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Acute impact of tetracycline and erythromycin on the storage mechanism of polyhydroxyalkanoates

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ABSTRACT

The study investigated acute impact of tetracycline and erythromycin on substrate storage under aerobic conditions. A fill and draw reactor fed with peptone mixture was maintained at steady-state at a sludge age of 10 days; the acclimated biomass was used in a series of batch runs. The first run served as control reactor with organic substrate alone and the others were started with antibiotic doses of 50 mg/L and 200 mg/L for assessing intracellular storage. Parallel batch reactors were also conducted for recording oxygen uptake rate profiles. Both antibiotics enhanced substrate storage, leading to higher levels of polyhydroxyalkanoates incorporated into biomass, but they impaired its internal utilization for microbial growth. The observed decrease in oxygen consumption under the acute effect of antibiotics could partially be related to substrate storage – except for 50 mg/L of erythromycin dosing – suggesting an additional substrate binding mechanism by antibiotics, leading to residual biodegradable substrate.

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1. Introduction

Storage of intracellular biopolymers is now recognized as a significant auxiliary process during substrate utilization by microbial cultures. It was mainly observed under transient feeding conditions, where sequential *feast* and *famine* phases trigger substrate storage [1]. In essence, storage results from an imbalance between removal of available substrate and microbial growth potential; while substrate may be removed, limitations on the metabolic reactions leading to growth may prevent consumption of all energy and divert a fraction of the substrate for generating intracellular biopolymers [2]. Batch reactors are often selected as the most suitable experimental tool for investigating different aspects of substrate biodegradation, mainly because they offer accurate evaluation of transient responses and resulting concentration profiles of major parameters [3,4]. Dynamic conditions sustained in batch reactors approximate pulse feeding and induce a physiological adaptation for the microbial community, often leading to substrate storage.

The storage mechanism is often studied with simple, readily biodegradable substrates like acetate or glucose, generating *polyhydroxybutyrate* (PHB) and glycogen as storage products. Reported results suggested that up to 70% of the simple substrate could be converted to storage products under pulse feeding [5,6]. They also indicated that the magnitude of storage was likely to exhibit significant variations depending on the nature of substrate, the feeding regime and culture history – i.e. sludge age of the microbial culture [7–14]. Expected storage would be substantially lower in complex substrate mixtures such as domestic sewage where only a small percent of the organic matter consist of readily biodegradable compounds favoring formation of intracellular biopolymers. In fact, in a study using a peptone-meat extract mixture as the organic carbon source with biodegradation characteristics similar to domestic sewage, storage of only 30 mg COD/L of intracellular biopolymers was observed, corresponding to 60% of the available readily biodegradable COD at a sludge age of 8 days [15].

The storage mechanism is also important when studying inhibition of substrate biodegradation under the impact of adverse chemicals and xenobiotics. Traditionally, inhibition was interpreted by means of enzyme analogy affecting only microbial growth [16,17], simply because corresponding substrate utilization was only characterized by a single overall parameter such as BOD or COD. After recognition and experimental assessment of COD fractions with different biodegradation characteristics in domestic sewage and industrial wastewaters [15], interpretation of biodegradation involved all these fractions and related biochemical processes [18]. While multi-component models incorporated all these processes, they initially disregarded substrate storage [19,20]. Later, these models were modified to account for simultaneous substrate utilization for growth and storage [21,22]. Thanks

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Nomenclature

C_s	initial biodegradable substrate (mg COD/L)
C_{SG}	fraction of substrate directly utilized for microbial growth (mg COD/L)
C_{SGP}	fraction of the initial substrate potentially available for microbial growth (mg COD/L)
C_{SR}	residual fraction of the initially available biodegradable substrate (mg COD/L)
C_{ST}	fraction of substrate diverted to storage (mg COD/L)
C_{STR}	substrate COD equivalent of residual PHA entrapped in biomass (mg COD/L)
C_{STU}	fraction of substrate stored and internally utilized (mg COD/L)
k_{STO}	maximum storage rate (1/day)
K_{STO}	half saturation coefficient for storage and internal growth (mg COD/L)
OUR	oxygen uptake rate (mg O_2/L h)
PHA	polyhydroxyalkanoates
PHB	polyhydroxybutyrate
PHV	polyhydroxyvalerate
S_O	oxygen consumed (mg/L)
S_S	readily biodegradable substrate (mg COD/L)
S_{OT}	theoretical oxygen demand (mg/L)
X_{STO}	stored PHA (mg COD/L)
X_{STR}	residual PHA entrapped in biomass (mg COD/L)
Y_H	yield coefficient (mg COD/mg COD)
Y_{STO}	storage yield (mg COD/mg COD)
3H2MV	3-hydroxy-2-methylvalerate

to these modeling tools, interpretation of inhibitory substances was no longer limited to substrate utilization for microbial growth, but it could also cover all related biochemical mechanisms such as hydrolysis, endogenous respiration and substrate storage [14]. In this context, the storage mechanism is particularly important, mainly because it is most likely to be affected under stress conditions, i.e. diversion of substrate from growth to storage under adverse impact and when disregarded it may significantly distort the kinetics of parallel biochemical processes, leading to misinterpretation of the inhibitory impact.

