The use of microalgae and their culture medium for biogas production in an integrated cycle

E. L. Formagini, F. R. Marques, M. L. Serejo, P. L. Paulo and M. A. Boncz

ABSTRACT

Vinasse is a residue produced in large quantities as a sub-product of ethanol production. Anaerobic digestion of vinasse can yield large amounts of biogas, but often difficulties arise in maintaining stable operation, due to the acidity of the material (which has a pH between 3.5 and 5) and a strong tendency to further acidification. Anaerobically digested vinasse can be used as part of a culture medium for microalgae cultivation, for the production of biodiesel and other compounds, whilst the excess CO_2 produced in the ethanol fermentation can be used to stimulate algal growth. During algae cultivation, the pH of the culture medium has a strong tendency to increase; therefore, recycling of the spent culture medium or the concentrated algae suspension to the anaerobic digester treating vinasse was considered an option for pH stabilization there. Batch tests, however, showed that alkalinity of the spent culture broth, in spite of its high pH, is too low (only 350 mg$CaCO_3$ L^{-1}) to help stabilise the pH of vinasse digestion. Alkalinity of the algae suspension is higher and digestion of a mixture of vinasse and a suspension of algae results in efficient biogas production, but still the alkalinity is insufficient to stabilise the pH in a range suitable for methanogenic microorganisms; hence, the addition of additional alkalinity, for instance as sodium bicarbonate or urea, remains necessary.

Key words | alkalinity, bioenergy, methane production, volatile fatty acids, vinasse digestion

E. L. Formagini
F. R. Marques
M. L. Serejo
P. L. Paulo
M. A. Boncz (corresponding author)
Federal University of Mato Grosso do Sul (UFMS),
Centre of Exact Sciences and Technology (CCET),
P.O. Box 549,
79070-900 Campo Grande-MS,
Brazil
E-mail: *marc.boncz@ufms.br*

INTRODUCTION

Brazil is renowned worldwide for its sugar-cane based ethanol production. This activity, however, generates large volumes of a range of subproducts, amongst which is a liquid effluent known as vinasse. For each m^3 of ethanol, 12 m^3 of vinasse are produced (van Haandel 2005). This vinasse is now mainly used as a fertilizer on the areas where sugar cane is cultivated, by means of a process called fertirrigation (Figure 1(a)). However, the high organic matter content and low pH demand some form of treatment, and anaerobic digestion is often suggested. This would result in the production of large amounts of biogas that can be used for additional energy production, whilst the nutrients (especially N, P and K) would remain available for fertilization (van Haandel 2005), changing the ethanol production cycle from option A to option B (Figure 1).

However, a further improvement might be obtained. As the product of anaerobic digestion is rich in nutrients, it might also be used as part of the culture medium for the production of microalgae (Cenciani *et al.* 2011), which could then be used for the production of biodiesel, or for the production of other compounds with a higher aggregated value (Koller *et al.* 2012). One of the characteristics of algae cultivation processes is the tendency of the algae to increase the pH of their culture medium, as a result of their continuous absorption of carbon dioxide (CO_2). At the same time, vinasse has a low pH, and inadequate control of the anaerobic digestion tends to result in further acidification, impeding biogas production. The easiest way to guarantee an uninterrupted process, in addition to effluent recycling, is by continuously dosing small amounts of alkalinity. It should thus be possible to combine these two processes and use the effluent of the anaerobic digester, which is already treating vinasse, to cultivate algae, whilst using (part of the) spent culture medium as an alkaline additive to be recycled to the anaerobic digester to control its pH. Alternatively, it should be possible to recycle (part of the) concentrated and alkaline algae slurry (obtained by filtration or flotation) to the anaerobic digester to increase biogas production and reduce acidification during digestion of the vinasse (Figure 1(c)). This

