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Pre-treatment of Distillery Spent Wash (Vinasse) with Vortex Based Cavitation and its Influence on Biogas Generation

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Abstract

Distilleries generate high COD (chemical oxygen demand) wastewater streams that are anaerobically digested to produce biogas with sub-optimal yields. In this work, for the first time, novel vortex-based hydrodynamic cavitation (HC) pre-treatment of these waste streams was investigated for significantly enhancing biogas yields. Molasses spent wash (120,000 ppm COD, India) and vinasse (27,000 ppm COD, Brazil) were pre-treated at varying number of passes (between 1 – 20) through the HC device at constant inflow, biochemical methanation potential (BMP) was measured and described using a first order model. Vortex-based HC pre-treatment led to 14% enhancement (2 passes) in BMP from spent wash (difficult to digest compared to vinasse) with a net energy gain of 1 GJ/ton COD. 10-22% increase in biogas yields from large-scale industrial spent wash digesters confirmed the laboratory findings. The work presented is useful and can be translated to recalcitrant feedstock for enhancing valorization using vortex-based HC pre-treatment.

Keywords: hydrodynamic cavitation, spent wash, vinasse, biomethane, energy gain.

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1. Introduction

Sugarcane industry is one of the largest agri-based industry globally and contributes to ~80% of world's sugar production. Worldwide sugar prices are falling due to surplus sugar stocks and production (International Sugar Association, 2018). It is therefore inevitable and critical that allied industries such as distilleries that often operate alongside sugar industries support the economic viability. Alcohol fermentation can generate revenue, however, to enhance the profits to support the parent sugar industries, effective waste management and a circular bioeconomy approach is necessary. For instance, a predominant by-product of the sugar industry - molasses is utilized by distilleries for alcohol fermentation, where these process generates 10-15 m³ distillery wastewater/m³ alcohol produced (Christofaletti et al., 2013). With the global ethanol production increasing annually for beverage and fuel purposes the associated wastewater produced is immense [e.g., >40 billion liters in India (Basu et al., 2015) and >300 billion liters in Brazil (da Silva Neto et al., 2020)]. Therefore, it is paramount to address the need to manage this wastewater and simultaneously support the sugar industry.

Distillery wastewater is known as spent wash, vinasse, slop, stillage or distillery effluent depending upon the source of sugar used for fermentation. If distilleries are solely based on molasses (as in India), typical chemical oxygen demand (COD) of waste water (called as spent wash) ranges between 80,000 – 160,000 ppm and pH in the acidic range (Basu et al., 2015). If the distilleries also use sugar cane juice (as in Brazil), typical COD of wastewater (called as vinasse) is much lower (España-Gamboa et al., 2011) and is around 30,000 ppm. Variation in the composition and COD is seen due to

its origin and additionally, presence of trace elements, heavy metals, anionic salts, phenolics and melanoidins are responsible for the characteristic dark brown color. These wastewater streams therefore require treatment prior to discharge.

Most common industrial practices for the disposal of spent wash are fertirrigation, biocomposting, incineration and anaerobic digestion (Cooper et al., 2020; Fito et al., 2019). Fertirrigation is the process of using spent wash as a water source to irrigate croplands. This can effectively reduce the water footprint on the agricultural sector and discharge load of the distillery. However, long term use of spent wash for irrigation was reported to affect the soil quality by altering the alkalinity and biodiversity of the soil (Mohana et al., 2009). Biocomposting offers the use of two waste streams generated by the sugar industry, namely, press mud and spent wash. Biologically treated spent wash is mixed with press mud and then biocomposted to produce fertiliser (Chowdhary et al., 2018). This could add value to the industry by selling the fertiliser or could be recycled to the farmlands associated with the industry. In either case, the concerns surrounding the handling of huge volumes of spent wash and required land area to produce the compost in addition to the slow rate of composting limits its use. Incineration is a popular option amongst distilleries, where the spent wash can be burnt to generate heat and electricity as well as recover condensate water for reuse within the facility. Problems associated with the energy cost and maintenance of the incinerators are the main limitations to this technology (Fito et al., 2019). Anaerobic digestion (AD) offers an attractive option, not only for its treatment but also low sludge generation compared to aerobic treatment, low operating costs, robustness to handle varying feed compositions and most importantly

energy recovery in biogas (Tewari et al., 2007). Biogas generated could then be upgraded to biomethane to meet the requirements of transportation fuel or could be used internally by the sugar industry to satisfy their energy needs and offset the electricity costs. Biomethanated spent wash (digestate from AD) would still not be suitable for discharge as it is not legally permitted in India due to the zero-discharge policy regulating the distilleries (Central Pollution Control Board India, 2015). Further treatment, eg, biological treatments may be necessary to further reduce the COD (Pant & Adholeya, 2009; Pant & Adholeya, 2007). In other cases, the digestate can be further composted or in occasional cases directly used as a fertilizer (Tewari et al., 2007).

AD, although a suitable technology and the current industrial standard for spent wash valorization, due to its complexity and recalcitrance, its COD conversion rarely exceeds 70%. However, with vinasse, higher conversions are reported mainly due to the higher bioavailability of COD (predominantly present as soluble COD) for digestion. To extract more value and maximize the biogas generation from spent wash by making the available COD more digestible, pre-treatment may be required. A wide range of pre-treatments, from physical, chemical, physico-chemical to biological pre-treatments are reported in literature for the spent wash and vinasse as shown in Table S1 in supplementary information. However, these pre-treatment methods are often expensive or energy intensive and may not be suitable at an industrial scale (typically with spent wash flow rates of 20 m³/h and above).

Scalability and net energy gains of wastewater pre-treatment are reported scarcely in literature. With most available publications it could be seen that a net positive energy gain could not be obtained with many reported pre-treatment

methods (Chang et al., 1994) (Alkan-Ozkaynak & Karthikeyan, 2011) (Martín et al., 2002) (Siles et al., 2011) (Schaefer & Sung, 2008) (Wu-Haan et al., 2010) (España-Gamboa et al., 2017) (Jiménez et al., 2006) (Mallick et al., 2010). It is therefore essential to develop a scalable pre-treatment method which leads to net energy gains at industrial scales. In this work, the use of a novel vortex based hydrodynamic cavitation (HC) was investigated for the pre-treatment of high COD containing spent wash and vinasse to enhance their biogas generation with a net positive energy gain.

