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# Impact of lignocellulosic-waste intermediates on hydrolysis and methanogenesis under thermophilic and mesophilic conditions

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## HIGHLIGHTS

- Furfural, HMF, vanillin and humic acid were chosen as potential inhibitors.
- Various thermophilic and mesophilic batch tests were done during SMA and CMP tests.
- Furfural and HMF fully inhibited the SMA assays at 2.0 g/L under both conditions.
- Vanillin and humic did not inhibit the SMA assays at 2.0 g/L under both conditions.
- Propionate accumulation was found in the thermophilic CMP tests for furfural and HMF.

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## ABSTRACT

Intermediates of anaerobic conversion processes have been identified to inhibit methanogenic biomass and to decrease process performance. The used concentrations of model intermediates furfural, 5-hydroxymethylfurfural (HMF) and vanillin, as well as the recalcitrant humic acid were 0.4, 0.8 and 2.0 g/L. These compounds were used to determine their impact on methanogenesis by specific methanogenic activity (SMA) assays and hydrolysis by cumulative methane production (CMP) tests under thermophilic and mesophilic conditions at a concentrations of 0.8 g/L, using lignocellulosic biomass as the substrate.

HMF showed inhibitory effects at a concentration of 0.8 g/L under thermophilic conditions during SMA tests. HMF and furfural completely inhibited the methanogenic activity at 2.0 g/L under both thermophilic and mesophilic conditions. The inhibitory effect was absent with vanillin and humic acid at concentrations $\leqslant$2.0 g/L and 0.8 g/L, during SMA and CMP tests, respectively. The thermophilic microbial consortia were found to be more sensitive to increased concentrations of the intermediates than mesophilic consortia, determined by the methane production rates and quantity in CMP tests.



## 1. Introduction

At several sewage treatment plants (STPs) in the Netherlands, coarsely screened (6 mm) sewage is directed through a fine sieve (Salsnes Filter, Norway) with a mesh size of 350 μm. These sieves can be implemented as a compact alternative to primary clarification. The fine sieved fraction (FSF) is a heterogeneous substrate, sequestered from raw sewage, which mainly consists of partly dissolved toilet paper (with a high cellulose fraction), hair, lignin-rich compounds such as leaves and shell of fruits, sands and undefined materials. Although the exact composition of our FSF substrate was not measured, an approximate composition can be deduced from Appliedcleantech (www.appliedcleantech.com, accessed on 22 December 2015): 60–80% of cellulose, 5–10% of hemi-cellulose, 5–10% of lignin, 5–10% of oil and the rest accounted for inorganic salts (5–10%).

Anaerobic digestion (AD) is an attractive sludge treatment practice in which both waste control and energy recovery can be achieved [1,2]. Many agricultural and industrial wastes are ideal candidates for anaerobic digestion because they contain high levels of easily biodegradable materials [3]. Despite the vast knowledge on AD processes, unexpected low methane yields and process

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instability are frequently observed. Waste based inhibitory compounds and/or accumulating intermediates can be responsible for reactor perturbation and instabilities in the digestion process [2,4–6]. The nature and degree of inhibition fully depends on the type of inhibitor present, slowing down or even blocking the enzymatic activity [7–9]. During the physicochemical pretreatment or microbial hydrolysis of lignocellulosic biomass, soluble sugars (mainly xylose) are produced but by-products such as furan and lignin derivatives (phenolic compounds) are also generated in the biomass hydrolysate [10,11]. Furfural and 5-hydroxymethylfurfural (HMF) are representative of the many inhibitory compounds from lignocellulosic hydrolysis. Furfural and HMF are mainly derived from pentose and hexoses dehydration [12,13], whereas the main inhibiting phenols and polyphenols originate from lignin polymers and/or lignin oligomers, vanillin and syringaldehyde, resulting from partial lignin degradation [14,15]. A recent study conducted by [16] reviewed the different routes of furfural and HMF formation from simple sugars. Rasmussen et al. [16] has identified at least three routes for the formation of furfural from xylose and four routes for HMF formation from glucose. For instance, both furfural and HMF can be transformed into less inhibitory compounds such as furfuryl and HMF alcohols and then be degraded by clostridial species and facultative anaerobes [17,18].

Phenolic compounds as vanillin have a significant impact on the fermentation of hydrolysates and could be toxic at certain concentrations because they compromise the integrity of biological membranes [19]. HMF can be further degraded, forming levulinic acid and formic acid. In addition, formic acid can be formed from furfural under acidic conditions at elevated temperatures [20,21]. It has been reported that at concentrations of 4.6 g/L, formic acid was more inhibitory than levulinic acid which, in turn, was more inhibitory than acetic acid in the process of bioethanol production [12]. Hence, furfural was found to be more inhibiting than HMF, due to lower molecular weight of furfural compared with HMF, which facilitates its diffusion into microbial cells [22]. On the other hand, it was reported that when furfural is present alone at lower concentrations, it can be efficiently converted and metabolised, however, if it is present with HMF, the conversion rates of both decreased significantly and HMF degradation only proceeded when the complete degradation of furfural occurred [23].