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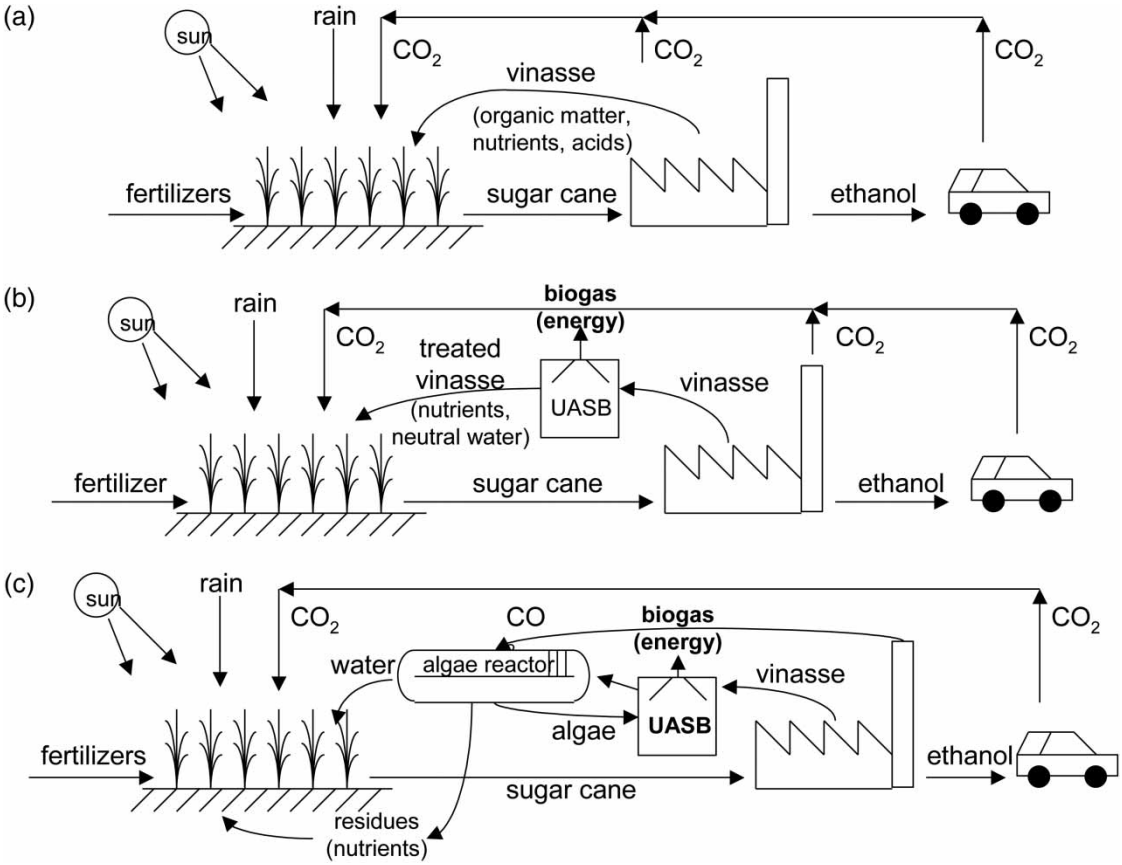

Figure 1 | Actual production cycle of sugar-cane alcohol (a), integration of anaerobic digestion for increased energy production and protection of surface waters and environment (b) and proposed scheme, with increased biogas production due to (partial) recycling of algae to the UASB reactor; algae cultivated with off-gas of fermentation process (c).

co-digestion of microalgae should result in a higher biogas production with relative ease, as the algae, unicellular organisms with a simple structure, contain only small amounts of complex organic compounds like lignins and cellulose, making them easily digestible (Gunaseelan 2004). In either method (converting the algae into biodiesel or recycling the dewatered algae to the upflow anaerobic sludge blanket (UASB) reactor for increasing its methane production), effectively, a large part of the CO_2 emitted during the fermentation process would be converted into algal biomass, which in turn, upon processing, would increase the energy production of the plant as a whole.