(Hydrodynamic) Cavitation is the phenomenon of formation of vaporous microbubbles or cavities in a flowing liquid experiencing local low pressures, its growth and collapse. These collapsing cavities generate localized hot spots with very high temperatures and pressures in addition to intense shear (Saharan et al., 2014). Consequently, highly reactive oxygen species namely hydroxyl radicals (OH) are formed. Hot spots, highly oxidizing radicals and shear generated by HC devices have been utilized for the physico-chemical pre-treatment of lignocellulosic biomass (but never for spent wash or vinasse) for enhancing the biomethane production via AD with a net positive energy gain. These include pre-treatment of agricultural residues (Garuti et al., 2018; Zieliński et al., 2019), grass silage and sugarcane bagasse (Nagarajan & Ranade, 2019), wheat straw (Patil et al., 2016) and waste activated sludge (Petkovšek et al., 2015) to name a few. Furthermore, HC has also been used to improve the aerobic biodegradability of biomethanated distillery wastewater (Padoley et al., 2012). Most commonly reported HC devices (typically for wastewater treatment) are linear flow devices such as venturi tubes or orifice plates with different throat geometries or multiple holes respectively. These conventional cavitation devices are prone to

clogging and severe erosion (since cavity collapse occur close to device walls). These problems have been overcome by a vortex based HC device disclosed by Ranade et al (Ranade et al., 2016) where no small holes or constrictions are present and the cavity collapse occurs in the core, away from the walls (Simpson & Ranade, 2019), thereby improving the life time of the devices.

Vortex based HC devices have been previously employed to successfully treat simulated wastewater (Sarvothaman et al., 2018) and pre-treat lignocellulosic biomasses (sugarcane bagasse and grass silage) to enhance its biochemical methanation potential (BMP) (Nagarajan & Ranade, 2019). Enhancement in BMP up to 24% upon 9 passes HC treatment with a net energy gain of ~2 GJ/ton total solids was reported. HC being energetically favorable offers scalability and therefore looks promising as a pre-treatment method for spent wash and vinasse.

The aim of this work is to evaluate vortex-based HC pre-treatment of distillery wastewater streams to significantly enhance its BMP and to quantify potential of net energy gain via pre-treatment. The effect of this pre-treatment on two types of distillery wastewater streams, namely, spent wash originating from sugarcane molasses based distillery (sourced from an industry in India) and vinasse (sourced from Brazil) were investigated. BMP tests of the untreated and HC treated feedstock were performed. Generated data were analyzed using a first order model following the example of Nagarajan and Ranade (Nagarajan & Ranade, 2019). The net energy gained due to enhanced BMP obtained using the pre-treatment is discussed. Results from large scale industry tests (from the industry collaborator in India) confirming the lab scale results are also presented. The novelty of this work not only lies in utilizing

hydrodynamic cavitation for the first time to pre-treat spent wash for enhancing BMP, but also effectively achieving analogous (to laboratory investigations) enhanced biogas generation at an industrial scale. The results will be useful to evaluate and recommend an energy efficient pre-treatment process for spent wash digesters.

2. Methodology

2.1 Materials

Spent wash (sourced from an Indian sugar mill) was air dried to concentrate it and then the concentrate was dried at 65 °C to a powder. The spent wash powder was then shipped from India to Belfast. Instead of sending in the slurry, dried spent wash powder was shipped for the ease of transportation. For cavitation experiments, the dry powder of required quantity was mixed with tap water present in the tank of the experimental rig to achieve an initial concentration of 162.6 g/L spent wash powder in water. Based on a calibration curve prepared (see supplementary information) this concentration corresponded to a COD of 120,000 ppm which is the typical concentration for spent wash in India. Tap water (also potable) characteristics in Belfast correspond to a total hardness of 125 ppm, with a slightly alkaline pH (Northern Ireland Water, 2020). Nitrates and nitrites as well as heavy metals are present in trace quantities below permissible limits. Therefore, this is not expected to affect the biogas generation. When using real spent wash slurry, the volatiles and residual alcohols present in the liquid will be retained to a greater extent as compared to the dried powder. This may also contribute to the organic fraction utilized towards biogas generation. Vinasse with an initial COD of 27,000 ppm was sourced from a distillery in Brazil and was used as received. Inoculum used for BMP tests were

obtained from a digester in Agri Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland, United Kingdom digesting cow slurry and grass silage.

2.2 Substrate and Inoculum Characterization

To determine the total solids (TS) present, 1 g, 20 g and 2 g of spent wash powder, vinasse and inoculum were taken in dry crucibles in triplicates respectively. These were then placed in a Carbolite Gero oven at 105 °C for 24 h. Upon drying, the crucibles were left to cool down to room temperature and weighed. The weight lost from the sample was attributed to moisture. The crucibles were then transferred to a SNOL 13/1100 LHM01 muffle furnace and held at 550 °C for 2 h. Upon combusting the samples, the crucibles were allowed to cool down to room temperature and then weighed. The weight lost was recorded as the volatiles solids (VS) content of the sample, whereas the weight of samples left behind in the crucibles were attributed to ash (Franco et al., 2018). COD of spent wash and vinasse were measured by first adding appropriate volumes (as recommended by the kit manufacturer) of the samples to the respective reagent kits with the ranges of 0–15,000 mg/L O₂ and 0–1,500 mg/L O₂ respectively purchased from either Hach UK or Merck UK. The mixtures were digested at 148 °C for 2 h and upon cooling to room temperature, the digested samples in the vials were measured using a Spectroquant Move 100 spectrometer against digested blanks.

