Monitoring Sugar Release during Pipeline Hydro-transport of Wheat Straw

Sonia Ghatora[a], Mayank Kumar[a], Mahdi Vaezi[a], Amit Kumar[a,1] and David Bressler[b]

[a]Department of Mechanical Engineering, 10-263 Donadeo Innovation Centre for Engineering, University of Alberta, Edmonton, AB, Canada

[b]4-10, Agriculture/Forestry Centre, University of Alberta, Edmonton, AB, Canada

Abstract

Pipeline transport of biomass is an economically viable and technically feasible approach to replace conventional truck delivery approach and make the biomass-based energy industry more competitive with fossil fuel-based plants. A 25 m long and 50 mm diameter closed-circuit pipeline facility was fabricated to experimentally investigate the mechanical and chemical feasibility of transporting agricultural residue biomass-water mixtures (slurries) through pipelines. This research used the pipeline facility to study the loss of sugars (glucose and xylose) while pipelining wheat straw-water mixtures. The release of similar sugars was also measured in shake-flask cultures under controlled conditions. The output of this research is important for bio-processing facilities as a high sugar content slurry would improve the yield of biofuels produced from pipelined lignocellulosic materials. After several hours of recirculating throughout the pipeline, as well as shaking in the flask, a drop in sugar concentration was detected. A microbiological analysis performed on both slurries proved the decline to be due to microbial proliferation. Accordingly, diethyl pyrocarbonate oxidizing antimicrobial agent and glutaraldehyde and bronopol non-oxidizing agents were alternatively tested to restrict microbial

[1] Corresponding author: Tel.: +1 (780) 492-7797, Email: amit.kumar@ualberta.ca (A. Kumar)

proliferation. These agents demonstrated reduced sugar loss and, in turn, showed an enhancement in the yield of glucose and xylose. This research aims at maximizing possible sugar release through mechanical action throughout the pipeline in the presence of antimicrobial compounds, which would increase the yield of biofuel produced from pipelined agricultural residue biomass.

Keywords: Pipeline; wheat straw slurry; antimicrobial agent; glucose; xylose

1. Introduction

The gradual decrease of fossil fuels and increase of GHG emissions, along with petroleum price hikes, have noticeably increased the global demand for alternative sources of energy such as biomass, wind, solar, and hydroelectric [1-3]. Among these energy sources, lignocellulosic biomass is the most promising due to its abundance, renewability, and carbon-neutral nature. However, biomass-based refineries are still in the early phases compared to well-established fossil fuel facilities. One of the main barriers to producing large quantities of biofuels in biorefineries is the high cost of truck delivery of feedstock [4, 5]. Transporting biomass-water mixtures (slurries) via pipelines (hydro-transport) can be considered an alternative approach to economically supply large amounts of biomass to bio-refineries [6]. Vaezi et al. [7-9] have extensively studied and proved the technical and economic feasibility of hydro-transporting lignocellulosic biomass through pipelines.

Lignocellulosic biomass is usually composed of carbohydrate polymers (50-60%) like cellulose and hemicellulose and non-carbohydrates like lignin (20-35%), which makes it a suitable candidate for the production of biofuels and other chemicals via fermentation [10]. However, these sugars might be released or lost prior to or during pipeline hydro-transportation as a result of chemical interactions or microbial proliferation [11-13], accordingly negatively impacting the

yield to be achieved at a biofuel production facility (i.e., a biorefinery) [14, 15]. Consequently, the release of sugars needs to be monitored and controlled during biomass pipeline hydro-transportation.

Throughout the pipeline, unsterilized untreated (ground) biomass, when it comes in contact with unsterilized water, requires the addition of antimicrobial agents to prevent microbial proliferation. Such contamination, if uncontrolled, reduces the biomass sugar yield during pipeline transport due to the uptake of sugar nutrients by the microbes and risks the potential formation of biofilms in the pipeline system. Since there are limited means of maintaining high temperatures in pipelines, agents that work at low temperatures were selected (diethyl pyrocarbonate, glutaraldehyde, and bronopol) [16-19]. These antimicrobial agents were first tested at the flask level to identify the best agent for further studies in a pipeline. The selected antimicrobial agents were then added to the mixing tank coupled with a pipeline system to prevent proliferation of microbes. The samples were taken at regular intervals to measure amount of sugars released during pipeline transport.