The possibility of recycling nutrients to the sugar cane fields would remain intact in all options, as no nutrients are lost in the products of the plant: ethanol, biogas and biodiesel. Thus, recycling either the treated vinasse (as in option B) or the solid residue of the algae after extraction of triglycerides for biodiesel production (option C) would satisfy the major proportion of the nutrient demand of sugar cane cultivation. Precisely because of this potential for nutrient recycling to sugarcane cultivation, we showed in an earlier paper that urea dosing to the anaerobic digester (rather than applying urea directly as a fertilizer on the fields) is a low-cost alternative for controlling alkalinity in the UASB reactor and stabilising the process (Boncz *et al.* 2012). By keeping the pH in that reactor well below the ammonia pK_B of 9.24 at all times, no significant part of the added nitrogen will be lost as NH_3.

Considering that during the fermentation process 755 kg of CO_2 is released for every 1 m^3 of ethanol produced, as well as approximately 6.45 kg of N and 1.65 kg of P (in the vinasse, although this number may vary, because large variations in vinasse composition exist, mainly depending on whether the distillery produces only ethanol, or both ethanol and sugar), and considering a global molecular formula of $C_{106}H_{181}O_{45}N_{16}P$ for the algal biomass (Larsdotter 2006), the limiting element amongst the available resources will be nitrogen. Thus, taking the amount of nitrogen as a starting point, the use of anaerobically digested vinasse for cultivating algae could produce enough biomass to yield another 26.9 kg of methane per m^3 of ethanol produced, meaning a 40% increase in energy production potential,

even without implementing any scheme to recycle nitrogen to the algae cultivation reactor. As the available amount of nitrogen is the limiting factor, this figure could be even bigger when urea is used for controlling the pH in the UASB reactor (Boncz *et al.* 2012). Dosing 33 mM_{urea}, P rather than N will become the limiting factor, and algal mass will correspond to 50 kg of methane per m^3 of ethanol produced, a 74% increase compared to scenario B, if a 95% removal efficiency can be obtained. In practice, this efficiency (corresponding to a biogas production of 0.54 L CH_4 g VSS^{-1}) will be smaller, as hardly any biological process operates at 100% efficiency. Golueke was quoted to have obtained a conversion of 0.17–0.32 L CH_4 g VSS^1, for instance (González-Fernández *et al.* 2012).

The objective of this work was to evaluate the use of the spent culture medium (supernatant) of algae production as an alkaline reagent in order to stabilize the pH during anaerobic digestion of vinasse as well as to evaluate the anaerobic digestion of a suspension of microalgae separately or during co-digestion with vinasse, to assess the potential of algae cultivation to increase biogas production based on the use of residues of ethanol distilleries.

METHODOLOGY

Algae (mainly *Chlorella* and *Scenedesmus* species, as identified by microscopy) were cultivated in a 40 L photobioreactor (operating in batch-mode) mounted on the roof of the effluents laboratory of UFMS in Campo Grande-MS, Brazil, using BBM cultivation medium. Growth was registered recording suspension turbidity with a Hanna 93414 turbidity meter, and the algae suspension was withdrawn from the reactor when growth stopped. Batch experiments to determine anaerobic digestion of vinasse an/or the algae suspension were carried out in duplicate at 30 °C in 500 ml dark glass bottles with 20% headspace and 4 gVSS/L of anaerobic biomass (from a 40 L UASB fed with vinasse), following standard methodology for Specific Methanogenic Activity assays (Owen *et al.* 1979). After filling, the bottles were sealed with a rubber stopper and aluminium crimp cap, and oxygen was purged using N_2 gas. Methane production was determined daily using the liquid displacement method, using Mariotte flasks with 18% NaOH in order to eliminate any CO_2 present in the biogas. Volatile fatty acids (VFA), chemical oxygen demand (COD) and pH were monitored according to the specific needs of each experiment, according to *Standard Methods* (APHA *et al.* 2005) while at the beginning and end of the experiment alkalinity was also determined. A first experiment was carried out to verify the possibility of using the supernatant (spent culture medium) of algae cultivation to help secure the pH during anaerobic digestion of vinasse, checking at the same time the effect of different types of separation process on the suitability of the material for this purpose. All supernatants were obtained by means of physico/chemical processes; the methods applied were filtration (supernatant denominated as 'filtered') and, using Jar-Test equipment, also coagulation/flocculation/sedimentation, using a natural (saline extract of *Moringa oleifera* seeds) and a chemical flocculant ($FeCl_3$). The assays were carried out using a mixture of 50% of the supernatant obtained before, and 50% of diluted vinasse. All experiments were carried out with and without additional buffering by 1.6 g/L of $NaHCO_3$ (equivalent to 0.2 gr$NaHCO_3$/gCOD). A second series of experiments was carried out using the same algal suspension as described before. In this case, vinasse (with or without the addition of additional $NaHCO_3$) and algae suspension were mixed in 50:50 (v/v) ratio.