2.3 HC Pre-treatment

A HC rig, similar to the one previously reported by Nagarajan and Ranade (Nagarajan & Ranade, 2019), hosting a vortex based cavitation device with a nominal capacity of 1.2 m³/h was used for the cavitation pre-treatment of spent wash and

vinasse. Details on rig construction and the schematic of the rig can be found in the supplementary information. For a given hydrodynamic cavitation device, the two operating parameters, namely, pressure drop across the device and number of passes through the device are the key performance governing parameters. It has been previously established for the vortex based HC device that pressure drop across the device does not have a significant impact on device performance (Sarvothaman et al., 2018). Operation with higher pressure drop across the device was therefore preferred because it offers higher flow rate (which reduces potential clogging risks) and higher number of generated cavities. Considering this, in the present work, it was decided to operate the pre-treatment at the maximum discharge pressure of the curve (3.9 bars). The influence of number of passes through the cavitation device on pre-treatment performance was investigated.

Spent wash pre-treatment was initiated by first filling the rig with tap water to a total volume of 12 L. A required quantity of spent wash powder (~1.95 kg) was then added to the holding and allowed to mix with tap water under non-cavitating flow conditions. Dissolution of the required quantity of spent wash powder in water did not lead to any significant increase in the volume of the solution. The solution volume of the spent wash therefore was almost the same as that of tap water taken initially (12 L). In the case of vinasse, 14 L working volume was used. The volume of the pipe sections, cavitation device and pump used in the experimental set-up was 3.5 L. This volume was initially filled with tap water. For making total working volume of 14L, 11.5 L of vinasse was added to the holding tank. Under non-cavitating conditions, the contents of the rig flowed through both the bypass and the main lines. An overhead

206 stirrer was also placed in the center of the holding tank to facilitate proper mixing and
207 to ensure no dead zones occurred. Upon mixing under the above-mentioned
208 conditions for 5 minutes, 500 ml sample was withdrawn from the holding tank and
209 named as untreated sample in the spent wash experiments. In case of vinasse
210 experiments, 1 L sample was taken. Then, the flow through bypass line was completely
211 shut and all flow was diverted through the main line. At this condition, the measured
212 flow rate was 1.54 m³/h and the inlet pressure was 3.9 barg. Cavitation inception for
213 the device used, occurs at inlet pressures slightly lower than 1 barg with the extent of
214 cavitation increasing with increase in inlet pressure. With the current configuration,
215 3.9 barg was the maximum achievable inlet pressure and hence used for all the
216 experiments. Upon setting up the cavitation conditions, 500 ml sample were then
217 taken from the holding tank after 2, 10 and 20 passes for the spent wash experiments.
218 With the vinasse experiments, 1 L samples were collected from the tank after 1, 2 and
219 5 passes. Withdrawal of samples will reduce working volume of liquid in the system.
220 Previously, for simulated wastewater treatment with the vortex based hydrodynamic
221 cavitation device, it was established that the cavitation performance is independent of
222 the working volume (9-14 L) (Sarvothaman et al., 2018). Only parameter that will be
223 affected by change in volume due to withdrawal of samples is the calculation of
224 number of passes through cavitation device. The number of passes per hour was
225 calculated as flowrate in m³/h/volume in m³. The corresponding time required to
226 achieve the reported number of passes were calculated appropriately by considering
227 the change in volume due to sampling. For instance, when 'X' L was the initial volume
228 for the HC experiments, and 1 L was sampled after 1 pass pre-treatment, the time

required to achieve another 1 pass pre-treatment (net treatment of 2 passes) was based on the working volume of 'X-1' L. Therefore, consistent sampling and time calculations were performed.

The initial temperature of the feed in the holding tank was $\sim 18^{\circ}\text{C}$. With the net pumping energy being 25.5 Wh, the theoretical maximum temperature increase was found to be $\sim 2^{\circ}\text{C}$. For spent wash, with the number of passes corresponding to a total pre-treatment time of < 9 min and for vinasse with the time < 3 min. Since the increase in temperature during the pre-treatment experiments was $\sim 2^{\circ}\text{C}$, no efforts were made to control the temperature. All the samples collected were then stored in a refrigerator at 4°C prior to BMP tests.

2.4 BMP Setup and Kinetics

Untreated and cavitated spent wash, as well as vinasse were subjected to BMP tests. An automatic methane potential test system, popularly known as AMPTS II and a gas endeavour system (Bioprocess control, Sweden) were used to perform the BMP tests. Since BMP was measured to quantify influence of pre-treatment, indirect means of quantifying this influence by measuring the change in BOD/COD ratio was not undertaken in the present work. The method of setting up a BMP test previously described by Nagarajan and Ranade (Nagarajan & Ranade, 2019) was used in this case. For each feedstock, 15 reactors were set up that was composed of triplicates of blank tests followed by untreated and cavitated feedstock at various number of passes. Overhead stirrers were fitted to all the reactors and connected in series. Mixers were operated at 50% of the maximum speed with a 10 sec on and off cycle. The stirrers mixed the contents of the reactor both in clockwise and counterclockwise directions

periodically. The reactors were then placed in a water bath that was kept constant at 41 °C. The gas generated as a result of anaerobic digestion, passed through reactor outlets to bottles containing 3 M NaOH and 0.4% thymolphthalein pH-indicator solution that stripped CO₂ and H₂S from the gas mixture. This enabled automatic quantification of the generated biomethane. The obtained results were normalized to standard temperature and pressure (STP, 0 °C and 1 atm). BMP tests were performed for 20 days with vinasse and 29 days with spent wash. BMP of each substrate was calculated using Equation 1 and reported as cumulative biomethane produced.

$$BMP_t = \frac{(V_{s,t} - V_{i,t})}{VS_s} \dots\dots\dots(1)$$

where BMP_t (ml CH₄/g VS) is the biochemical methanation potential of the substrate at time t (days), V_{s,t} (ml CH₄) is the volume of biomethane produced by the substrate at time t, V_{i,t} (ml CH₄) is the volume of biomethane produced by the inoculum at time t, from the blank tests and VS_s (g VS) is the amount of volatile solids present in the substrate fed to the reactor. Triplicates within the sample were used for BMP tests and hence the average of the replicates was used to produce the plots which are reported here. The standard deviation between the replicates for each time point from its mean was calculated and plotted as error bars of the experimental data. Otherwise, generally, potential errors can occur due to differences in the composition of inoculum in each reactor, the degassing time of the inoculum before inoculation, the solids content of the inoculum, use of different batches of inoculum or substrate and sampling from an unmixed holding tank.