For the purpose of this study, the release of sugar was assessed while pumping wheat straw-water mixtures through pipelines. This involved qualitative and quantitative detection of glucose and xylose in both pipeline and flasks. In addition, various anti-microbial agents were tested to identify the best agent for long-time pumping of wheat straw slurries in large-scale long-distance pipelines. This study aims towards preventing the loss of sugar through microbial proliferation while pipelining wheat straw-water mixtures. Retaining the wheat straw sugar content in the slurry will improve the yield in biofuel production processes that receive biomass by pipeline.

2. Materials and methods

2.1. Feedstock properties and preparation

Wheat straw (Triticum sativum; dry stalks of wheat) collected from farms in southern and northern Alberta, Canada, was first milled using a commercially available cutting mill (SM-100; Retsch Inc., Newtown, PA, USA) and then classified using a chip classifier (BM&M Inc., Surrey, BC, Canada). The wheat straw fibrous particles 6.4 mm in length (d_{50}=5 mm) were selected as the biomass substrate to conduct this experiment.

2.2. Experiments in flasks

Shaking flask experiments were carried out using Erlenmeyer flasks (500 ml) with wheat straw, and distilled water (6.4% dry matter). All the preliminary experiments with flasks were performed during a 24-hour time period, under unsterilized conditions, and without any antimicrobial agent. In the next step, both the Erlenmeyer flasks and the water were sterilized under autoclave conditions at 15 psi and 121°C for 20 min prior to the addition of unsterilized wheat straw. The oxidizing antimicrobial agent, DPEC (Sigma Aldrich, St. Louis, MO, USA), and non-oxidizing antimicrobial agents, gluteraldehyde (Sigma Aldrich, St. Louis, MO, USA) and bronopol (OSP Microcheck, Calgary, AB, Canada), were used separately at a concentration of 0.005% individually. The flasks were incubated at 25ºC for 48 hours, and the samples were made at 0, 2, 4, 8, 24, 28, 32, and 48 hours. All the experiments were performed in triplicate.

2.3. Experiments in a pipeline facility

The pipeline test circuit consisted of a 25.5 m long and 50 mm diameter steel pipe. The mixing tank, 0.8 m in diameter with a 455 L capacity, attached to the pipeline was agitated by a 0.37 kW centrally placed vertical mixer carrying a motor-boat propeller (EV6P50M; Lightning Inc., Rochester, NY, USA). Flow in the system was created by centrifugal slurry pump of 7.45 kW (CD80M; Godwin Pumps Ltd., Bridgeport, NJ, USA) connected to a 7.45 kW induction electric motor (CC 068A; Madison Industrial Equipment, Vancouver, BC, Canada), and the flow was

controlled by a 14.91 kW variable frequency drive (VFD) controller (MA7200-2020-N1, TECO-Westinghouse Co., Round Rock, TX, US). Further details on the system configuration can be found elsewhere [7-9, 20]. The temperature of the mixture downstream from the pump was partially controlled at 20ºC by a double tube heat exchanger. The mixture bulk velocity and mixture temperature were measured using an electromagnetic flow meter (FMG-401H; Omega Eng., Stamford, CT, USA) and a resistance temperature detector (RTD-E; Omega Eng., Stamford, CT, USA) [7, 9].

Prior to the slurry preparation in the tank, the closed pipeline circuit was washed thoroughly with water to ensure sterilization and facilitate deaeration. While slurry was pumped through the pipeline, batches of 6.4 mm long wheat straw were gradually added to the mixing tank to reach the maximum solid mass content of 6.4% dry matter. Following the addition of wheat straw, the non-oxidizing sterilization agent bronopol was added to the tank at a concentration of 0.005 wt% of raw material. The temperature in the pipeline was maintained at ~20ºC and slurry samples of 50 ml volume were made from the pipeline discharge valve at time intervals of 0, 2, 4, 8, 12, 24, 28, 32 and 48 hours. The samples were centrifuged at 8000 rpm for 20 min and the supernatant was used for sugar analysis.

