals from the time of excretion. They have stated that the virus is active for up to 7 days at 25– C and 1-2 days at 33-37– C. SARS-CoV-2 lost infectivity at extreme pH value of 2-3 and 11-12 in 24 hours.

Given the large variations in the compositions in addition to the likely presence of a viral load of the SARS-CoV-2, they commonly exist as a result of an individual's diet, i.e., food and fluid ingested which is dependent on the regional diet patterns and would be similar across that region. In terms of composition fractions, faecal matter is potentially a rich source of protein with the bacterial biomass accounting for about 25-54% of dry solids (Rose *et al*., 2015) and proteins accounting for about 50% of the dry weight of those bacterial cells. This has been a well-known subject matter and several attempts have been made in the past to recover proteins from sewage sludge and studies comparing the effectiveness of different methods of protein extraction. Ras *et al.* (2008) investigated the efficiency of combinations of mechanical and chemical treatment on protein extraction and quantification from activated sludge collected at two different wastewater treatment plants. Lerch *et al.* (1993) investigated on the suitable method for protein extraction from sewage sludge where they employed mechanical treatment such as grinding followed by chemical treatment where protein was extracted using three extractants namely, H_2O, NaOH and Triton X-100 followed by quantification of the extracted protein. These mechanical and chemical methods of extraction aid in sludge disintegration releasing the protein in the solution for their recovery.

Materials and Methods

Preliminary work

Sample of faecal sludge was collected from the Devanahalli, Karnataka-based Faecal Sludge Treatment Plant (FSTP). Due to the current situation, faecal sludge sample is likely to include evidence of Covid-19 causing pathogen SARS-CoV-2. Considering the pandemic and hence the probable presence of the pathogen, additional care and precautions were taken into account during sample handling and preparation.

The faecal sludge sample was oven-dried at two different temperatures namely 50 °C and 90 °C. These samples were labelled as DFS-50°C and DFS-90 °C denoting the temperatures at which the sludge samples were dried. After complete drying, the samples were reduced to fine powder using a mortar and pestle. To avert protein denaturation, the powdered sludge samples were kept in a refrigerator under inert conditions.

Protein extraction and quantification

The samples DFS-50 °C and DFS-90 °C were subjected to alkali treatment. For this, the samples were treated with 1M NaOH and were stirred for 2 hours. The resulting samples were subjected to centrifugation at 3000 rpm for 40 minutes. The supernatant and pellet from sample DFS-50 °C was labelled as S-50 and P-50 respectively. Similarly, supernatant and pellet from sample DFS-90 °C was labelled as S-90 and P-90 respectively. S-50, P-50, S-90, P-90 was analysed for protein content. P-50 and P-90 were suspended in pH 7 buffer and then centrifuged. The resulting supernatants containing the crude protein were collected and stored at 4 °C until further analysis. The samples were quantified for protein by Lowry assay. The protein standard used was Bovine Serum Albumin (BSA).

Results and Discussion

Extraction and Quantification

For protein extraction alkali treatment was used as alkali increases the protein solubility in the solution. Alkali breaks down the protein into peptides reducing their molecular weight and hence increasing the solubility of protein (Jamdar *et al.,* 2010; Erkan Yalçýn *et al.,* 2007). Alkali also helps in disintegration of cell resulting in higher protein yield. The resulting proteins were quantified (Table 1) by Lowry's Assay due to its simplicity and sensitivity. The protein content decreased at a higher temperature of 90 °C when compared to 50 °C (Figure 1). The trend describes temperature is a key component to extract the protein at lower thermal vitality.

Conclusion

The present work, designed to extract the proteins from faecal sludge that could be recovered by a simple extraction. For every 0.5 g of faecal sludge, we were able to extract 658.375 µg and 478.45 µg of protein from sludge dried at different temperatures.