RESULTS AND DISCUSSION

The spent algae cultivation medium used in the experiments, after separation of the algae, shows comparable characteristics, independent of the method used for separating the algae (Table 1). Except in the case where the algae were removed using $FeCl_3$, the pH of the supernatant is high, but the alkalinity much lower than expected. As a consequence, the results shown in Figure 2 demonstrate that adding this supernatant, even in the same amount as the

Table 1 | Characterization of the different types of supernatant (spent algae cultivation medium) used in the experiments

Sample (triplicate)	pH	Alkalinity (mg$CaCO_3$/L)	COD (mgO_2/L)	VFA (mg/L)
Filtrated	9.26 ± 0.04	348.2 ± 2.1	112.9 ± 21.0	Not detected
Coagulated with *Moringa Oleifera* extract	9.15 ± 0.06	352.0 ± 3.0	244.0 ± 54.9	Not detected
Coagulated with Iron(III)chloride	7.43 ± 0.10	158.2 ± 2.6	118.3 ± 29.3	Not detected

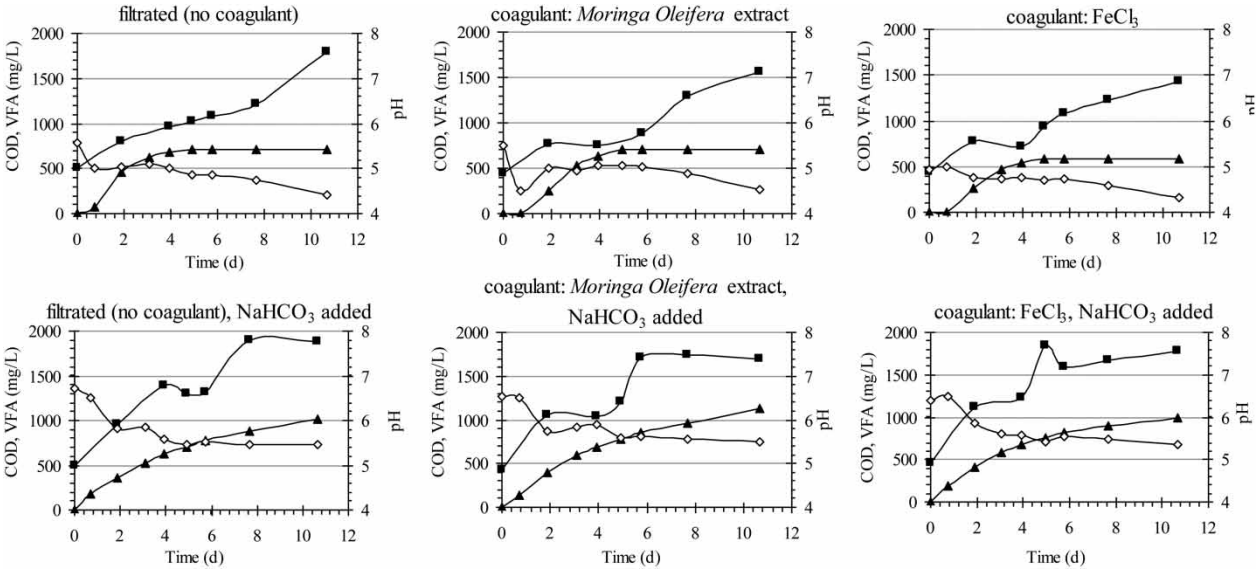

Figure 2 | Production of methane (as COD equivalents, ▲) and VFA (VFA, as $mg_{acetate}$/L, ■), and resulting pH (◇), during tests in which alkaline spent algae cultivation medium was used to stabilise the pH of vinasse during anaerobic digestion. Top graphs: supernatant (50%) and vinasse (50%) without $NaHCO_3$ addition and bottom graphs: supernatant (50%) and vinasse (50%) with 1.6 g/L $NaHCO_3$ added to further increase initial alkalinity.

vinasse, does not result in neutralization of the vinasse and cannot prevent quick acidification. Thus, the main result of all experiments is the production of VFA rather than methane gas.