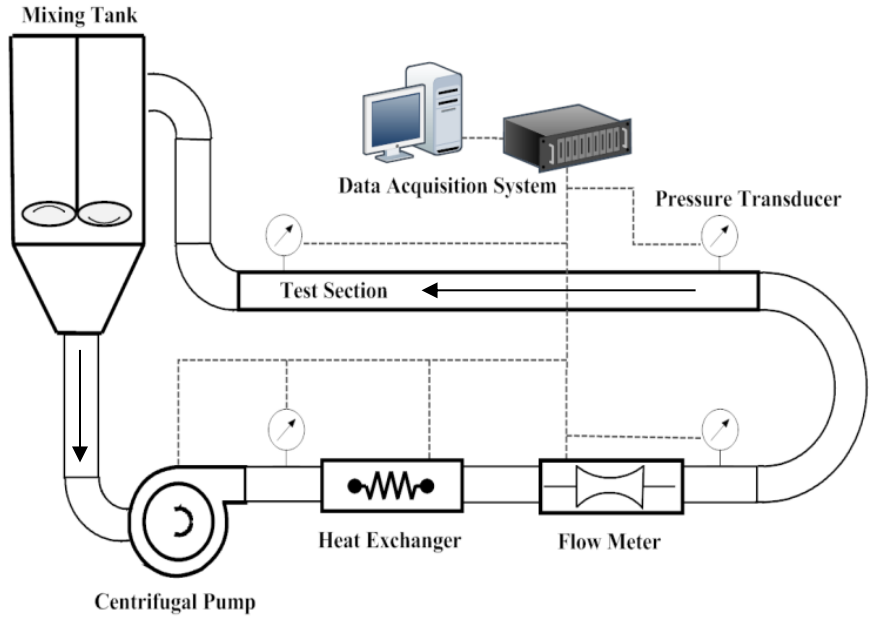


Figure 1: Schematic diagram of the laboratory-scale closed-circuit pipeline facility set-up (modified from Vaezi and Kumar [9]).

2.4. High Pressure Liquid Chromatography (HPLC) analysis to determine sugar and bronopol concentrations

2.4.1. Sugar analysis: The initial concentrations of glucose and xylose in g kg^{-1} of biomass were calculated based on cellulose and xylan amounts from the literature [21-23]. The glucose and xylose content in the samples, from both pipeline and flask, taken at different time points were analyzed using an Agilent 1200 series HPLC (Agilent Technologies, Inc., CA, USA) equipped with a refractive index detector, a Bio-Rad HP87H column (300×7.8 mm) at 60°C, and 5 mM sulfuric acid mobile phase at 0.6 mL min^{-1} [24].

2.4.2. Bronopol analysis: A set of experiments was conducted to monitor the degradation of bronopol 48 hours after being pumped through the pipeline. The results were later used to explain the variation in sugar concentration within hours of pumping. With respect to the use of HPLC to determine the residual bronopol, the methodology outlined by Wang et al. [25] was

adopted with some modifications. The HPLC grade reagents (acetonitrile, methanol, and orthophosphoric acid) as well as the HPLC water (Chromasolv) used for sample preparation were obtained from Fisher Scientific Co. (Pittsburgh, PA, Canada) and Sigma Aldrich Co. (St. Louis, MO, USA), respectively. Bronopol standard was prepared following standard preparation approaches. Samples were made out of wheat straw-water-bronopol mixtures at 0, 24, and 48 hours. The samples were then centrifuged and filtered through a 0.45 μm nylon membrane filter (Mandel Scientific, Guelph, Canada).

An Agilent (1200 series) HPLC system with an Agilent 5 μm C18 column (dimensions 150×4.6 mm) at 30°C, methanol/water/orthophosphoric acid (5:95:0.05, volume-based) mobile phase at 1.5 ml min^{-1}, and a variable wavelength detector (VWD - wavelength of 210 nm) were employed to develop chromatograms for the standard and mixture samples and obtain the corresponding concentrations.

