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## Research article

## Start-up of an anaerobic fluidized bed reactor treating synthetic carbohydrate rich wastewater

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## ABSTRACT

The present work studied the start-up process of a mesophilic ( $37 \pm 2$  °C) anaerobic fluidized bed reactor (AFBR) operated at a hydraulic retention time (HRT) of 20 days using synthetic carbohydrate rich wastewater. Anox Kaldness-K1 carriers were used as biofilm carrier material. The reactor performance and biofilm formation were evaluated during the process. The start-up process at lower liquid recirculation flow rate enhanced the biofilm formation and reactor performance. The organic substrate composition had a major impact on early colonization of methanogenic archaea onto the surface of the Kaldness carriers during the start-up process. Specific organic substrates favouring the growth of methanogenic archaea, such as acetate, are preferred in order to facilitate the subsequent biofilm formation and AFBR start-up. The supply of 'bio-available' nutrients and trace elements, in particular iron, had an important role on optimal methanogenic activity and speeding-up of the biofilm development on the Kaldness carriers. This paper provides possible strategies to optimize the various operational parameters that influence the initial biofilm formation and development in an AFBR and similar high rate anaerobic reactors, hence can be used to reduce the long time required for process start-up.

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## 1. Introduction

At present, the global energy demand and environmental concerns on fossil fuel utilization are demanding alternative energy generation options, aimed at combining economic well-being with a green future (Van Lier et al., 2015). Bioenergy production made an impressive progress in addressing the energy demands with sustainable perspectives (Bialek et al., 2012). In particular, anaerobic digestion (AD) has been recognized as a promising technology for future biofuel production whilst protecting the environment in a cost effective way (Aydin et al., 2015; Minale and Worku, 2014). Accordingly, intensified practice of the process led to technological modifications and development of high rate AD reactors over conventional typologies (Habouzit et al., 2014; Wang et al., 2010). The latter are slow rate AD reactors operated at a longer hydraulic retention time (HRT), and hence impose larger reactor volumes. Instead, the high rate AD reactors are designed to reduce the HRT

and increase the biogas production rate (Van Lier et al., 2015; Yeshanew et al., 2016b). These prominent advantages were achieved via immobilization of microbial biomass in the reactor, i.e. decoupling of a short HRT and long solid retention time (SRT) (Habouzit et al., 2014). Depending on the biomass retention techniques, various types of high rate AD reactors have been developed, such as upflow anaerobic sludge blanket (UASB) reactors, anaerobic packed bed reactors (APBR), anaerobic fluidized bed reactors (AFBR) and anaerobic membrane bioreactors (AnMBR) (Van Lier et al., 2015).

An AFBR configuration has attracted an increasing attention due to the retention of biomass onto a solid inert biofilm carrier material with a large specific surface area (Karadag et al., 2015). The reactors have been widely used for the treatment of many industrial and municipal wastewaters since the last two decades (Van Lier et al., 2015). The carrier materials are fluidized by the influent flow and/or through recirculation of the supernatant liquid (Barca et al., 2015). The bed fluidization provides adequate mixing and mass transfer compared to the other reactor types (Karadag

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et al., 2015), thus facilitates the proliferation of microbial biomass and improves treatment efficiency (Barca et al., 2015). In spite of these benefits, some common shortcomings such as the longer start-up period remain the major deterrent for the extensive application of this technology (Cresson et al., 2007).

The start-up period of an AFBR is an important economic aspect of the system, as the desired stable operation and satisfying reactor performance rely on an effective start-up process (Escudie et al., 2011; Yeshanew et al., 2016b). Its main task is to retain a proper amount of active and well adapted microbial biomass on the surface of the carrier materials in a possible shorter period of time (Habouzit et al., 2014; Cresson et al., 2007; Escudie et al., 2011; Michaud et al., 2002). The presence of this matured and well-balanced biofilm (e.g. in terms of the ratio of methanogenic archaea to fermentative bacteria) is vital, particularly considering the syntrophic bioconversion of organic matter in the AD process (Bialek et al., 2012). Due to the very low growth rate of AD microbial biomass, especially methanogenic archaea, the typical long start-up period with the spontaneous development and maturation of the biofilm is a drawback of the AFBR system (Cresson et al., 2008; Encina and Hidalgo, 2005). It might take several months (2–9 months) to obtain a matured biofilm and stable process (Escudie et al., 2011). To address these concerns, several studies have focused on the fundamentals of biofilm formation and enhanced development (Bialek et al., 2012; Escudie et al., 2011; Habouzit et al., 2014; Cresson et al., 2007).

The biofilm formation process involves three basic successive stages: initial attachment of microbial cells on the surface of the carrier material, irreversible attachment by producing extracellular polymeric substances (EPS) and maturation of the biofilm (Escudie et al., 2011; Bialek et al., 2012). In these dynamic processes, various physico-chemical and biological parameters as well as operational conditions are of great relevance, such as hydrodynamic conditions (Cresson et al., 2007), types and nature of carrier material (Habouzit et al., 2014), substrate composition (D'Acunto et al., 2015b), source of inoculum (Habouzit et al., 2014) and availability of trace metals (Cresson et al., 2006; D'Acunto et al., 2015a). A further understanding of the various factors affecting the start-up process is essential for the development and success of the AFBR configuration (D'Acunto et al., 2015b).