Table 1. Protein quantification by Lowry's Assay

S.No.	Protein Content	Temperature 50 °C	Temperature 90 °C
1.	Total protein in Supernatant	240.7 µg	213.45 µg
2.	Total protein in Pellet	417.675 µg	265 µg
3.	Total protein in 0.5 g sludge	658.375 µg	478.45 µg
4.	*Total protein in 1 g sludge	1.31675 mg	0.9569 mg
5.	*Total protein in 290 Bn Kg	381857500 kg	27750100 kg

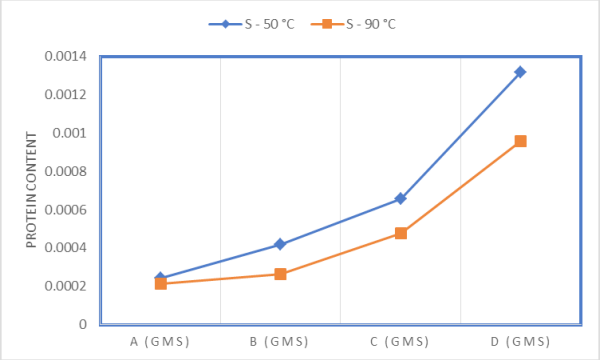

Fig. 1. Relation between protein content and temperature

This present process facilitates to recover the protein unutilised in rural and urban regions. The scope of the work suggests the possibility of improving recovery of protein from the simple way for various applications such as bio-component of natural fertilizer and base material of animal food products.

References

Chan, K.H., Peiris, J.M., Lam, S.Y., Poon, L.L.M., Yuen, K.Y. and Seto, W.H. 2011. The effects of temperature and relative humidity on the viability of the SARS coronavirus. *Advances in Virology*. 2011.

Chan, K.H., Sridhar, S., Zhang, R.R., Chu, H., Fung, A.F., Chan, G., Chan, J.W., To, K.W., Hung, I.N., Cheng, V.C. and Yuen, K.Y. 2020. Factors affecting stability and infectivity of SARS-CoV-2. *Journal of Hospital Infection.* 106 (2) : 226-231.

Denecke, M. 2006. Protein extraction from activated sludge. *Water Science and Technology*. 54(1) : 175-181.

Guo, M., Tao, W., Flavell, R.A. and Zhu, S. 2021. Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. *Nature Reviews Gastroenterology & Hepatology.* 1-15.

Hansa, K., M.C. Sampath Kumarand Priya, V. 2022. Ecology, Environment and conservation Journal, Issue Vol 28, Feb Suppl. Issue 2022, "Detection of valuable material-protein in faecal sludge.

Jamdar, S.N., Rajalakshmi, V., Pednekar, M.D., Juan, F., Yardi, V. and Sharma, A. 2010. Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. *Food Chemistry*. 121(1) : 178-184.

Lerch, R.N., Barbarick, K.A., Azari, P., Sommers, L.E. and Westfall, D.G. 1993. *Sewage sludge proteins: I. Extraction Methodology*. 22(3) : 620-624. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Mallory, A., Holm, R. and Parker, A. 2020. A Review of the Financial Value of Faecal Sludge Reuse in Low-Income Countries. *Sustainability.* 12(20) : 8334.

Paul, D., Kolar, P. and Hall, S.G. 2021. A review of the impact of environmental factors on the fate and transport of coronaviruses in aqueous environments. *NPJ Clean Water*. 4(1): 1-13.

Pervaiz, M. and Sain, M. 2011. Protein extraction from secondary sludge of paper mill wastewater and its utilization as a wood adhesive. *Bio Resources*. 6(2) : 961-970.

Ras, M., Girbal-Neuhauser, E., Paul, E., Spérandio, M. and Lefebvre, D. 2008. Protein extraction from activated sludge: an analytical approach. *Water Research*. 42(8-9) : 1867-1878.

Redmile-Gordon, M.A., Armenise, E., White, R.P., Hirsch, P.R. and Goulding, K.W.T. 2013. A comparison of two colorimetric assays, based upon Lowry and Bradford techniques, to estimate total protein in soil extracts. *Soil Biology and Biochemistry*. 67 : 166-173.

Rose, C., Parker, A., Jefferson, B. and Cartmell, E. 2015. The characterization of feces and urine: a review of the literature to inform advanced treatment technology. *Critical Reviews in Environmental Science and Technology*. 45(17) : 1827-1879.

TIME, 2015. How Poop Can Be Worth $9.5 Billion. Available at https://time.com/4098127/human-waste-energy-recycling/ (2015).

Xiao, K. and Zhou, Y. 2020. Protein recovery from sludge: a review. *Journal of Cleaner Production.* 249 : 119373.

Yalçýn, E. and Çelik, S. 2007. Solubility properties of barley flour, protein isolates and hydrolysates. *Food Chemistry*. 104(4) : 1641-1647.