The previous works investigated the impact of selected antibiotics on either heterotrophic or autotrophic bacteria in terms of general process kinetics. The main objective of this part of the study was to evaluate the acute impact of two selected inhibitors on the mechanism of substrate storage in a nitrifying microbial community. Two major antibiotics, namely *tetracycline* and *erythromycin* were selected as the inhibitor compounds, due to the fact that they take place in our daily life, discharged into the wastewaters and to the environment [23] and well studied for their inhibitory effects [24–26]. The acute impact was evaluated on the biodegradation of peptone-meat extract mixture – called thereafter *peptone mixture* for simplicity – under aerobic conditions. It was selected as the complex organic carbon source because it is prescribed as the standard substrate by ISO 8192 [27] in similar inhibition studies [28] and it approximates the COD fractionation and biodegradation characteristics of domestic sewage [29].

2. Materials and methods

2.1. Experimental rationale

The experiments were conducted as part of a comprehensive study investigating the impact of antibiotics on the heterotrophic and autotrophic communities in single sludge systems operated under aerobic conditions. The first part involved running a fill and draw reactor with biomass taken from a domestic wastewater treatment plant. The system was maintained at steady-state at a sludge age of 10 days, a value compatible with the level selected for biological treatment systems targeting combined organic carbon removal and nitrification [30].

Table 1
Operating characteristics of batch experiments [33].

Runs	Antibiotic	Biomass (mg VSS/L)	S_0/X_0 (mg COD/mg VSS)	T (°C)
Run 1	–	920	0.55	21
Run 2.1	50 mg TET/L	1005	0.50	21
Run 2.2	200 mg TET/L	995	0.51	24
Run 3.1	50 mg ERY/L	995	0.51	25
Run 3.2	200 mg ERY/L	1035	0.49	25

Peptone mixture having 506 mg COD/L was added in all runs.

The acclimated biomass from the fill and draw reactor was used for starting a series of batch runs, each run including two parallel batch reactors, one for the assessment of intracellular biopolymers and the other, for the respirometric analysis of oxygen uptake rate (OUR) profiles. The initial COD concentration of the peptone mixture was adjusted to around 500 mg/L. The first run served as the control reactor; the other two runs included in a feed having initial dose of 50 mg/L and 200 mg/L of the antibiotic for its acute impact, primarily on substrate storage. The highest antibiotic level was selected mainly to approximate effluent discharges of pharmaceutical industries and hospitals which were reported to reach up to 100–500 mg/L [23,31]. As an example, tylosin antibiotic was detected in the range of 20–200 mg/L in an antibiotic wastewater [32]. The lowest concentration was selected based on the respirometric studies previously conducted on three different antibiotics indicating oxygen uptake rate reduction by more than 50% for the same substrate [17]. Batch experiments were conducted separately for tetracycline and erythromycin.

2.2. Batch experiments

Batch experiments were conducted in aerated 2 L cylindrical benchtop reactors having 30 cm diameter. The dissolved oxygen was supplied air supplying diffusers connected to airline. The oxygen level in the reactors was maintained above 3.0 mg/L (33% oxygen saturation) by mixing with mechanical impellers to prevent any oxygen limitations on biochemical reactions. In order to determine the effects of selected antibiotics on substrate storage mechanism, related parameters such as COD and storage compounds were monitored. Batch reactors were supplied with 50 and 200 mg/L of TET or ERY solutions in addition to peptone mixture. Initial food to microorganisms ratio (S_0/X_0) was adjusted to 0.49–0.55 mg COD/mg VSS where macro (320 g/L K_2HPO_4, and 160 g/L KH_2PO_4) and micro nutrients (15 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L $FeSO_4{\cdot}7H_2O$, 0.5 g/L, $ZnSO_4{\cdot}7H_2O$, 0.41 g/L $MnSO_4{\cdot}H_2O$, 2.65 g/L, $CaCl_2{\cdot}2H_2O$ g/L) were also fed to support microbial activity. The system was fed once on a daily basis and continuously aerated to have a hydraulic retention time of 1 day. Activated sludge was settled for 1 h and effluent was decanted to 2 L. COD removal efficiencies and internal storage biopolymers, polyhydroxyalkanoates (PHAs) including polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and 3-hydroxy-2-methylvalerate (3H2MV) were monitored during the experiments for the determination of biological activity [33]. The tests lasted for 24 h and pH remained at neutral levels for this time period. Information related to batch experiments was given in Table 1. Repeated experiments yielded the same profiles of related parameters.

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