A simple first order model (Equation 2) was used to describe the BMP data for most of the samples. However, a linear combination of two first order models was

used to process the BMP data showing diauxic digestion (untreated spent wash) as shown in Equation 3.

$$G = G_{max1} [1 - e^{-k1(t-t_0)}] \text{ for } t \leq t_{cross} \dots\dots\dots(2)$$

$$G = G_{max1} [1 - e^{-k1(t-t_0)}] + G_{max2} [1 - e^{-k2(t-t_0-t_{cross})}] \text{ for } t > t_{cross} \dots\dots\dots(3)$$

where G_{max1} and G_{max2} are the maximum biomethane that could be generated from the stage 1 and stage 2 (ml CH₄/g VS) respectively. Overall G_{max} was calculated as the sum of G_{max1} and G_{max2} , $k1$ and $k2$ are the rate constants for biomethane generation in stage 1 and 2 respectively (day⁻¹), t_0 is the lag time (days) and t_{cross} is the time (days) at which stage 2 becomes active (after the lag phase). In order to obtain the value of t_0 unambiguously, a line was fitted through the first four data points post lag time. The slope and intercept of the linear fit of the first four data points were used to obtain the value of t_0 . The five model parameters namely, G_{max1} , G_{max2} , $k1$, $k2$ and t_{cross} were obtained using non-linear regression (using the solver tool included in Microsoft Excel). Unlike untreated spent wash, the BMP data obtained for HC treated spent wash as well as untreated and HC pre-treated vinasse did not exhibit diauxic digestion. Therefore, for all the BMP data except untreated spent wash, a simple first order model shown in Equation 2 was used to process the BMP data (by setting t_{cross} to a value higher than the period of BMP tests).

2.5 Statistical Analysis

Confidence intervals (95%) of the fitted parameters were obtained using the curve fitting tool in MATLAB. The average confidence intervals for parameter G_{max} were found to be $\pm 7\%$ and $\pm 12\%$ for spent wash and vinasse respectively. The average

confidence intervals for parameter k were found to be $\pm 10\%$ and $\pm 21\%$ for spent wash and vinasse respectively. The error bars shown on all the plots are the standard deviation values calculated from triplicates. The correlation coefficient (R^2) for all the fits were greater than 0.99. These parameters obtained by processing experimental BMP data are reported and discussed in the Section 3.1.

3. Results and Discussion

3.1 BMP measurement and Kinetics

The total inlet COD of spent wash and vinasse used for the BMP tests were 120,000 ppm and 27,000 ppm respectively. The measured VS content of these feedstocks were used to establish the relationship between VS and COD. 1 g COD was found to be equal to 1.02 g VS of spent wash and 0.72 g VS of vinasse. The theoretical maximum CH_4 production from one gram of COD corresponds to 0.35 L CH_4 at STP assuming an equimolar ratio of CO_2 to CH_4 in biogas. BMP of untreated and HC treated spent wash and vinasse are shown in Figure 2(a) and Figure 2(b) respectively. A striking similarity at the first instance in both the cases is an analogous initial gas production stage. Biomethane yields in this initial stage were similar for the same type of untreated and HC treated samples (for both the substrates) and could be due to the presence of easily utilizable fractions such as carbohydrates, alcohols or volatile fatty acids (España-Gamboa et al., 2011; Wilkie et al., 2000). The maximum biomethane yield in this phase for vinasse was almost twice as that of spent wash, indicating a higher availability of easily digestible organics in vinasse. The ease of vinasse digestion was clearly seen with a higher BMP yield even with the untreated sample. This initial stage was termed as the lag phase, lasted up to ~5 days for untreated spent wash, but

decreased to ~4 days upon HC pre-treatment. With vinasse samples, the untreated and 1 pass samples had an initial lag time of ~7 days, and the rest had a lag time of ~8 days. Lag phase seen in both the cases could have been due to the use of a non-acclimatized inoculum. An acclimatization stage might have helped to overcome or shorten the lag phase observed with the digestion of spent wash and vinasse.

The BMP data (net generation per g of VS – Equation 1) obtained from untreated spent wash in the present work is shown in Figure 1. It can be seen that the BMP data clearly exhibits three distinct regions. The first day produced significant biomethane which was subsequently decreased. This initial gas production stage was also found for both the untreated and pre-treated spent wash as well as vinasse. This initial gas production phase occurred because of the presence of easily utilizable fractions such as alcohols, fatty acids or soluble sugars in the feedstock. Once the easily digestible organics were utilized, the net biomethane generation stopped before starting again with the breakdown of complex organics. Since the interest is in understanding digestion of complex COD contained in the feedstocks, the initial data obtained during this phase (called as lag phase here) was not considered for processing the BMP data. Instead, the data was processed by shifting the time coordinate by t_0 , lag time (days).

The BMP data of untreated spent wash shows diauxic digestion (digestion in multiple stages) after the lag phase. This could have been due to the presence of recalcitrant organics in untreated spent wash. Presence of these complex organics affect the overall rate of digestion as well as the cumulative biomethane generation. Similar cases of diauxic digestion from a variety of substrates such as paper wastes

(Walter et al., 2016), food waste (Ashekuzzaman & Poulsen, 2011) and activated sludge (Roebuck et al., 2019) have been reported in the past. With such diauxic digestion, a simple first order model is not appropriate and hence the linear combination of two first order models shown in Equation 3 was used to describe the BMP data obtained with untreated spent wash. Upon pre-treatment, no diauxic gas production was observed with all the other spent wash samples or any of the vinasse samples and hence a simple first order model represented in Equation 2 was used.