The present work studied the start-up process of an AFBR, investigating various operational parameters that influence the initial biofilm formation and reactor performance during treatment of synthetic carbohydrate rich wastewater at mesophilic ( $37 \pm 2$  °C) temperature. Throughout the experiment, the daily methane yield was used as a main indicator of the dynamic steps of biofilm formation and reactor start-up, as indicated by Michaud et al. (2002). The methane yield could be correlated to the AD biomass activity and development of biofilms since it is the result of the balance between the organic carbon flows to anabolism (biofilm growth) and catabolism (methane and other product formation) in the AD process (Michaud et al., 2002; Cresson et al., 2006). Theoretically, the methane yield of an AD biomass is 350 mL CH<sub>4</sub>/g COD<sub>removed</sub>. Reaching this value would indicate that the majority of the carbon is used by the biofilm for anaerobic respiration and maintenance (Cresson et al., 2006). Moreover, the reactor performance and stability were evaluated by monitoring several parameters such as total organic acids, total alkalinity, pH, chemical oxygen demand (COD), total ammonium and individual volatile fatty acids (VFAs) concentration.

## 2. Materials and methods

### 2.1. Substrate

Synthetically prepared carbohydrate-rich wastewater containing

glucose as the sole carbon source was used as the substrate for 57 days. Afterwards, the substrate was changed to a mixture of acetate and propionate at a ratio of 3:1 (v:v). The necessary nutrients and trace elements (TEs) for the AD biomass were supplied according to Table 1. The concentration of nutrients and TEs added was based on literature reports (Arnaiz et al., 2006; Fermoso et al., 2008). In order to avoid precipitation in the storage vessels prior to reactor feeding, each solution was prepared separately and mixed based on the proportion given in Table 1. Ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>) was used as a source of nitrogen and alkalinity. All reagents were of analytical grade (Sigma-Aldrich®).

### 2.2. Inoculum

The inoculum was obtained from a full scale AD plant located in Capaccio-Salerno (Italy), treating buffalo manure and dairy wastes at mesophilic conditions. An inoculum from a running full scale AD plant under suspended biomass system has been previously used by several authors (Encina and Hidalgo, 2005; Yeshanew et al., 2016b). Larger particles and small sticks of the inoculum were removed by centrifugation at 4500 rpm for 10 min (IEC Centra GP8R, USA) and filtered through a sieve with pore size of 1.0 mm. Approximately 800 mL, 62% of the active volume of the reactor, of the centrifuged inoculum was added to the reactor at the start of the experiment. The main physico-chemical characteristics of the inoculum are given in Table 2.

### 2.3. Biofilm carrier material

Kaldness-K1, developed by Anox-Kaldness (Veolia, Sweden), was used as biofilm carrier material. Its use has been widely reported for different types of wastewater and is one of the most commonly employed in moving bed biofilm reactors (Rusten et al., 2006; Aygun et al., 2008). The Kaldness-K1 carrier is a small cylinder of low density polyethylene, lower than the density of water, with a cross inside and “fins” in the external surface. It has a specific weight of 145 kg/m<sup>3</sup> and specific surface area of 500 m<sup>2</sup>/m<sup>3</sup>. To achieve a good substrate removal without crowding the reactor, 20% of the reactor was filled with the Kaldness carriers (volume basis) according to Wang et al. (2010).

### 2.4. AFBR configuration and operation

The AFBR reactor consisted of a cylindrical glass column having a dimension of 34 cm height and internal diameter of 24 mm, with an active volume of 1.3 L (Fig. 1). The temperature of the reactor was maintained at mesophilic conditions ( $37 \pm 2$  °C) by immersing it in a water bath connected to a thermostatic controller. Prior to starting of the operation, the AFBR was purged with argon gas to ensure an initial anaerobic environment. Dark conditions were maintained by wrapping the reactor and tubes with black plastic covers. Liquid recirculation (LR) was performed in up-flow mode using a peristaltic pump (type 505 L, Watson and Marlow, Fal-mouth, England).

Initially, the reactor was operated in batch feeding mode. This was done to initiate the initial contact between the inoculum and the carrier materials as described by Escudie et al. (2011), as well as to make sure that the reactor and pipes were leak proof. Afterwards, the batch feeding was switched to semi-continuous mode, i.e. adding the substrate once a day. The semi-continuous operational conditions were set at an OLR of 1 gCOD/L. day and HRT of 20 days, corresponding to an influent COD concentration of 20 g/L. Before substrate addition, manual withdrawing of the effluent was conducted at the feeding inlet.

The biogas produced was led to pass through a concentrated

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