3. Results and discussion

3.1. Assessment of sugar release

The release of glucose and xylose, both during the slurry circulation in the pipeline and shaking in the flask, was observed over periods of 24 and 48 hours under unsterilized and sterilized conditions. A decline in the concentrations of glucose and xylose in the slurry was observed within 4 hours, and sugar loss continued for 24 hours of slurry circulation (Figure 2).

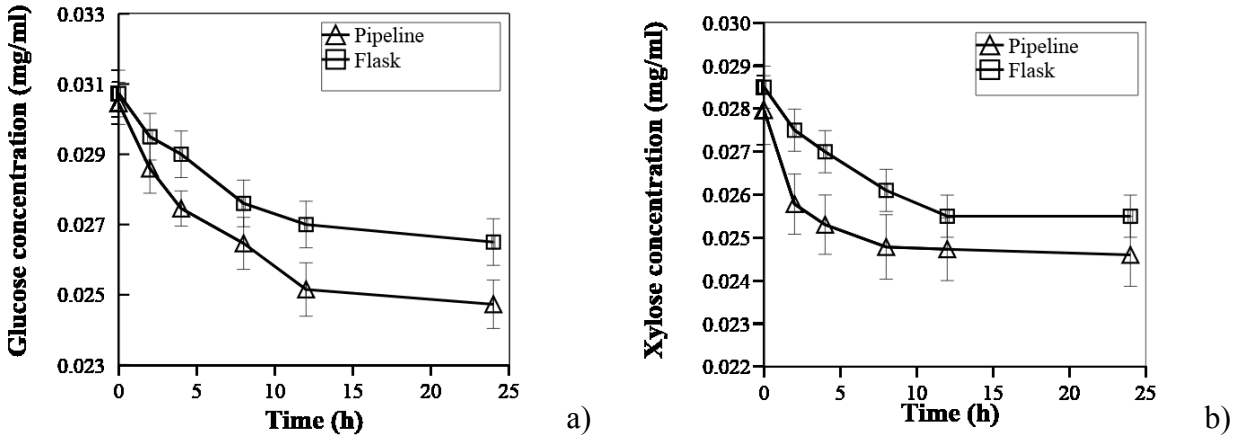


Figure 2: Release of glucose (a) and xylose (b) in pipeline and flask under unsterilized conditions.

Monitoring the change in glucose within the first 24 hours in the flask and the pipeline revealed an overall glucose loss of ~0.25 g kg^{-1} of biomass in the flask compared to a loss of ~0.33 g kg^{-1} of biomass in the pipeline. The loss in sugar could be attributed to the proliferation of microbes sourcing from unsterilized water, feedstock, and environmental exposure. The microbes have regulating mechanism for their catabolic activity with subject to environmental growth nutrients. Metabolic pathways in bacteria have evolved for easy utilization of hexoses and as they come in contact with hexoses like glucose, they tend to show rapid proliferation and cause a decrease in sugar content. Similarly, overall decreases of ~0.31 and ~0.35 g kg^{-1} of biomass of xylose concentration were observed in the flask and the pipeline, respectively (see Table 1). This finding demonstrates that under the experimental conditions tested, at least a subset of the microbial population showed the ability to use pentose sugars. The loss in glucose and xylose concentration in the flask was relatively less than that observed in the pipeline.

3.2. Contamination studies

The micro-plate assay of the filtrate from both flask and pipeline showed that there was microbial proliferation in both flask and pipeline, and the initial pH of 7 at zero hour decreased

to pH 5 after 24 hours, confirming possible microbial metabolism and growth [26]. The release of monosaccharides from wheat straw as a result of particle size reduction using mechanical grinding has been documented in the literature [11-13]. However, the grinding processes used in these studies resulted in very fine grinding of the wheat straw, and therefore the amounts of sugars released were also high. In our study, the initial levels of sugars released, found at zero hour, were very low, which may be due to the grinding of wheat straw to 6.4 mm. The decline in sugar concentration observed in pipeline and flask resulted from microbial proliferation from the unsterilized straw, water, and interior of the pipeline and flask [27]. Therefore, various antimicrobial agents were added to the slurry to restrict microbial growth.

