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Autotrophic nitrogen assimilation and carbon capture for microbial protein production by a novel enrichment of hydrogen-oxidizing bacteria

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ABSTRACT

Domestic used water treatment systems are currently predominantly based on conventional resource inefficient treatment processes. While resource recovery is gaining momentum it lacks high value end-products which can be efficiently marketed. Microbial protein production offers a valid and promising alternative by upgrading low value recovered resources into high quality feed and also food. In the present study, we evaluated the potential of hydrogen-oxidizing bacteria to upgrade ammonium and carbon dioxide under autotrophic growth conditions. The enrichment of a generic microbial community and the implementation of different culture conditions (sequenced batch resp. continuous reactor) revealed surprising features. At low selection pressure (i.e. under sequenced batch culture at high solid retention time), a very diverse microbiome with an important presence of predatory *Bdellovibrio* spp. was observed. The microbial culture which evolved under high rate selection pressure (i.e. dilution rate $D = 0.1\ h^{-1}$) under continuous reactor conditions was dominated by *Sulfuricurvum* spp. and a highly stable and efficient process in terms of N and C uptake, biomass yield and volumetric productivity was attained. Under continuous culture conditions the maximum yield obtained was 0.29 g cell dry weight per gram chemical oxygen demand equivalent of hydrogen, whereas the maximum volumetric loading rate peaked 0.41 g cell dry weight per litre per hour at a protein content of 71%. Finally, the microbial protein produced was of high nutritive quality in terms of essential amino acids content and can be a suitable substitute for conventional feed sources such as fishmeal or soybean meal.

1. Introduction

Primary producers - autotrophic microorganisms - are essential for carbon and nutrients cycling. While fixing inorganic CO_2 into organic biomass they recycle nutrients (N and P) and provide food for higher life forms (Elser et al., 2000). Primary producers such as algae and autotrophic bacteria can serve as alternative protein source in the form of microbial protein (MP) for livestock but also for human consumption (Anupama and Ravindra, 2000; Verstraete, 2015; Walsh et al., 2015). Besides protein, microbes can also accumulate considerable amounts of biocompatible prebiotics such as PHB (Defoirdt et al., 2007), thereby enhancing the nutritional value of the microbial biomass.

After being extensively studied in the past, mainly as means to upgrade fossil fuel (e.g. paraffin, natural gas) to protein supplements (Westlake, 1986), the use of bacteria for microbial protein (MP) production has nowadays re-gained significant interest (Aas et al., 2006; Marit Berge et al., 2005) with natural gas based MP production entering the market economy (Strong et al., 2015). Innovative approaches implementing bacteria to produce MP within the context of resource recovery from used water have also been recently proposed (Lee et al., 2015; Liu et al., 2016; Matassa et al., 2015a). Indeed, the production of MP can allow the up-cycling of nitrogen and carbon dioxide recovered from used water streams, converting them into protein-rich feed and food substances. Different physico-chemical techniques can be implemented in the recovery of N and C substrates. Air stripping or pervaporative processes can recover N from concentrated streams

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such as anaerobic digestate, whereas pressure swing adsorption (PSA) can concentrate CO_2 from biogas, thus providing the building blocks which are at the base of MP biosynthesis.

Among the various metabolic pathways suitable for MP production, including both eukaryotic and prokaryotic microorganisms (Anupama and Ravindra, 2000), autotrophic hydrogen-oxidizing bacteria (HOB) constitute a special and thus far unexplored metabolic niche with potential for novel applications in resource recovery and upgrade. Even if ubiquitous, autotrophic HOB have only received limited attention, with previous studies focusing on the use of axenic cultures comprising bacteria such as *Alcaligenes eutrophus*, *Ralstonia eutropha*, *Seliberia carboxydohydrogena* (Ishizaki and Tanaka, 1990; Repaske and Mayer, 1976; Volova and Barashkov, 2010). The metabolic features of autotrophic HOB allow them to grow on hydrogen (electron donor) and oxygen (electron acceptor) while fixing carbon dioxide into cell material and assimilating nitrogen into high quality protein (Parkin and Sargent, 2012; Pohlmann et al., 2006). MP produced by autotrophic HOB is characterized by all the essential amino acids, having an amino acid profile closer to high-quality animal protein rather than to vegetable protein (Volova and Barashkov, 2010). Given this interesting feature, autotrophic HOB were already proposed as possible protein source within biological life support systems for space missions (Bartsev et al., 1996), as well as for human and animal nutrition (Volova and Barashkov, 2010).

An attractive characteristic of MP production with autotrophic HOB is the possibility to exploit the increasing potential of renewable energy generation. A clear example is the use of hydrogen gas produced from water electrolysis, powered by e.g. wind or solar energy, or also from biomass gasification (Ni et al., 2006) Recently, biomethane has also been proposed as possible renewable feedstock for hydrogen production by means of a combined heat, hydrogen and power generation unit (CHHP) (Agll et al., 2013; Hamad et al., 2014). The possibility to implement such technologies on-site and produce hydrogen on demand might enable the direct up-cycling of mineral nitrogen and carbon dioxide recovered from wastewater treatment plants, as previously mentioned. Moreover, upcoming technological developments and the decrease of hydrogen prices (Ball and Weeda, 2015) justify further research efforts towards the application of autotrophic HOB within resource recovery and up-cycling.