When $NaHCO_3$ is added to further increase the alkalinity, the results look similar again; in these cases, biogas production is improved and acidification reaches a maximum, but the main product remains VFA rather than methane, and COD conversion remains low (Table 2).

As with the case of the first series of experiments, the cause is the inability to neutralise the acidity of the vinasse; addition of only the supernatant of algae cultivation increases initial pH to 5.7 at most, whereas addition of $NaHCO_3$ results in an initial pH of 6.5, but the quick accumulation of VFA results in a final pH of below 4.5 (without addition of $NaHCO_3$) or 5.5 (with addition of $NaHCO_3$), too low to sustain methanogenic activity for an extended period of time.

Table 3 and Figure 3 present the results obtained with codigestion of vinasse and the suspension of microalgae. It can be observed that, just like in the previous experiments, the algae suspension, even though alkaline, is unable to stabilize the pH of the vinasse.

The algae suspension and the diluted vinasse had a comparable COD, but digestion of only algae produced relatively less biogas. Codigestion of vinasse and the algae suspension resulted in more or less the same quantity of methane produced, but dosing of additional alkalinity (as $NaHCO_3$) still proved necessary.

In all cases (digestion of algae, vinasse, or a mixture of these), initially a reduction of the pH occurs. However, in the case of digestion of only algae, no accumulation of

Table 2 | Parameters evaluated at the beginning and end of the experiments with supernatant

Experiment	$Alk_{initial}$ (mg/L)	Alk_{final} (mg/L)	COD_0 (g/L)	$COD_{removed}$ (%)	CH_4–COD (g/L)	% of COD, removed as CH_4
Vinasse			6.81	10.8	0.255	34.8
Vinasse + Filtrated	118	250	6.02	31.5	0.716	37.8
Vinasse + Filtrated + $NaHCO_3$	1,672	1,698	6.26	34.0	1.02	47.8
Vinasse + *Moringa oleifera* extract	108	394	6.30	26.1	0.71	42.9
Vinasse + *M. O.* extract + $NaHCO_3$	1,626	1,740	6.24	30.7	1.13	49.4
Vinasse + $FeCl_3$	60	176	6.27	35.3	0.58	26.1
Vinasse + $FeCl_3$ + $NaHCO_3$	1,492	1,540	6.50	33.9	0.99	45.1

Table 3 | Initial and final values of some parameters

Experiment	$Alk_{initial}$ ($mgCaCO_3/L$)	Alk_{final} ($mgCaCO_3/L$)	$COD_{initial}$ (gO_2/L)	$COD_{removed}$ (%)	CH_4–COD (g/L)	CH_4 recovery[a] (%)
100% Algae	603	1,160	8.69	33.2	2.35	81.6
50% Algae/50% Vinasse	206	370	8.21	37.4	9.13	29.7
50% Algae/50% Vinasse + $NaHCO_3$	1,962	2,190	7.87	54.7	3.83	89.1
100% Vinasse + $NaHCO_3$	1,547	1,650	7.48	67.3	4.12	82.0

[a]% of COD removed that was converted into CH_4.