All the experiments in the flask were performed under aseptic conditions. Since the wheat straw added to the flask was unsterilized, an antimicrobial agent (DPEC, glutaraldehyde, or bronopol) was added to the slurry. The aim of this was investigate the transport the biomass slurry in a pipeline system, which are usually at low temperatures in Western Canada. Therefore, in addition to DPEC, which is an oxidizing antimicrobial agent, less temperature sensitive non-oxidizing glutaraldehyde and bronopol were also selected. The glucose concentration in the flask at zero hour was 0.029, 0.027, and 0.031 mg ml^{-1} in the presence of DPEC, glutaraldehyde, and bronopol, respectively (Figures 3A-C). More glucose was released in the solution over time, and glucose levels reached their maximum at 28 hours in the presence of DPEC (0.062 mg ml^{-1} of glucose concentration). However, in the presence of glutaraldehyde, 0.052 mg ml^{-1} of glucose was observed after 24 hours and in the case of bronopol, glucose levels of 0.066 mg ml^{-1} were detected. A decline in glucose levels was observed in the slurry containing DPEC after 28 hours of incubation, where glucose concentration decreased to 0.032 mg ml^{-1} at the 48-hour time point. No such decline in glucose levels was observed in the presence of glutaraldehyde and bronopol,

though a slight increase in glucose levels was observed after 48 hours of incubation (Figures 3B, 3C). Similarly, an increase in xylose levels was observed in the slurry in the presence of DPEC, glutaraldehyde, and bronopol after 48 hours of incubation. In the presence of DPEC as a sterilant, the xylose concentration increased from 0.029 to 0.035 mg ml^{-1} in 32 hours of incubation; however, it decreased after this point to 0.030 mg ml^{-1} at 48 hours of incubation (Figure 3A). When glutaraldehyde and bronopol were used as sterilants, a decline in xylose concentration was not observed. Xylose concentration increased from 0.028 to 0.036 mg ml^{-1} and from 0.029 to 0.038 mg ml^{-1} in the presence of glutaraldehyde and bronopol, respectively (Figures 3B, 3C). The increase in concentrations of glucose and xylose may be attributed to the mechanical pumping of the biomass supported by the antimicrobial effect of DPEC, glutaraldehyde, and bronopol [28-30], which stopped the microbial growth on the available sugars. A drop in sugar concentration after 28 hours, when DPEC was used as antimicrobial agent, may be because of the rapid oxidizing behavior of DPEC. Simply, after initial treatment, the residual amount of available DPEC was insufficient to continue to protect the system from microbial growth in the presence of the available sugar solution.

Table 1: Release of glucose and xylose in flask and pipeline during sterilization and unsterilization (with and without bronopol) experiments:

	Flask		**Pipeline**	
Time	Glucose (g kg^{-1})*	Xylose (g kg^{-1})	Glucose (g kg^{-1})	Xylose (g kg^{-1})
Unsterilized				
0 hour	2.02 ± 0.20	2.96 ± 0.28	1.97 ± 0.26	2.91 ± 0.29
24 hours	1.77 ± 0.18	2.65 ± 0.23	1.64 ± 0.19	2.56 ± 0.21
Sterilized**				
0 hour	2.04 ± 0.25	2.99 ± 0.31	2.17 ± 0.22	3.02 ± 0.25
24 hour	3.94 ± 0.33	3.95 ± 0.28	4.79 ± 0.35	5.20 ± 0.51

48 hour	4.47 ± 0.36	3.92 ± 0.34	1.90 ± 0.21	3.64 ± 0.32
Gain (inhibited sugar loss)				
24 hour	2.17	1.3	3.15	2.64