The fitted model parameters for untreated and HC pre-treated spent wash samples are shown in Table 1. As seen, HC pre-treatment has facilitated in lowering the lag time as compared to the untreated sample. Furthermore, a 14% increase in G_{max} was observed upon HC treatment after just 2 passes. G_{max} of 10 passes HC treated spent wash was found to be similar to that of the 2 passes sample, however in the case of 20 passes HC treated spent wash, a lower G_{max} compared to 2 passes was observed. Additionally, no diauxic gas production was observed with any of the HC treated spent wash samples indicating that the recalcitrant organics were broken down due to the physico-chemical effect of HC thereby making the COD more digestible (or enhanced soluble COD). Similar effect of cavitation (ultrasonication) on the number of stages in BMP of activated sludge were reported by (Roebuck et al., 2019).

Compared to spent wash, a longer lag time was observed with the vinasse samples, but no diauxic gas production was observed, suggesting that the organics were already in its bioavailable form, i.e. as soluble COD. Therefore, a simple first order model (Equation 2) was used to process the BMP data. The data for untreated vinasse showed much larger error bars around day 10. Couple of data points in that

region were therefore not considered while fitting the Equation 2. The fitted results are shown in Figure 2(b) and the corresponding model parameters are listed in Table 2. Unlike spent wash, no significant influence of HC pre-treatment on BMP was observed for vinasse. Comparing the observed $G_{\max}$ with the theoretical value of BMP, one can calculate the COD used (or COD conversion) for biogas generation. It can be seen that HC pre-treatment enhanced COD conversion from 59% (for untreated spent wash) to 65-68% (for the HC treated spent wash). With the case of vinasse, the COD conversion ranged between 78-95% suggesting the presence of easily digestible COD.

Low COD removal rates could be primarily due to the use of non-acclimatized inoculum for digestion and possibly also due to no use of nutrients or trace elements to support microbial activity, unadjusted pH prior digestion, and seasonal variability of feedstock. The inoculum used in our study was obtained from a digester operating on cattle slurry and grass silage as feedstock and was not adapted to digest spent wash. Hence, a lag phase was clearly seen, where only the easily available organics were utilized first (both in the cases of vinasse and spent wash) followed by the recalcitrant organics. HC pre-treatment helped in breaking down the recalcitrant compounds to an extent thereby also leading to a lower lag time, lack of diauxic digestion from pre-treated samples and higher rates compared to untreated samples. An acclimatization phase prior to BMP tests might have possibly resulted in higher COD removal. Bories et al. (1988), for instance performed high COD stillage digestion studies with a range of inocula to determine its effect on biomethane production (Bories et al., 1988) and reported the need for inoculum acclimatization. Another additional important factor to consider is the inherent variability within the feedstock itself. The effect of seasonal

and compositional differences of vinasse on BMP has been reported earlier where significantly different values of BMPs was observed for vinasse samples containing similar COD (Janke et al., 2015; Leite et al., 2015). It is evident from these reports that even with a narrow range of COD across samples, differences in the underlying composition of the vinasses may lead to significantly different BMP values.

Spent wash and vinasse are known to possess melanoidins which are recalcitrant sugar-amine complexes that are resistant to microbial degradation (Chandra et al., 2008). These refractory complex organic polymers possess a surface charge and interact with metal ions in the active centers of enzymes leading to protein precipitation, thereby making them inactive and unavailable for biomass breakdown. In addition, melanoidins are known antioxidants that can be bactericidal. This may have also led to a lower biomethane yield from the untreated samples. In the case of pre-treated samples, melanoidins could have been partially broken down by the action of HC. Depending on its original chain length, composition of the side chain and molecular weight, a variety of intermediates might have formed due to pre-treatment. With optimal pre-treatment, the available COD may become digestible however with over treatment intermediate inhibitors may be produced thereby lowering the overall biomethane yield. An increase in concentration of toxic intermediates as a result of sonication pre-treatment causing an inhibition to the aerobic biodegradability of distillery spent wash has been reported previously by (Sangave & Pandit, 2006). In the case of phenolics present in the samples, the type of phenolics present and its solubility in water determines its inhibitory effect. For example, (Hernandez & Edyvean, 2008) reported an inhibitory concentration of phenolics for biomethane

production (from anaerobic sludge) to be between 800 – 1,600 mg C/L. Back of the envelop calculations based on these numbers indicate that an inhibitory effect looks more plausible than the loss of organics due to over treatment. However, further investigations into identification and quantification of inhibitors generated by HC pre-treatment and their influence on BMP are needed to draw definitive conclusions.

Acoustic cavitation studies and its effect on the BMP of stillage was reported by Wu-Haan et al (Wu-Haan et al., 2010). They used a 2.2 kW 20kHz ultrasonic system to pre-treat stillage with an initial COD of 110,000 ppm. Combinations of various pre-treatment times ranging from 10-50 s and sonication amplitudes ranging from 33-100% was used. Batch BMP of the pre-treated and untreated samples were set up and compared under mesophilic conditions. Stillage upon 10 and 20 s sonication at 33% amplitude showed BMP's lower than the untreated stillage samples, whereas all the other samples showed an increased BMP in comparison. The highest enhancement in BMP of 29% was observed upon sonication at 66% amplitude for 10. Addition of inorganic nutrients and adjusted alkalinity prior digestion could have positively influenced the microbial activity for biomethane generation in Wu-Haan et al's work. In our work however, no trace elements or nutrients were added nor was the alkalinity adjusted. As the inoculum to substrate ratio (on the basis of VS) was 2:1 in our study, the higher quantity of inoculum used was assumed to provide the required buffering capacity. At an industrial scale, addition of trace elements or nutrients are often not favored and hence could support this argument.

