Aquaculture feed from *Thauera* sp. cells obtained from the acidogenic fermentation of agricultural wastes

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Keywords: Single cell proteins, *Thauera*, VFAs, PHAs, acidogenic fermentation, aquaculture feed
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Single cell proteins (SCP), is a term that describes the application of dried microorganisms (bacteria, algae, fungi and yeasts) as food and feed (Saeed *et al*, 2016). Indeed, due to the ever-growing population, the demands of protein-rich food for human and animal nutrition is continually increasing (FAO, 2018). The current use of plant-based proteins for such scope is not environmentally sustainable because of the high CO_2 footprint of agriculture (Vermeulen *et al*, 2012) and is also problematic because of the scarcity of arable land (Mekonnen and Hoekstra, 2014). SCP, being rich in proteins, vitamins and lipids, can be used as supplements in human food and as animal feed. Furthermore, SCP can be obtained by aerobic and anaerobic fermentation of inexpensive feedstocks such as agricultural residues and animal manure, making them more economical than traditional sources of proteins, and helping with the environmental burden created by the disposal of such wastes (Nasseri *et al*, 2011). The use of polyhydroxyalkanoates (PHAs) producing microbes in the anaerobic reactors can be advantageous because of the added value provided by PHAs in feed, since they have been shown to help protect aquatic animals from pathogenic bacteria (Saguna *et al*, 2014; Laranja *et al*, 2017); PHAs producing organisms can be used directly as SCP without the need of expensive extraction and purification procedures, which are at present hindering their use as bio-plastics (Chee *et al*, 2019).

This study aimed at a preliminary assessment of potential feed for aquaculture originating from cells of the gram-negative proteobacterium *Thauera* sp., obtained from bio-based VFAs (volatile fatty acids) produced by acidogenic fermentation of agricultural and animal wastes. The bacterial strain, namely Thauera sp. Sel9, was previously isolated from a mixed microbial culture (Conca *et al*., 2020), has a 99% similarity to *Thauera butanivorans* NBRC 103042T (Botturi *et al*., 2021) and is a producer of PHAs (Sabapathy *et al*, 2020). In order to assess the productivity of such system, the fermentation was initially performed in batch reactors, and then evaluated in fed-batch mode in a stirred reactor. The culture was supplied with oxygen, kept at 30˚C, stirred at 60 rpm and fed with digestate twice a day for 15 minutes at the rate of 3 ml/minute (Figure 1). Initial experiments were performed with artificial feed, containing VFAs at 1 g COD_{VFA}/L in different concentration (100% acetic acid or 100% propionic acid, or 50:50, v/v) and different carbon/nitrogen ratios (C/N), in order to assess *Thauera* growth kinetics and identify the best one for optimal production. Subsequently, the real fermentation liquid (coming from the anaerobic digestion plant "La Torre" located in Isola della Scala, Verona, Italy) was characterised, and was used to feed the bacterial culture (after filtration at 0.20 μm); a synthetic feed imitating the concentration of the three main VFAs (acetic, propionic and butyric acid) found in the mixture was used as reference. Different hydraulic retention times (HRT) were tested, with steady-state conditions reached when MLSSs (mixed liquor suspended solids) variations were less than 5% for 3 times the value of the HRT. The biomass obtained from the *Thauera* culture was collected for analyses aimed at determining the yield and its centesimal composition, with particular focus on the amino acid composition, for the evaluation of its suitability as fish feed. In the coming months, feeding trials with Zebrafish have been planned, aimed at assessing the digestibility and the growth rate of the fish when compared to the control feed.

