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Short- and long-term temperature effects on aerobic polyhydroxybutyrate producing mixed cultures

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ABSTRACT

Short- and long-term temperature effects on polyhydroxybutyrate (PHB) producing mixed cultures enriched in feast–famine sequencing batch reactors (SBRs) were investigated in a temperature range of 15–35 °C and 15–30 °C, respectively. After short-term temperature changes (i.e. 1 cycle) from the steady state temperature of 20 °C, reaction rate changes in the famine phase could be described over the whole temperature range with the Arrhenius equation with one temperature coefficient. For the feast phase different temperature coefficients were identified for acetate uptake, PHB production and growth. These were only valid for temperatures 5 °C higher or lower than the steady state temperature.

Long-term temperature changes (i.e. new steady states) influenced not only the reaction rates but also the selective pressure in the SBR. At higher temperatures (30 °C) the SBR feast phase was short and the rates of acetate uptake and PHB storage were very high. This culture was characterized by a storage strategy with high yields of PHB and low yields of biomass in the feast phase. The PHB storage capacity of this culture was 84 wt% as evaluated in fed-batch experiments. At lower temperatures (15 °C) the feast phase was longer due to a lower rate of acetate uptake and the culture followed a strategy of direct growth on acetate rather than on PHB. This culture had a low maximal PHB storage capacity (about 35 wt%). The SBR culture enriched at 20 °C was able to store up to about 70 wt% PHB. The temperature at which fed-batch experiments were conducted did not influence the maximal PHB storage capacity. The SBR temperature was found to be an important factor to consider when designing a mixed culture PHB production process.

1. Introduction

Mixed culture biotechnology is a promising alternative to pure culture biotechnology for the production of the bioplastic polyhydroxyalkanoate (PHA). Mixed culture biotechnology employs open undefined mixed cultures and ecological selection principles to produce a product such as bioplastics, ideally from a waste stream. It therefore combines the methodology of environmental biotechnology with the goals of industrial biotechnology. Optimization of the mixed culture PHA production process has led to cellular PHA contents and PHA production rates comparable or superior to those of pure cultures including genetically modified organisms (Johnson et al., 2009a). One of the important factors influencing PHA production is the process temperature, which was the subject of this study.

The critical step in the PHA production process with open mixed cultures is the enrichment of superior PHA producing bacteria in a mixed culture. This can be achieved by using a selective pressure for PHA production based on the

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Nomenclature

A	pre-exponential factor in Arrhenius equation
E_a	activation energy
$\tilde{f}_{PHB}$	modeled fraction of PHB
k	rate constant of a reaction
k_T	rate constant at temperature T
k_{293K}	rate constant at the reference temperature of 20 °C (293 K)
k_{PHB}	rate constant of PHB degradation
L_N	gas volume in liters at standard (normal) conditions (273 K, 1013 mbar)
m_{ATP}	maintenance ATP requirement
q_{Ac}	average biomass specific acetate uptake rate
$\tilde{q}_{Ac}^{max}$	maximum biomass specific acetate uptake rate in the model
q_{CO_2}	average biomass specific carbon dioxide evolution rate
q_{NH_3}	average biomass specific ammonia uptake rate
q_{O_2}	average biomass specific oxygen uptake rate
q_{PHB}	average biomass specific PHB production or consumption rate
$\tilde{q}_{PHB}^{fam}$	modeled biomass specific PHB consumption rate
R	gas constant
R^2	coefficient of determination
t	time
T	absolute temperature
$Y_{CO_2/Ac}^{obs}$	observed yield of carbon dioxide on acetate
$Y_{CO_2/PHB}^{obs}$	observed yield of carbon dioxide on PHB
$Y_{PHB/Ac}^{obs}$	observed yield of PHB on acetate
$Y_{X/Ac}^{obs}$	observed yield of active biomass on acetate
$Y_{X/PHB}^{obs}$	observed yield of active biomass on PHB
θ	temperature coefficient in simplified Arrhenius equation
μ	average biomass specific growth rate
$\tilde{\mu}^{max}$	maximum biomass specific growth rate in the model

Abbreviations

Ac	acetate
ATP	adenosine triphosphate
DGGE	denaturing gradient gel electrophoresis
DO	dissolved oxygen
GAO	glycogen accumulating organism
HRT	hydraulic residence time
PAO	polyphosphate accumulating organism
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
SBR	sequencing batch reactor
SRT	sludge residence time
TSS	total suspended solids

ecological role of PHA as a microbial storage material (van Loosdrecht et al., 1997). Two different strategies can be applied: (i) alternating periods of presence and absence of the final electron acceptor (aerobic and anaerobic periods) with substrate being supplied during the absence of the final electron acceptor, or (ii) alternating periods of presence and absence of the carbon source (feast and famine periods) (Reis et al., 2003). The first strategy selects for polyphosphate- and/or glycogen-accumulating organisms (PAOs, GAOs). The temperature effects on PAO and GAO cultures have been studied extensively (Brdjanovic et al., 1997, 1998; Lopez-Vazquez et al., 2007, 2008, 2009a,b). In these studies the temperature was found to influence the metabolic reaction kinetics as well as the competition between these two different types of organisms.

In contrast to the PAO and GAO cultures, temperature effects on PHA storing cultures enriched with a feast–famine strategy have hardly been studied. Krishna and van Loosdrecht (1999) investigated the influence of temperature on acetate-fed feast–famine cultures and also found a strong influence of temperature on the kinetics of the process. However, these experiments were performed on cultures with an acetate uptake rate that was relatively low and limited by the acetate addition rate in the feast phase. Kinetics in dynamic processes with faster feeding and absence of substrate limitation will be more dominated by PHA storage and the impact of temperature on the kinetics will likely be different.

Neither the mentioned PAO and GAO studies nor the study by Krishna and van Loosdrecht (1999) explored the influence of the temperature on the maximum PHA content that can be established depending on the temperature at which the mixed cultures were enriched.

When working with open mixed cultures a temperature change can have different effects depending on whether the temperature is changed for a short time or for a long time. Short-term changes are expected to mainly influence the kinetics of the metabolism of the organisms that are present in the reactor; long-term changes, however, would likely also lead to an adaptation of the present organisms and changes in the community structure (Brdjanovic et al., 1998; Lopez-Vazquez et al., 2009a). A change in the PHA storage capacity could therefore occur particularly after long-term temperature changes.

The impact of the temperature on PHA storage is particularly relevant when aiming at using industrial wastewaters, which are produced at a wider temperature range than communal wastewaters. For study purposes usually a synthetic wastewater with acetate as the sole carbon source is used. Once the mechanisms are understood for this simplified system, more complex media and wastewaters can be explored.

In this study we investigated both the effect of short- and long-term temperature changes on PHA accumulating cultures enriched with the feast–famine strategy. Additionally we examined the influence of temperature on the maximum PHA storage capacity of the enriched cultures. Sequencing batch reactors (SBRs) were used to enrich PHA storing bacteria with a feast–famine regime. Acetate was supplied as the sole carbon source and the polymer produced was consequently pure polyhydroxybutyrate (PHB). The sludge residence time (SRT) of the SBR was chosen as 1 day in order to aim for a high biomass productivity, which would be desirable for a commercial system. The SBRs were operated at temperatures of 15, 20 and 30 °C until a steady state was reached (long-term temperature change experiments). The steady state obtained at 20 °C was used as the base for the short-term