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Evaluating the effect of enzymatic pretreatment on the anaerobic digestibility of pulp and paper biosludge

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ABSTRACT

Anaerobic digestion of biosludge has not yet been implemented in pulp mills due to low biogas yields. Enzymatic pretreatment of biosludge has shown improvements in biogas yields but results are varied. A key limitation of previous studies is that they fail to consider the COD contribution from the enzyme solutions. The aim of this study was to systematically investigate the potential for enzymatic pretreatment on the anaerobic digestibility of pulp mill biosludge. Out of the six enzymes tested, four enhanced the anaerobic digestibility of biosludge. At the end of the BMP, a maximum improvement of 26% in biogas yield was observed with protease from *B. licheniformis*. There was no correlation between enzymatic activities on standard substrates and/or on biosludge and the effect of enzymes on biogas yields. Enzymes have potential for improving biosludge anaerobic digestibility but more research on optimal conditions and potential synergies with other pretreatment is needed.

1. Introduction

There is increasing interest in developing technologies to reduce biomass produced during wastewater treatment processes in pulp and paper (P&P) mills. Sludge management accounts for up to 60% of treatment costs [1]. Anaerobic digestion of sludge is extensively used in municipal wastewater treatment but its implementation in pulp mills is still limited. The mass and volume reduction afforded by anaerobic digestion translates into savings associated with sludge handling and disposal, and the recovery of energy from biogas make this a very attractive process. However, the use of anaerobic digestion for P&P mill biosludge has not been industrially established because of low methane yields, reportedly due to the complexity and recalcitrance of pulp and paper mill biosludge and the presence of toxic chemicals [2]. As discussed in recent reviews by Elliott & Mahmood (2007) and Meyer & Edwards (2014), several biosludge pretreatment approaches have been investigated for improving the feasibility of anaerobic digestion of biosludge in P&P mills [2,3].

Enzymatic pretreatment of biosludge can potentially enhance methane yields. Hydrolysis is widely accepted as the limiting step in the anaerobic conversion of the complex organic matter in biosludge. Enzymes that can speed-up hydrolysis are gaining attention because of their catalytic activity and potential to be produced from renewable and/or waste sources [4]. Discovery of novel enzymes, enzyme engineering, and the reduction of production costs is driving the development of many enzyme-based technologies. As discussed in Parawira (2012), enzymes are recognized for their potential to hydrolyze biosludge, resulting in improved anaerobic digestion. However, the effects of enzymatic pretreatment are poorly understood [5]. To date, studies have concentrated primarily on municipal biosludge with conflicting findings. While some authors have reported a substantial improvement in biogas production, methane yield, and/or chemical oxygen demand (COD) solubilization [6–9], others found improvements only in lab-scale experiments but not in pilot scale [10] and still others found no improvement [11].

Proteases and glycosidases are the obvious first enzyme candidates for pretreatment, because biosludge is mainly composed of microbial biomass comprising proteins and complex carbohydrates. In addition, the particles in biosludge are embedded in a gel-like matrix of extracellular polymeric substances (EPS) comprising different biopolymers, including proteins, carbohydrates, lignin, DNA, and RNA [12,13]. Proteins and carbohydrates account for up to 70% of the organic matter present in P&P biosludge [2]. Accordingly, previous studies employed proteases, glycosidases, or a combination thereof for biosludge treatment [6,9–11].

In reviewing the work done in previous studies on the enzymatic pretreatment of biosludge we identified three possible confounding factors, which we addressed in this study. First, in only two studies does

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the chemical oxygen demand (COD) contributed by the enzymes appear to be taken into account [10,11]. Second, enzymes are polypeptides and, as polymers, could have an effect on biosludge flocculation and possibly digestibility that is not related to their enzymatic activity. For example, proteins can induce flocculation of sludge particles as has been reported previously [14]. Accordingly, the use of inactivated enzyme controls is needed to confirm a catalytic mechanism to a pretreatment. Finally, deconvoluting biogas produced from biosludge from biogas produced by digestion of the inoculum is important to quantify the effect of enzymes on biogas yield from the intended substrate (biosludge). Reducing the "background" biological activity of the system (i.e. biogas from inoculum) enables quantification of the true impact of the enzymatic pretreatment on biosludge. Addressing these issues will lead to a more accurate assessment of the potential of enzymatic pretreatment for enhanced anaerobic digestibility of biosludge at larger scale. With these considerations, the specific objectives of this study were:

- To develop an experimental methodology that evaluates the effect of enzymatic pretreatment on anaerobic digestibility separately from any effects related to the enzymes as organic additives.
- To test hydrolytic enzymes from two groups, proteases and glycosidases, for their potential to enhance the anaerobic digestibility of biosludge.
- To measure enzymatic activity using standard substrates added into biosludge, to detect possible inhibitions or synergies.
- To measure changes in soluble COD content during enzymatic pretreatment of biosludge to better characterize the process.