3.2 Application to large scale digesters

Based on the bench scale results, scale up of the vortex based device was performed and retrofitted in four distilleries in west and central India with the help of our industry collaborator to pre-treat the incoming spent wash streams for enhanced biomethane generation (Figure 3) (Utikar, 2019). Due to confidentiality, the names of the industries are not disclosed. The average COD loads to these digesters were in the range of 120,000 – 190,000 ppm and the spent wash inflow rates were in the range of 13 – 50 m³/h. The vortex-based HC device installed upstream of the digester was designed to match the inflow rates thereby achieving one pass HC treatment before being digested. With just one pass through vortex-based cavitation device, an increase in biogas yield in the range of 10 – 22% was observed on these industrial scale digesters. This is for the first time, a fully scaled-up hydrodynamic cavitation-based pre-treatment has been shown to enhance biogas generation by effectively pre-treating spent wash at industrial scale. Illustrative energy calculations (gained and required for HC pre-treatment) were therefore performed for 1 and 2 passes HC pre-treatment for typical industrial spent wash inflow rates of 20 m³/h and 50 m³/h for 150 days digester operation in a year. These calculations were based on the G_{max} enhancement observed upon HC treatment at 2 passes and reported in Table 1. The energy required for pre-treatment; E_{pre-treatment} can be calculated using Equation 4.

$$E_{pre-treatment} = \frac{0.1 \Delta P n_p}{C_f \eta} \frac{MJ}{ton COD} \dots\dots\dots(4)$$

where, ΔP is the pressure drop across the vortex-based HC device (bars), n_p is the number of passes, C_f is the concentration of COD in the feed stream (ton COD/m³) and η is the pump efficiency (assumed to be 0.66). The enhanced biomethane generation, G_{gained} as a result of HC treatment can be calculated and translated to E_{gained}, energy

gained using Equation 5 and Equation 6. The net energy gain can therefore be calculated using Equation 7.

$$G_{gained} = ([G_{max}]_{pre-treated} - [G_{max}]_{untreated}) \frac{m^3}{ton\ COD} \quad \dots\dots\dots(5)$$

$$E_{gained} = \Delta H_{cal} G_{gained} \frac{MJ}{ton\ COD} \quad \dots\dots\dots(6)$$

$$Net\ Energy\ Gain = E_{gained} - E_{pre-treatment} \frac{MJ}{ton\ COD} \quad \dots\dots\dots(7)$$

where ΔH_{cal} is methane's calorific value (MJ/m³), $[G_{max}]_{pre-treated}$ and $[G_{max}]_{untreated}$ are the G_{max} of pre-treated and untreated samples respectively in m³ CH₄/ton COD.

At an inflow rate of 20 m³/h for a COD load of 120,000 ppm, for 1 and 2 passes HC treatment, the corresponding $E_{pre-treatment}$ were calculated to be 5.8 MJ/ton COD and 11.9 MJ/ton COD respectively and the corresponding net energy gain of ~1 GJ/ton COD at 1 and 2 passes were calculated respectively. In a commercial scenario however, a broad range of COD loads are expected in the feed stream across distilleries all over the country. Hence, for illustrative purposes the influence of HC on net energy gain and G_{gained} for various COD loads at an inflow rate of 20 m³/h was calculated and shown in Figure 4. Similar plot was also prepared for a higher inflow rate of 50 m³/h and shown in the supplementary information. It can be seen from Figure 4 that even at a minimum COD load of 0.25 ton COD/h (900 ton COD/year), the net energy gained upon 1 and 2 passes HC treatment was 0.98 GJ/ton COD and 0.93 GJ/ton COD respectively. Enhanced spent wash BMP upon pre-treatment obtained in the laboratory was also observed with large scale industrial spent wash digesters with a calculated net positive energy gain. This clearly indicates that the novel vortex-based

HC device can pre-treat spent wash energy efficiently at scale with an enhanced biogas generation. This opens up the possibility to extend its application to other biomass and waste streams that needs pre-treated prior bioprocessing.

4 Conclusion

Vortex based HC pre-treatment of spent wash at an inlet pressure of 3.9 barg and 2 passes (initial COD of 120,000 ppm) was found to enhance BMP by 14%. Similar pre-treatment for vinasse (initial COD of 27,000 ppm) was however insignificant due to its already 'easy to digest' COD. Net energy gains due to the increased biomethane produced from HC treated spent wash was ~1 GJ/ton COD. Scaled-up applications of single pass HC pre-treatment on industrial spent wash digesters was found to enhance biogas generation (10-22%) with a range of inlet COD loads (120,000 – 190,000 ppm).

Supplementary Information

E-supplementary data of this work can be found in online version of the paper.

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Declaration of Interest

One of the authors (VVR) is a co-founder and Director of Vivira Process Technologies Pvt Ltd, India, which commercially offers cavitation devices used in this study.

References

1. Alkan-Ozkaynak, A., Karthikeyan, K.G., 2011. Anaerobic digestion of thin stillage for energy recovery and water reuse in corn-ethanol plants. *Bioresour. Technol.*, 102(21), 9891-9896.
2. Ashekuzzaman, S.M., Poulsen, T.G., 2011. Optimizing feed composition for improved methane yield during anaerobic digestion of cow manure based waste mixtures. *Bioresour. Technol.*, 102(3), 2213-2218.
3. Basu, S., Mukherjee, S., Kaushik, A., Batra, V.S., Balakrishnan, M., 2015. Integrated treatment of molasses distillery wastewater using microfiltration (MF). *J. Environ. Manage.*, 158, 55-60.
4. Bories, A., Raynal, J., Bazile, F., 1988. Anaerobic digestion of high-strength distillery wastewater (cane molasses stillage) in a fixed-film reactor. *Biological Wastes*, 23(4), 251-267.
5. Central Pollution Control Board India. 2015. Guidelines on techno-economic feasibility of implementation of zero liquid discharge for water polluting industries. Ministry of Environment, Forests & Climate Change. New Delhi, India.