In the present study, we aimed to experimentally determine the feasibility of nitrogen and carbon upgrade into MP by means of a microbial community enriched in HOB using a lab-scale gas. Along the experimental investigation different culture conditions were imposed to the enriched HOB culture (i.e. sequenced batch and continuous). This was done in order to establish how the microbial community was shaped by the process conditions and how this affected the overall biological performance of the system, aiming at maximizing MP production (i.e. biomass yield and volumetric productivities). Nitrogen under the form of ammonium salt and gaseous CO_2 represented the N and C substrates needed for the production of MP protein by means of autotrophic HOB. The study started with the enrichment of a generic aerobic microbial mixed culture with autotrophic HOB under sequencing batch reactor operations. Consequently, the enriched mixed community was cultured in a continuous reactor configuration, resulting in the ongoing evolvement of a highly specific bacterial culture dominated by the genus *Sulfuricurvum*. The efficiency of the process in terms of gas utilization and by-product formation was monitored along the time course of the selective enrichment process. The microbial community analyses of the HOB microbiome under batch and continuous culture systems allowed delineating the evolution of the mixed bacterial community towards a quasi-monoculture dominated by *Sulfuricurvum* spp. Finally, the MP produced was characterized in terms of crude protein content and amino acid profile in order to assess its nutritional value.

2. Material and methods

2.1. Enrichment of hydrogen-oxidizing bacteria

Aerobic sludge from a local food (potatoes) processing plant (Nazareth, Belgium) was used as an initial mixed culture for the enrichment of autotrophic HOB community. The enrichment was carried out in a 1 L gas fermentor. The fermentor was connected to 3 gas bags supplying a gas mixture composed by $H_2/O_2/CO_2$ with the following composition: 65/20/15 (vol/vol). Prior to use, each gas bag was flushed with Alphagaz 2-grade H_2, O_2 and CO_2 gasses (Air Liquide, Belgium). The gaseous $H_2/O_2/CO_2$ atmosphere was constantly recirculated between the culture vessels and the gas bags by means of a peristaltic pump adapted to gas recirculation (Sci-Q 300, Watson Marlow, Belgium). The reactor was placed in a 28 °C temperature controlled room and shaken at 150 rpm. A volume of 500 mL of mineral media inoculated with 10% of inoculum was used at start. The mineral medium was prepared accordingly to Yu et al. (2013) for HOB isolation and culturing. The growth of HOB was followed by monitoring the increase of cell dry weight (CDW) over the course of the experimental run. When ammonium nitrogen was depleted, 50 mL of bacterial culture was withdrawn and diluted into 450 mL of fresh medium to restart the enrichment. After a stable and reproducible growth was attained in terms of CDW concentrations (2–3 g CDW/L before medium replenishment), the culture was considered enriched and used to start the experimental phase in the final reactor setup.

2.2. Reactor operations and controls

A completely stirred tank reactor (CSTR) (Biostat A plus, Sartorius, Belgium) was used during batch as well as continuous experiments. The 5 L glass vessel, with a working volume of 3 L, was stirred at 700 rpm with a 3-blade segment impeller to ensure completely mixed conditions. Hydrogen gas was produced on site by means of a lab-grade hydrogen generator (Alphagaz™ Flo H2, Air Liquide, Belgium), while CO_2 from gas bottles was of the same grade of the one used during the initial enrichment of the culture. Compressed air was used to provide the oxygen. Gases were fed separately by means of 3 micro-spargers (Sartorius, Belgium) submerged in the reactor. Gas flows were monitored using gas rotameters (Omega, USA) and kept at H_2: 120 mL/min; CO_2: 25 mL/min; Air: 180 mL/min. The gas collected in the headspace was constantly recirculated by means of a peristaltic pump adapted to gas recirculation (Sci-Q 300, Watson Marlow, Belgium) using a fourth micro-sparger. Utilized gas by the bacteria, was bubbled through an external water lock (imposing an overpressure of 20 mbar) and subsequently vented to the atmosphere by means of a fume hood. Temperature and pH were automatically controlled and kept at 35 ± 1 °C and 6.7, respectively.

2.3. Sequencing batch and continuous reactor culture systems

Sequencing batch reactor (SBR) tests were started by transferring 300 mL of fully grown bacterial culture into 2.7 L of fresh mineral medium, allowing an initial cell dry weight Cell Dry Weight (CDW) concentration of 300–500 mg CDW/L. Each sequencing batch test was allowed to evolve for an average of 5–6 days before transferring the culture into fresh medium, corresponding to a solid retention time (SRT) of 6 ± 0.5 days. Additional NH_4Cl was added to the standard mineral medium composition in order to achieve initial NH_4^+–N concentration of 1.2 g/L, and simulate higher N