2. Materials and methods

The approach used to meet these objectives involved three biochemical methane potential (BMP) assays, enzymatic and compositional analyses. A flow diagram of the general approach is provided in Fig. 1.

2.1. Biosludge samples

Waste activated sludge, or biosludge, from a secondary clarifier was obtained from a Canadian P&P mill that produces a variety of pulp, paper and specialty products using sulfite pulping and mechanical pulping (bleached chemi-thermomechanical pulp − BCTMP). Samples were kept at 4 °C in the laboratory prior to the experiments and for a maximum of two weeks. Before use in experiments, biosludge samples were allowed to settle overnight in a cold room at 4 °C, and the supernatant was discarded to obtain a thickened sludge.

Gamma irradiated biosludge was used in one experiment (BMP 3) to inactivate microorganisms in the biosludge to enable testing enzyme activity without confounding effects from microbial activity inherent to biosludge. Sludge was irradiated at a dose of 25 kGy produced from a cobalt source (Co-60) using a Gamma Cell (G.C. 220). Previous studies have reported a > 99% inactivation of common pathogens present in sewage sludge at a dose of 5 kGy [15].

2.2. Anaerobic inoculum (Granules) preparation

Anaerobic granules were used as the inoculum for the biochemical methane potential (BMP) assays described in section 2.6. Granules were obtained from the anaerobic wastewater treatment reactor of a Canadian pulp and paper mill and were maintained in the laboratory under anaerobic conditions at 4 °C. Two weeks before the BMP set up, anaerobic granules were diluted (1:2) in a synthetic medium described in [16]. The diluted granules suspension was then incubated at 37 °C and fed with the synthetic feed (0.4% v/v) previously reported by [17]. The anaerobic activity of the inoculum was confirmed by measuring biogas production. The inoculum was left incubating until the day of the experiment. This two-week incubation period reduced the easily digestible COD, minimizing the background biogas produced in the BMP assays.

2.3. Enzyme preparations

The enzymes used in this study were hydrolases from two subgroups: proteases (EC 3.4) and glucosidases (EC 3.2.1). A preliminary screening of proteases and glucosidases was conducted in our laboratory. Based on their activity on standard substrates six enzymes were selected. Four of the enzymes were available commercially and two were produced in our laboratory. General information about the

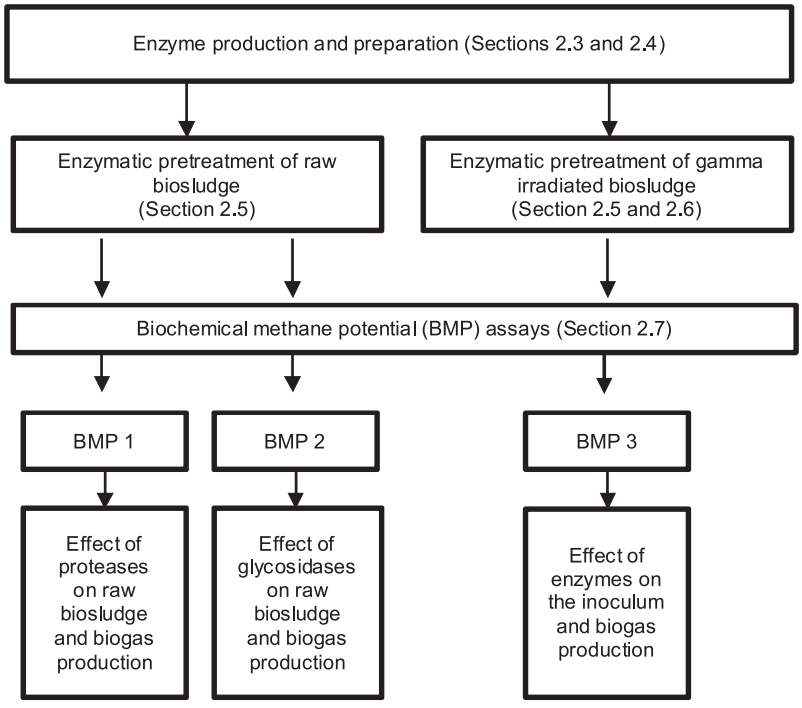

Fig. 1. General approach for investigating the effect of enzymatic pretreatment on biosludge anaerobic digestibility.