- 524 6. Chandra, R., Bharagava, R.N., Rai, V., 2008. Melanoidins as major colourant in
525 sugarcane molasses based distillery effluent and its degradation. *Bioresour. Technol.*, 99(11),
526 4648-4660.
- 527 7. Chang, I.S., Choo, K.H., Lee, C.H., Pek, U.H., Koh, U.C., Kim, S.W., Koh, J.H., 1994.
528 Application of ceramic membrane as a pretreatment in anaerobic digestion of alcohol-distillery
529 wastes. *J. Membr. Sci.*, 90(1), 131-139.
- 530 8. Chowdhary, P., Raj, A., Bharagava, R.N., 2018. Environmental pollution and health
531 hazards from distillery wastewater and treatment approaches to combat the environmental
532 threats: A review. *Chemosphere*, 194, 229-246.
- 533 9. Christofolletti, C.A., Escher, J.P., Correia, J.E., Marinho, J.F.U., Fontanetti, C.S., 2013.
534 Sugarcane vinasse: Environmental implications of its use. *Waste. Manag.*, 33(12), 2752-2761.
- 535 10. Cooper, J., Kavanagh, J., Razmjou, A., Chen, V., Leslie, G., 2020. Treatment and
536 resource recovery options for first and second generation bioethanol spentwash – A review.
537 *Chemosphere*, 241, 124975.
- 538 11. da Silva Neto, J.V., Gallo, W.L.R., Nour, E.A.A., 2020. Production and use of biogas from
539 vinasse: Implications for the energy balance and GHG emissions of sugar cane ethanol in the
540 brazilian context. *Environ. Prog. Sustain.*, 39(1), 13226.
- 541 12. España-Gamboa, E.I., Mijangos Cortes, J., Barahona Perez, L., Dominguez Maldonado,
542 J., Hernández Zarate, G., Alzate Gaviria, L., 2011. Vinasses: characterization and treatments.
543 *Waste Manage. Res.*, 29(12), 1235-1250.
- 544 13. España-Gamboa, E.I., Vicent, T., Font, X., Dominguez Maldonado, J., Canto Canché, B.,
545 Alzate Gaviria, L., 2017. Pretreatment of vinasse from the sugar refinery industry under non-
546 sterile conditions by *Trametes versicolor* in a fluidized bed bioreactor and its effect when
547 coupled to an UASB reactor. *J. Biol. Eng.*, 11, 6-6.

548 14. Fito, J., Tefera, N., Van Hulle, S.W.H., 2019. Sugarcane biorefineries wastewater:
549 bioremediation technologies for environmental sustainability. *Chem. Biol.*, 6(1), 6.

550 15. Franco, R.T., Buffière, P., Bayard, R., 2018. Co-ensiling of cattle manure before biogas
551 production: Effects of fermentation stimulants and inhibitors on biomass and methane
552 preservation. *Renew. Energy.*, 121, 315-323.

553 16. Garuti, M., Langone, M., Fabbri, C., Piccinini, S., 2018. Monitoring of full-scale
554 hydrodynamic cavitation pretreatment in agricultural biogas plant. *Bioresour. Technol.*, 247,
555 599-609.

556 17. Hernandez, J.E., Edyvean, R.G.J., 2008. Inhibition of biogas production and
557 biodegradability by substituted phenolic compounds in anaerobic sludge. *J. Hazard. Mater.*,
558 160(1), 20-28.

559 18. International Sugar Association. 2018. The World sugar economy in 2018.
560 <https://www.isosugar.org/sugarsector/sugar>.

561 19. Janke, L., Leite, A., Nikolausz, M., Schmidt, T., Liebetrau, J., Nelles, M., Stinner, W.,
562 2015. Biogas Production from Sugarcane Waste: Assessment on Kinetic Challenges for Process
563 Designing. *Int. J. Mol. Sci.*, 16(9), 20685.

564 20. Jiménez, A.M., Borja, R., Martín, A., Raposo, F., 2006. Kinetic analysis of the anaerobic
565 digestion of untreated vinasses and vinasses previously treated with *Penicillium decumbens*. *J.*
566 *Environ. Manage.*, 80(4), 303-310.

567 21. Leite, A.F., Janke, L., Harms, H., Zang, J.W., Fonseca-Zang, W., Stinner, W., Nikolausz,
568 M., 2015. Assessment of the Variations in Characteristics and Methane Potential of Major
569 Waste Products from the Brazilian Bioethanol Industry along an Operating Season. *Energ.*
570 *Fuels.*, 29(7), 4022-4029.

- 571 22. Mallick, P., Akunna, J.C., Walker, G.M., 2010. Anaerobic digestion of distillery spent
572 wash: Influence of enzymatic pre-treatment of intact yeast cells. *Bioresour. Technol.*, 101(6),
573 1681-1685.
- 574 23. Martín, M.A., Raposo, F., Borja, R., Martín, A., 2002. Kinetic study of the anaerobic
575 digestion of vinasse pretreated with ozone, ozone plus ultraviolet light, and ozone plus
576 ultraviolet light in the presence of titanium dioxide. *Process Biochem.*, 37(7), 699-706.
- 577 24. Mohana, S., Acharya, B.K., Madamwar, D., 2009. Distillery spent wash: Treatment
578 technologies and potential applications. *J. Hazard. Mater.*, 163(1), 12-25.
- 579 25. Nagarajan, S., Ranade, V.V., 2019. Pretreatment of Lignocellulosic Biomass Using
580 Vortex-Based Devices for Cavitation: Influence on Biomethane Potential. *Ind. Eng. Chem. Res.*,
581 58(35), 15975-15988.
- 582 26. Northern Ireland Water. 2020. Water Quality Results.
583 <https://www.niwater.com/water-quality-results.aspx>.
- 584 27. Padoley, K.V., Saharan, V.K., Mudliar, S.N., Pandey, R.A., Pandit, A.B., 2012.
585 Cavitationally induced biodegradability enhancement of a distillery wastewater. *J. Hazard.*
586 *Mater.*, 219-220, 69-74.
- 587 28. Pant, D., Adholeya, A., 2009. Concentration of fungal ligninolytic enzymes by
588 ultrafiltration and their use in distillery effluent decolorization. *World J. Microbiol. Biotechnol.*,
589 25(10), 1793-1800.
- 590 29. Pant, D., Adholeya, A., 2007. Identification, Ligninolytic Enzyme Activity and
591 Decolorization Potential of Two Fungi Isolated from a Distillery Effluent Contaminated Site.
592 *Water, Air, Soil Pollut.*, 183(1), 165-176.
- 593 30. Patil, P.N., Gogate, P.R., Csoka, L., Dregelyi-Kiss, A., Horvath, M., 2016. Intensification
594 of biogas production using pretreatment based on hydrodynamic cavitation. *Ultrason.*
595 *Sonochem.*, 30(Supplement C), 79-86.

