Journal of Biotechnology 192 (2014) 161–169

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Enrichment of *Plasticicumulans acidivorans* at pilot-scale for PHA production on industrial wastewater

Jelmer Tamis[a,*], Kätlin Lužkov[a,b], Yang Jiang[a], Mark C.M. van Loosdrecht[a], Robbert Kleerebezem[a]

[a] Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, the Netherlands
[b] Department of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 7 October 2014
Accepted 13 October 2014
Available online 23 October 2014

Keywords:
Polyhydroxyalkanoates (PHA)
Pilot-scale
Industrial wastewater
Bioplastic
Resource recovery

ABSTRACT

A PHA producing microbial culture dominated by *Plasticicumulans acidivorans* was enriched in a pilot plant using fermented wastewater from the Mars candy bar factory. The pilot plant comprised (1) anaerobic fermentation, (2) enrichment of a PHA-producing microbial community and (3) accumulation for maximization of the cellular PHA content. After anaerobic fermentation, the wastewater contained mainly VFAs (0.64 ± 0.15 gCOD/gCOD) and ethanol (0.22 ± 0.13 gCOD/gCOD). In the enrichment reactor (cycle 12 h, SRT 24 h) a feast-famine pattern was established with a feast phase of around 35 ± 5 min. The culture was able to accumulate 0.70 ± 0.05 gPHA/gVSS. The difference with previous lab-scale results from *P. acidivorans*, in which a PHA content of 0.90 gPHA/gVSS was achieved, could be attributed to the presence of solids in the influent, the growth of a side population and the accumulation of non-PHA storage compounds that appeared to be related to ethanol consumption.

1. Introduction

The biobased, biodegradable polyhydroxyalkanoate (PHA) polymer can replace petroleum based feedstock in e.g. packaging applications and chemical industry but is until now too expensive for large scale application (Chen, 2009; Crank and Patel, 2005). PHA production from waste streams using microbial enrichment cultures is a promising option for cost reduction of both PHA polymers and treatment of industrial wastewater (Reis et al., 2003; Kleerebezem and van Loosdrecht, 2007; Chen, 2009). Integration of waste-based PHA production into industry would encompass direction of a suitable waste stream towards a PHA production facility, likely in the proximity of the waste source. Here, the organic compounds in the waste stream are converted to a sludge with ideally a high PHA content and at the same time a clean effluent is produced for discharge to surface waters in accordance with local legislation. The PHA containing sludge may be further processed to purified, marketable PHA polymer product or used as feedstock for other added value processes (Chen, 2009).

Although many types of wastewater can be used for the production of PHA, high concentrations of fermentable COD, relatively low nitrogen and solid concentrations and low toxicity promote process feasibility. In this perspective, food and paper industry effluents may be considered the most suitable substrates for waste-based PHA production. Other waste streams that may be interesting for PHA production include leachate from the composting industry and municipal wastewater, but it should be noted that these streams pose additional challenges due to e.g. the relatively high nitrogen content and the presence of solids.

There are a large number of factors that influence the feasibility of a waste-based PHA production process (Chen, 2009), but in general, the downstream processing represents a major cost factor (Gurieff and Lant, 2007). Apart from optimization of the downstream process itself, the process feasibility can be improved by increasing the PHA content of the biomass, resulting in a reduced chemicals and energy demand for the downstream process, less waste solids and a higher overall process yield (Choi and Lee, 1999; Van Wegen et al., 1998).

The crux of enriching biomass with superior PHA-storing capacity in an open reactor system (an environment in which myriad species constantly invade the system e.g. by being present in the

Abbreviations: ATU, allylthiourea; Cmol, carbon mole; COD, chemical oxygen demand; DGGE, denaturing gradient gel electrophoresis; DO, dissolved oxygen; FISH, fluorescent in situ hybridization; HPLC, high performance liquid chromatography; GC, gas chromatography; MCFA, medium chain length fatty acids; PHA, polyhydroxyalkanoate; PHB, polyhyrdoxybutyrate; PHV, polyhydroxyvalerate; rpm, rounds per minute; SBR, sequencing batch reactor; sCOD, soluble COD; SRT, solid retention time; TSS, total suspended solids; USB, upflow sludge blanket; VFA, volatile fatty acid; VSS, volatile suspended solids.