*g kg^{-1} of biomass
**Experiments performed in the presence of bronopol

Bronopol, the non-oxidizing antimicrobial agent, was selected for further experimental work in the pipeline. In the presence of bronopol, the amount of glucose and xylose released were relatively higher in the pipeline compared to that observed in the flask immediately following 24 hours of incubation. However, the concentrations of both glucose and xylose started to decrease afterwards up to 48 hours. The concentration of glucose released in the pipeline (4.79 ± 0.35 g kg^{-1} of biomass) was found to be higher than that released in the flask (3.94 ± 0.33 g kg^{-1} of biomass) after 24 hours of incubation. The higher glucose release in the pipeline could be attributed to the continuous mechanical movement of the slurry in the pipeline. This is due to the degradation in bronopol concentration during the course of the experiment and was proven by measuring the residual bronopol within the straw-water sample within 48 hours. Figures 4(b) shows a 22% reduction in bronopol content within the first 24 hours and an increase of 42% in the next 24 hours [25].

An earlier study showed that particle size during wheat straw slurry transportation by pipeline decreases with time [31]. The amount of glucose and xylose released in the pipeline was relatively higher than that released in the flask, which can be attributed to the fact that pipeline provides higher shear effect as compared to flask. The mixing in pipeline is caused by the flow (velocity) while flask uses a magnetic stirrer beads for stirring. The internal environment of the pipeline and the operating conditions may have led to the degradation of wheat straw particles that resulted in the release of sugars. That the amount of sugar released in the pipeline declined after 24 hours when bronopol was used as a sterilant indicates that another dose of bronopol may

be required to restrict microbial growth and save sugars. When an appropriate antimicrobial agent is used, pipelines can transport untreated and pretreated biomass free of microbial contamination, and further when enzyme is added to the slurry, some biomass hydrolysis may be achieved [32, 33].