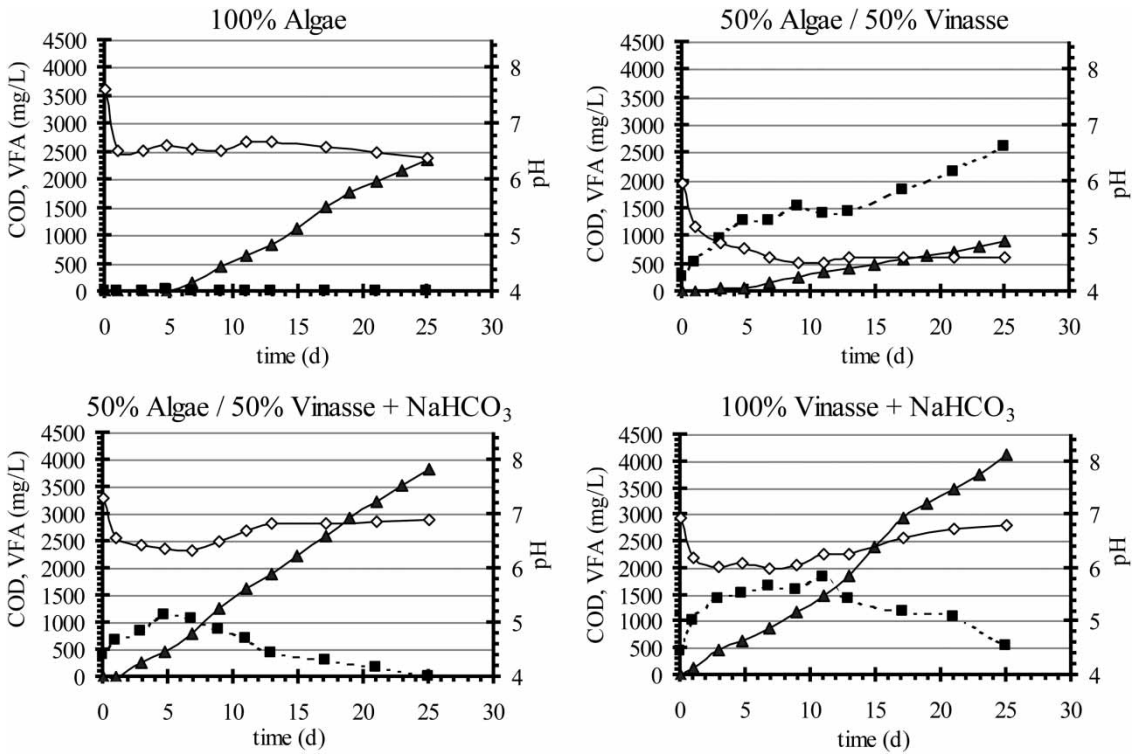

Figure 3 | Production of methane (as COD equivalents, ▲) and VFA (VFA, ■), and resulting pH (◇), during tests of methanogenic activity of anaerobic biomass fed with vinasse, a suspension of algae, or a mixture of these two, with or without correction of initial alkalinity.

VFA is observed in the experiment, and the pH stabilizes around 6.5. In the other batches, the pH varies, depending on the presence of VFA, which initially accumulate as an intermediate product, to be converted into CH_4 later on. In the case of digestion of only algae, overall removal of COD was low (33%) but as observed in the other conditions, almost all COD removed is converted into CH_4. Considering data from Sialve *et al.* (Sialve *et al.* 2009) on overall algae composition, production of 0.8 L of CH_4 per gram of volatile suspended solids (VSS) (algae) (0.24 $gCH_4/gCOD$) digested can be estimated. Thus, in the present experiment 1.09 L of CH_4 can be expected to be produced, but only half of this amount was obtained, a result similar to the result obtained by Verstraete, who reported a yield of between 0.24 and 0.36 L of CH_4 per gramme of volatile solids (algal biomass) digested (Verstraete *et al.* 2010).

CONCLUSIONS

The recycling of the basic spent culture medium from algae cultivation as a source to control alkalinity in anaerobic digestion of vinasse turned out to be unrealistic. The alkalinity of this material is too low to make any difference in the rate or yield of the anaerobic digestion of this acidic waste. Anaerobic digestion of a suspension of microalgae

(cultivated in a culture medium, partially composed of vinasse), however, can be a viable alternative for increasing biogas production in Brazilian alcohol/bioenergy plants; a methane yield of 0.5 L/gVSS-algae can be obtained (at STP). Thus, a system consisting of two bioreactors: a UASB reactor converting vinasse and algae into methane, and a photobioreactor for cultivating algae to be co-digested in this UASB reactor may be a viable system for additional biogas production in the Brazilian bioenergy sector.

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