- 596 31. Petkovšek, M., Mlakar, M., Levstek, M., Stražar, M., Širok, B., Dular, M., 2015. A novel
597 rotation generator of hydrodynamic cavitation for waste-activated sludge disintegration.
598 Ultrason. Sonochem., 26, 408-414.
- 599 32. Ranade, V.V., Kulkarni, A.A., Bhandari, V.M., 2016. Vortex diodes as effluent treatment
600 devices. US9422952B2.
- 601 33. Roebuck, P., Kennedy, K., Delatolla, R., 2019. Ultrasonic pretreatment for anaerobic
602 digestion of suspended and attached growth sludges. Water Quality Research Journal, 54(4),
603 265-277.
- 604 34. Saharan, V.K., Pinjari, D.V., Gogate, P.R., Pandit, A.B., 2014. Chapter 3 - Advanced
605 Oxidation Technologies for Wastewater Treatment: An Overview. in: Industrial Wastewater
606 Treatment, Recycling and Reuse, (Eds.) V.V. Ranade, V.M. Bhandari, Butterworth-Heinemann.
607 Oxford, pp. 141-191.
- 608 35. Sangave, P.C., Pandit, A.B., 2006. Ultrasound and enzyme assisted biodegradation of
609 distillery wastewater. J. Environ. Manage., 80(1), 36-46.
- 610 36. Sarvothaman, V.P., Nagarajan, S., Ranade, V.V., 2018. Treatment of Solvent-
611 Contaminated Water Using Vortex-Based Cavitation: Influence of Operating Pressure Drop,
612 Temperature, Aeration, and Reactor Scale. Industrial & Engineering Chemistry Research,
613 57(28), 9292-9304.
- 614 37. Schaefer, S.H., Sung, S., 2008. Retooling the Ethanol Industry: Thermophilic Anaerobic
615 Digestion of Thin Stillage for Methane Production and Pollution Prevention. Water. Environ.
616 Res., 80(2), 101-108.
- 617 38. Siles, J.A., García-García, I., Martín, A., Martín, M.A., 2011. Integrated ozonation and
618 biomethanization treatments of vinasse derived from ethanol manufacturing. J. Hazard.
619 Mater., 188(1), 247-253.

39. Simpson, A., Ranade, V.V., 2019. 110th Anniversary: Comparison of Cavitation Devices Based on Linear and Swirling Flows: Hydrodynamic Characteristics. *Industrial & Engineering Chemistry Research*, 58(31), 14488-14509.
40. Tewari, P.K., Batra, V.S., Balakrishnan, M., 2007. Water management initiatives in sugarcane molasses based distilleries in India. *Resour. Conserv. Recycl.*, 52(2), 351-367.
41. Utikar, R.P., 2019. Private communication on behalf of Vivira Process Technologies Pvt Ltd, Pune, India.
42. Walter, A., Silberberger, S., Juárez, M.F.n.-D., Insam, H., Franke-Whittle, I., 2016. Biomethane potential of industrial paper wastes and investigation of the methanogenic communities involved. *Biotechnol. Biofuels.*, 9(1), 21.
43. Wilkie, A.C., Riedesel, K.J., Owens, J.M., 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass. Bioenerg.*, 19(2), 63-102.
44. Wu-Haan, W., Burns, R.T., Moody, L.B., Hearn, C.J., Grewell, D., 2010. Effect of ultrasonic pretreatment on methane production potential from corn ethanol coproducts. *T. ASABE*, 53(3), 883-890.
45. Zieliński, M., Rusanowska, P., Krzywik, A., Dudek, M., Nowicka, A., Dębowski, M., 2019. Application of Hydrodynamic Cavitation for Improving Methane Fermentation of *Sida hermaphrodita* Silage. *Energies*, 12(3), 525.

Figure Captions

Figure 1: Experimental biomethane generation and fitted data from untreated spent wash showing lag phase.

Figure 2: Influence of number of passes in HC on BMP of (a) spent wash and (b) vinasse. Symbols denote experimental data and lines denote fitted data.

Figure 3: Enhancement in biogas yields from large scale spent wash digesters upon 1 pass vortex-based HC pre-treatment (Utikar, 2019).

Figure 4: $E_{\text{pre-treatment}}$ (continuous and dashed lines) and E_{gained} (dotted line) as a result of HC pre-treatment of spent wash at 1 and 2 passes at an inflow rate of $20 \text{ m}^3/\text{h}$.

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Table 1: Fitted parameters used in the model to describe the BMP profiles of spent wash shown in Figure 2(a). * denotes k_1 and $^{\$}$ denotes k_2 observed in untreated spent wash. 95% confidence intervals for $G_{\max}$ and k are $\pm 7\%$ and $\pm 10\%$ of the respective fitted values.

Parameters	Untreated spent wash	Spent wash – 2 passes	Spent wash – 10 passes	Spent wash – 20 passes
$G_{\max}$ (ml $\text{CH}_4/\text{g COD}$)	207	236	237	228
k (day^{-1})	0.106^* and $0.097^{\$}$	0.054	0.046	0.050
t_0 (days)	5.1	4.1	4.2	4.1

Table 2: Fitted parameters used in the model to describe the BMP profiles of vinasse shown in Figure 2(b). 95% confidence intervals for $G_{\max}$ and k are $\pm 12\%$ and $\pm 21\%$ of the respective fitted values.

Parameters	Untreated vinasse	Vinasse – 1 pass	Vinasse – 2 passes	Vinasse – 5 passes
$G_{\max}$ (ml $\text{CH}_4/\text{g COD}$)	333	329	322	275
k (day^{-1})	0.114	0.124	0.119	0.158
t_0 (days)	7.3	7.2	7.9	8.0