* Corresponding author. Tel.: +31152782359; fax: +31152782355.
E-mail address: j.tamis@tudelft.nl (J. Tamis).

http://dx.doi.org/10.1016/j.jbiotec.2014.10.022
0168-1656/

Nomenclature

k	specific PHA degradation rate constant ($gPHA^{1/3}\ gX^{-1/3}\ h^{-1}$)
PHA^{max}	maximum PHA storage capacity ($gPHA\ gVSS^{-1}$)
$q_S(VFA)^{max}$	maximum biomass specific uptake rate for VFA ($gCOD\ gX^{-1}\ h^{-1}$)
$q_S(CODo)^{max}$	maximum biomass specific uptake rate for other COD ($gCOD\ gX^{-1}\ h^{-1}$)
X	active biomass (g)
$Y_{PHA/S}$	yield of PHA on substrate ($gPHA\ gCOD^{-1}$)
$Y_{X/PHA}$	yield of active biomass on PHA ($gX\ gCOD^{-1}$)
$Y_{X/CODo}$	yield of active biomass on other COD ($gX\ gCOD^{-1}$)

wastewater substrate) is the establishment of a selective environment. The cyclic presence and subsequent absence of volatile fatty acids (VFA) provides a competitive advantage for PHA storing species (Bengtsson et al., 2008; Dionisi et al., 2007; Johnson et al., 2009; Serafim et al., 2008). Based on this principle a three-step process was proposed: (1) anaerobic fermentation to direct the myriad organic compounds in wastewater to VFA (Temudo et al., 2007), (2) enrichment of biomass with superior PHA-producing capacity in a selective environment and (3) maximization of the PHA content of the biomass in an accumulation step by feeding the enriched biomass with VFA in fed-batch mode in absence of a nitrogen source (Reis et al., 2003).

Although PHA accumulation is a common trait in bacteria, the highest PHA contents reached by microbial enrichment cultures were achieved in reactor systems dominated by a genus identified as *Plasticicumulans*. Lab-scale experiments with synthetic substrates resulted in a biomass with around 0.90 gPHA/gVSS on acetate (Johnson et al., 2009) or lactate (Jiang et al., 2011b). Lab-scale experiments with wastewater resulted generally in lower PHA content: e.g. 0.75 gPHA/gVSS on fermented molasses (Albuquerque et al., 2010) and 0.77 gPHA/gVSS on fermented paper mill wastewater (Jiang et al., 2012). Nevertheless, the above reports show that microbial enrichment cultures can reach similar PHA contents as genetically modified pure cultures that are conventionally used in industrial biotechnology for PHA production. To further assess the industrial relevance of the waste-based PHA process, pilot-scale experiments are required, especially concerning the product quality (separability from the water phase, PHA content of the biomass, and PHA quality) under industrial conditions (variable influent, complex substrate, less strict pH control) and the identification of potential bottlenecks in the process, such as the use of chemicals (e.g. acid, base, and nutrients) and the effluent quality of the process.

Only a limited amount of literature was found related to pilot-scale waste-based PHA production. Morgan-Sagastume et al. (2013) used municipal wastewater (with 17–60% of the soluble COD present in the form of VFA) to establish a PHA producing biomass in a pilot reactor with a volume of 500 l. The PHA storing capacity of this biomass was evaluated by feeding it with acetate and this way a maximum PHA content up to 0.34 gPHA/gVSS was achieved. Chakravarty et al. (2010) tested a concept to harvest a PHA rich biomass and meet effluent discharge regulations at the same time. A pilot system treating 60–65 l per day of dairy industry wastewater produced a sludge with an estimated 0.4–0.5 gPHA/gVSS content. Anterrieu et al. (2014) demonstrated the use of different VFA feedstock for PHA production while treating a food industry wastewater. A biomass that was cultivated on a food industry effluent (Procordia Food, Eslov, Sweden) was fed with effluent from a sugar refinery (Suikerunie, Groningen, The Netherlands). A batch experiment performed on 1000 l scale resulted in a maximum PHA content of 0.60 gPHA/gVSS.