Furthermore, furanic compounds (i.e. furfural and HMF) were reported to have detrimental effects on microorganisms by inhibiting cell growth, inducing DNA damage and inhibiting several enzymes of the glycolysis pathway [24,25]. Phenolic compounds damage microbial cells by altering selectively the membrane permeability, causing leakage of intracellular components and inactivation of essential enzymatic systems [26,27].

Humic substances, including humic acids and fulvic acids, are the main components of sludge organic substances (sewage sludge and compost) [28] that are recognized to be recalcitrant compounds and hardly degradable in biological treatment processes [29]. They become enriched in oxygen functional groups and aromatic rings in the digestion chamber [30] and probably no two humic acid molecules will be identical. Humic acid can be formed also from the phenolic compounds released during lignin decomposition (residues) as well as from reducing sugars and amino acids formed as results of microbial metabolism. At certain concentrations, humic compounds can have inhibitory effects on the methane production during the anaerobic digestion of organic waste [7,9,17,31]. Fernandes et al. [9] showed that humic acid like and fulvic acid like compounds may seriously impact the hydrolytic enzymatic activity. Azman et al. [31] did not only find lower biomethane potential (BMP) of the tested microcrystalline cellulose (Avicel) substrate at presence of humic acids, but also additional VFA accumulation, indicating the inhibition of methanogens rather than hydrolysis as suggested earlier by others [7,9]. There are plenty of researches conducted towards the effect of these inhibitor compounds on the fermentation of lignocellulosic materials for bioethanol production [8,32,33]. However, their effects on the anaerobic digestion under both thermophilic (55 °C) and mesophilic (35 °C) conditions hardly have been studied. Fig. 1 schematically presents possible FSF (as a lignocellulosic material) conversion during (pre-)treatment, producing recalcitrant humic matter, furans (furfural and HMF) and aromatic compounds [15,17].

Hence, the purpose of this study was to assess the impact of representative intermediates such as furfural, HMF, vanillin and humic acid sodium salt on anaerobic consortia from digesters operated under both thermophilic and mesophilic conditions. Influence of these potentially inhibiting intermediates on the hydrolysis and methanogenesis was studied employing specific methanogenic activity (SMA) assays and cumulative methane production (CMP) tests.

## 2. Materials and methods

### 2.1. Substrate

FSF was collected from a 350 μm mesh fine sieve (Salsnes, Norway) at sewage treatment plant (STP) Loenen, the Netherlands, and stored at 4 °C prior to conduct the CMP tests. Total solids (TS) and volatile solids (VS) were measured on weight base (g/L) according to the standard methods for the examination of water and wastewater [34]. Chemical oxygen demand (COD) was measured using Merck photometric cell tests (500–10,000 mg/L, Merck, Germany). All analyses were done in triplicate. Furfural and 5-hydroxymethyfurfural (HMF), Vanillin and humic acid sodium salt were purchased from Sigma Aldrich (98% purity, Germany).

### 2.2. Inoculum

Four water jacketed laboratory mixed fed-batch digesters operated as a fed-batch reactor (FBR) with a working volume of 8 L were operated in duplicate to digest FSF under both thermophilic (55 °C) and mesophilic (35 °C) conditions over a period of 718 days, prior to harvest the inoculates. The inoculum was directly taken from the digesters. At the time of sampling, thermophilic and mesophilic digesters were operated at organic loading rates (OLR) of 5.5 and 2.5 kg COD/$m^3$ d, respectively. The inoculates were characterized in the same way as the substrate. Prior to the experiments, sludge pH was determined at 7.4 ± 0.2 and 7 ± 0.1 for thermophilic and mesophilic sludge, respectively.

### 2.3. Volatile fatty acid (VFA)

Volatile fatty acids (VFAs) were quantified by Gas Chromatograph (GC, Agilent Technology 7890A), using a flame ionization detector (FID) and a capillary column type HP-FFAP Polyethylene Glycol (25 m × 320 μm × 0.5 μm) with helium as the carrier gas at a total flow of 67 mL/min and a split ratio of 25:1. The GC oven temperature was programmed to increase from 80 min to 180 °C in 10.5 min. The temperatures of injector and detector were 80 °C and 240 °C, respectively, and the injected volume was 1 μL. Prior to GC analysis 10 mL of digested sample was first centrifuged at 15,000 rpm for about 15–20 min. Then, the supernatant was filtrated over 0.45 μm filter paper. The filtrated liquid was diluted 2 and 3 times with pentanol as internal solution (300 ppm) for mesophilic and thermophilic digestion samples, respectively. Finally, 10 μL of formic acid (purity >99%) was added into the 1.5 mL vials.