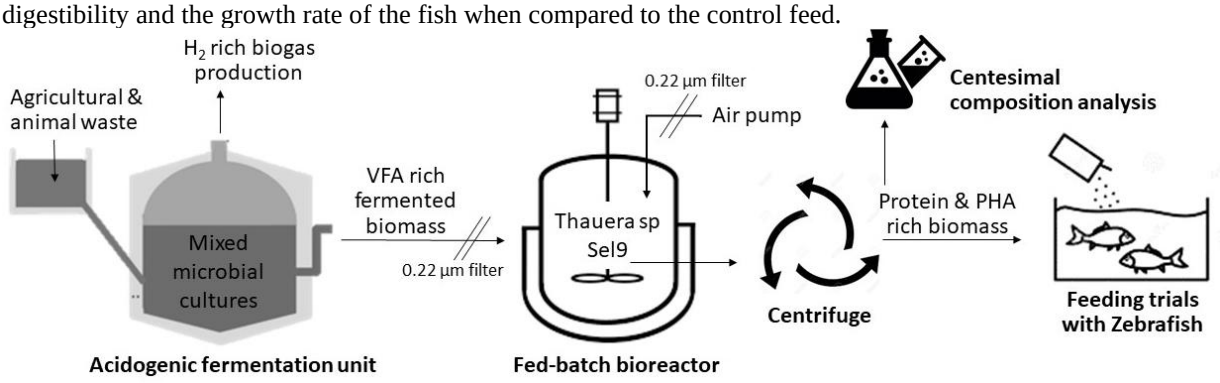

Figure 1. Diagram of the experimental fed-batch mode set-up for the production of SCP from *Thauera* sp. Sel9 to be used as aquaculture feed.

Table 1 presents the results of the analysis of the fermentation fluid, which has a total soluble COD of 26.93 g/L, to which the VFAs contribute with 18.44 g/L. Among the VFAs, acetic acid is the most abundant (43.8%), followed by butyric (21.2%) and propionic acid (20.2%), while pentanoic, isobutyric and isopentanoic make up together to the remaining 14.8%.

Table 1. Characterisation of the fermentation liquid used for the growth of *Thauer*a sp. Sel9 in the bioreactor.

Parameter	Acronym	Unit	Value
Soluble COD	sCOD	gCOD/L	26.93
Volatile Fatty Acids	VFAs	gCOD/L	18.44
Total solids	TS	%	13.30
Ammonium	NH_4^+	g/L	1.64
Phosphates	PO_4^{3-}	mg/L	258.50

Volatile fatty acid	%
Acetic	43.8
Butyric	21.2
Propionic	20.2
Pentanoic	8.1
Isobutyric	3.7
Isopentanoic	3.0

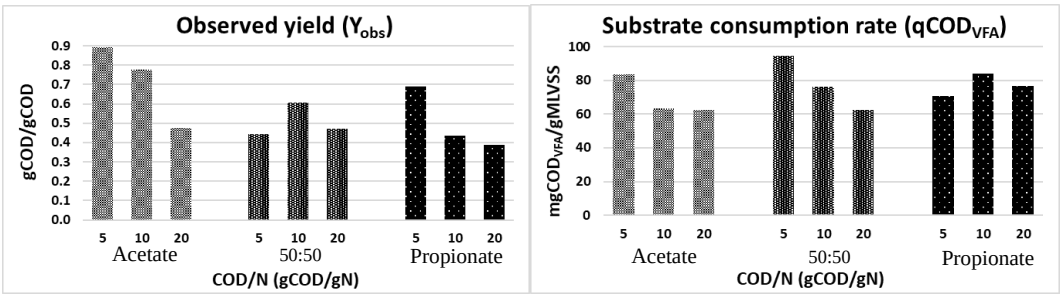

Figure 2. Thauera's growth kinetics when fed with different concentration of VFAs and with different COD/N ratios.

Thauera's growth kinetics show that the best yield is obtained when fed with acetic acid and with a COD/N ratio of 5; the latter is also the best one for propionic acid, while a 50:50 mixture of the two acids gives the lowest values (Figure 2). The COD/N ratio of 5 also gives the highest rate of removal of VFA by the bacteria, which normally use it up within 48 hours after inoculation for the different types of artificial feed; the rates however are different, and normally faster for propionic acid. NH_4^+ concentration decreases in a similar way for the different feeds (data not shown), indicating an uptake of nitrogen, an important value in light of the interest in the aminoacidic composition of the bacteria to use as SCP.

Limitations on the use of SCP as fish feed depend on the presence of high amounts of nucleic acids and toxins (Anupama and Ravinda, 2000); further work is therefore needed to quantify the presence of such unwanted compounds in *Thauera*-derived SCP.

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