It would be interesting to understand why the reported pilot-scale experiments resulted structurally in a biomass with a lower PHA content than lab-scale experiments. However, the above publications are difficult to compare to earlier published lab-scale experiments because of the use of uncharacterized biomass (Anterrieu et al., 2014), a more difficult waste stream resulting in relatively low PHA contents (Morgan-Sagastume et al., 2013), or a conceptually different process setup (Chakravarty et al., 2010).

In this paper, we present results on the enrichment of a PHA producing microbial enrichment on industrial wastewater (Mars, Veghel, the Netherlands) in a pilot installation set-up similar to previous lab-scale studies (Johnson et al., 2009; Temudo et al., 2007; Jiang et al., 2012). In this study we aimed to characterize the enrichment of PHA-producing biomass in a pilot reactor operated under less strictly controlled conditions compared to lab-scale and using variable wastewater characteristics as encountered in industrial practice. In order to compare the performance of the biology in the system, characteristic process parameters were derived using a model based on earlier publications (Tamis et al., 2014). Additionally, the research was aimed at identification of key limiting factors of PHA production at pilot-scale and explaining the difference in PHA content between lab-scale (Johnson et al., 2009; Jiang et al., 2011b) and pilot-scale experiments in general.

2. Materials and methods

Experiments were conducted with wastewater from a candy bar factory (Mars, Veghel, the Netherlands). The pilot plant was designed as a three-step process comprising anaerobic fermentation, enrichment and accumulation steps; a schematic representation of the pilot plant layout is provided in Fig. 1.

2.1. Wastewater and pilot-scale anaerobic fermentation reactors

The wastewater from the Mars factory was pretreated in a flotation-based fat separation unit before entering the influent tank of the pilot installation. Subsequently, maximization of the VFA concentrations in the wastewater was pursued by application of two anaerobic reactors, operated in series. Firstly, the wastewater was fed to an upflow sludge blanket (USB) type reactor with a working volume of 60 l that was maintained at $30 \pm 1\ ^{\circ}C$ and a pH of 4.5 ± 0.1 by addition of controlled amounts of 1 M NaOH. The USB reactor was inoculated with sludge from a lab-scale reactor performing acidification of sugar molasses. The hydraulic retention time (HRT) of the reactor was 4 h and the solid retention time (SRT) was maintained around 4 days by manual sludge removal. To keep the reactor effluent nitrogen depleted (favorable for use in the accumulation reactor later in the process) the target COD:N mass ratio was around 300:1. A nutrient solution containing 3 M nitrogen in the form of urea, 0.3 M phosphate, 0.3 M $MgSO_4$, 0.2 M K_2SO_4, and trace elements (64 mM $FeCl_3$, 3 mM $ZnSO_4$, 2.7 mM H_3BO_3, 2.1 mM $NiCl_2$, 1.5 mM $CoSO_4$, 0.6 mM $CuSO_4$, 0.8 mM Na_2MoO_4) was provided to the reactor.

To buffer the volumes of available VFA substrate for the enrichment and accumulation processes, and to secure full conversion of the fermentable COD to VFA, a second anaerobic fermentation reactor was included in the system, comprising an anaerobic tank with a liquid volume of 1500 l with a hydraulic retention time of 4 days, maintained at a temperature of 40 (± 1) °C and a pH of 4.5 (± 0.1) by addition of 1 M NaOH. After this second step the fermented wastewater was used as a substrate for the enrichment and accumulation reactors.