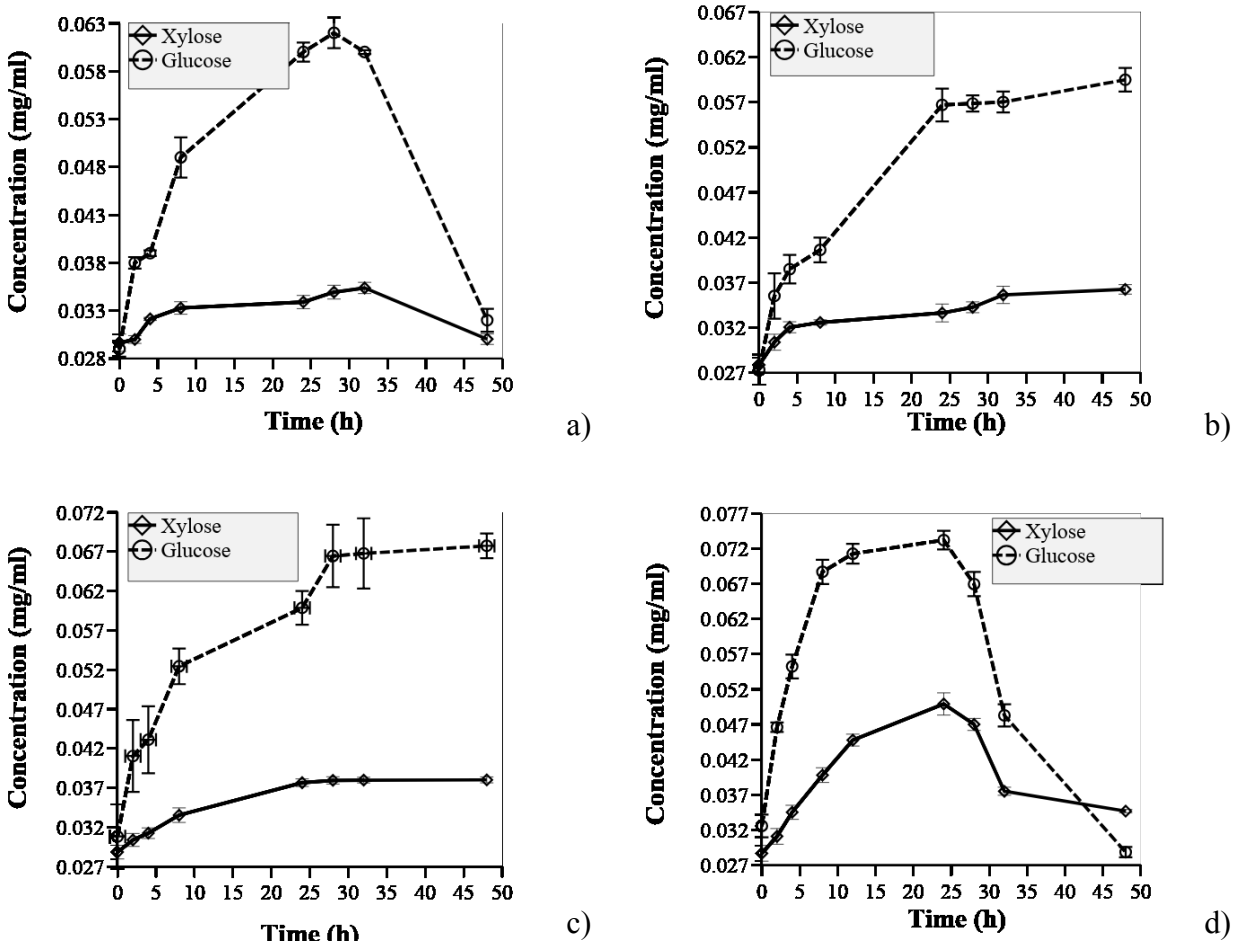
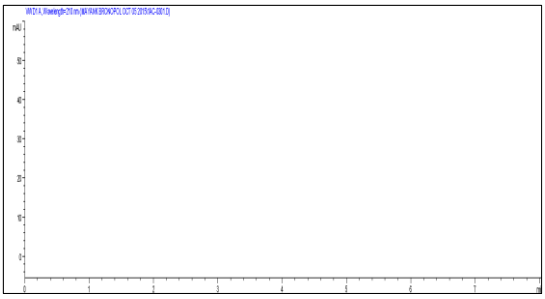
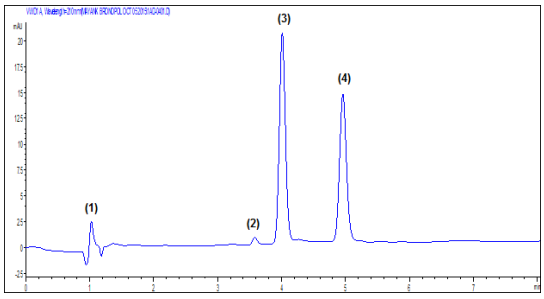


Figure 3: Release of glucose and xylose in flask in the presence of (a) DPEC, (b) gluteraldehyde, (c) bronopol and (d) in pipeline in the presence of bronopol.



a)



b)

Fig 4: Chromatogram of samples measured at (a) 0 hr and (b) 48 hr. Chromatographic peaks refer to (1): 2-bromoethanol, (2): bromonitromethane, (3): bronopol, and (4) bromonitroethanol [25].

4. Conclusion

The release of glucose and xylose sugars was monitored while pumping wheat straw-water mixtures (slurries) throughout a closed-circuit pipeline. The release of similar sugars was also measured in shake-flask cultures under controlled conditions. Sugar contents in the biomass slurry dropped within a couple of hours of pumping and shaking. This sugar loss was attributed to microbial proliferation sourcing from unsterilized wheat straw, water, or equipment. To restrict microbial growth and prevent biofilm formation, both oxidizing and non-oxidizing antimicrobial agents were tested in both the experimental setups where an increase in sugar concentration was observed with the use of non-oxidizing agents, indicating successful inhibition of microbial growth. Bronopol non-oxidizing agents inhibited the loss of glucose and xylose sugars by 2.64 and 3.15 g per unit mass of wheat straw, respectively, within 24 hours. However, the bronopol was effective for one day when sugar loss was observed afterwards.

The results obtained here could apply to most agricultural residue biomass-water mixtures in various environments and would help improve biorefinery conversion yields from pipelined wheat straw.

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