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Short Communication

Nutritional and Protein Characterization of Leachate from the São Paulo Zoo composing Unit

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Introduction

In recent years the, Foundation Zoological Park of São Paulo (FZPSP) implemented and put into operation the Organic Composting Production Unit (OCPU). The main goal of this action was to give a new destination to the organic waste collected daily in the park since before that sustainable attitude, this waste had the same fate as the usual household waste: landfills. Currently, the composting produced in OCPU serves as fertilizer for the park gardens and food cultivation in the Rural Production Unit (UPR), where much of the food served to the animals in the Zoo (ZOO) and Zoo Safari comes from Bernal et al [1].

Leachate is a liquid residue of high organic contents and strong color produced during the percolation of water through the composted organic waste. During the passage of this water, various chemical, physico- chemical and fermentation processes occur concurrently and, for this reason, various organic and inorganic compounds, besides that microorganism and its metabolites can be transferred to the leachate. Thus, leachate from different sources can be considered as a distinct matrix with specific characteristics.

Currently, what is known of leachate are those generated in landfills that have a high toxic potential. But it can be considered that the one produced during the composting in FZPSP is different, due to the nature of the organic material and also the water used to cool the composters, which runs several times by the composting systems. Therefore, because there is very few information on leachates from different sources other than municipal landfills, by the differentiated characteristics of the process and also of the materials used during composting at OCPU there was interest in studying this particular leachate, the potential of this matrix in bringing nutritional and proteomic information.

Material and Methods

Digestion of the leachate sample

The materials used for composting are shredded tree branches and leaves from the surrounding Atlantic rain forest, manure, waste food and carcasses from small and large animals (previously reduced in the room necropsy).

The leachate sample collected for the experiments circulated by the cooling system of the production of composting for a period of 40 days. After this period, about 5 L of this leachate was collected in an amber bottle and stored at 4˚C.

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Determination of macro and micronutrients

The digestion of the leachate for analysis of macro and micronutrients was done according to the literature Carrilho et al. [2]. After digesting the sample, the nitrogen was analyzed according to the specifications of the spectrophotometer protocol HACH, DR 6000 (Loveland, CO, USA).The nutrients As, Cr, Pb, Cd, Ca, K, Mg, Mn, Cu, Zn, P, Fe, Na, and Al were determined inductively coupled plasma optical emission simultaneous spectrometer with radial view ICP OES VISTA RX (Varian - now part of Agilent Company - Mulgrave, Australia) was used for elements determination according to the conditions cited in literature Carrilho et al. [2].

Protein extraction and analysis

The proteins from leachate were extracted using the method outlined by Wang and colleagues Wang et al. [3].Digested peptides were subjected to analysis by nano-LC/MS-MS using a nano-LC system (EASY-NLC II, Thermo Scientific) coupled online to a hybrid ion trap linear-Orbitrap (LTQ Orbitrap Velos, Thermo Scientific) mass spectrometer, through a nanospray source Nano-Flex II nanospray ion (Thermo Scientific). The mobile phases used were: A) 0.1% formic acid in water and B) 0.1% formic acid in ACN. The pre-column used was (C18, 100 µm ID x 2 cm, Thermo Scientific) and C18 column (10 cm × 75 µm ID, 3 µm, 120 Å, Thermo Scientific). The gradient used was: 5% B isocratic, 0-5 min; 5% - 35% B, 5-65 min; 35 - 90% B, 65-80 min, 5% B isocratic, 80-90 min. The total analysis time, from column equilibration to the analysis, was approximately 105 min. All LC/MS-MS data were acquired using X Calibur software, version 2.0.7 (Thermo Fisher Scientific). LC-MS data files (MS2 centroided) were used for database searching with MASCOT (Matrix Science, version 2.3.0.0).

Results

The results of N present in the FZPSP leachate can be seen in (Table 1), together with published data from other leachates. Currently, there aren't studies in the literature reporting amounts of nutrients in leachates with the same characteristics of those produced by FZPSP. There are only studies on leachates from landfills, piggeries, poultry farms, among others.

Table 1: Macro and micronutrients analysis of the FZPSP leachate.

Parameters	Concentration (mg L^{-1})*	Parameters	Concentration (mg L^{-1})*
Total N	106	Ca	136
Ammoniacal N	0.86	Mg	88
P	16	Zn	0.45
K	1397	Pb	< LOD**
Na	452	Cd	< LOD**
Cu	0.14	Cr	0.02
Fe	7.51	As	2.6
Mn	0.3		

*n = 4; ** Limit of Detection. The standard deviation of all means of the samples analyzed was below 7%.

The nutrients found in FZPSP leachate can be observed in (Table 1). The use of leachate as an adjunct source of nutrients to the crop occurs mainly to reduce costs in agriculture. However, a preliminary analysis of this material is necessary as there may be excess of certain nutrients that cause damage to the soil and crops.

The results obtained from the shotgun of the leachate proteomics yeld a low score, which was expected since the leachate is a waste from composting and has much interference that make it difficult to extract and identify the proteins. Therefore, few proteins could be determined. Only those with a score equal to or greater than 30% (acceptable value for the shotgun technique reference [1] were considered. Thus, 16 proteins were identified, all belonging to bacterial genera as described below: MEMO1 family protein APE_1771 (*Aeropyrum pernix*); Uncharacterized protein AF_1654 (*Archaeoglobus fulgidus*); Protein translation factor SUI1 homolog (*Cenarchaeum symbiosum*); tRNA-guanine(15) transglycosylase (*Methanobrevibacter smithii*); Phosphoenolpyruvate guanylyltransferase and L-fuculose phosphate aldolase (*Methanococcus aeolicus*); L- lysine 2,3-aminomutase (*Methanococcus maripaludis*); Probable L-aspartate dehydrogenase (*Methanospirillum hungatei JF-1*); 50S ribosomal protein L1 (*Pyrobaculum islandicum)*; Adenosylhomocysteinase (*Saccharolobus solfataricus*); DNA double-strand break repair Rad50 ATPase (*Saccharolobus solfataricus)*; S- adenosylmethionine decarboxylase proenzyme (*Sulfolobus acidocaldarius*); Phosphoribosylaminoimidazole-succinocarboxamide synthase (*Sulfurisphaera tokodaii*); NAD kinase (*Sulfurisphaera tokodaii*); Maltodextrin phosphorylase (*Thermococcus litoralis);* Probable tRNA pseudouridine synthase B (*Thermococcus onnurineus*). It is interesting to note that the bacteria related to the proteins found live in extreme environments (such as extremophiles, hyperxtrmophiles, acidophiles, etc) . and/or those capable of generating energy from sulfur. That is, they are able to survive in the leachate, which is an extreme condition. In general, the proteins identified have the function of obtaining energy to maintain the bacteria.

Conclusion

Observing the results presented, it can be concluded that the leachate produced from the FPZSP composting process can be used in fertilizing plants as an organic fertilizer in addition to being a potential source of molecules to be explored for different applications.

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