# EXPLORING OPTIMAL FEED TO MICROBES RATIO FOR ANAEROBIC ACIDOGENIC FERMENTATION OF CASSAVA RESIDUE FROM BREWERY

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Cassava residue from breweries is being generated in large amounts in Guangxi Province of China, and this has potential to cause serious environmental problems if disposed of improperly. Two-stage anaerobic fermentation is a promising method for the treatment of such residue. In this study, the effect of feed to microbes ratio (F/M ratio) on the anaerobic acidogenic fermentation of cassava residue was studied to determine the optimal F/M ratio and to maximize the performance in a subsequent methanogenic stage. The experiments were carried out at the F/M ratios of 0.2, 0.61, 1.02, 2.05, 3.07, and 4.09 g cassava-TS/g sludge-VSS in six laboratory-scale, completely stirred, tank reactors (CSTR) at mesophilic temperature (35°C). An F/M ratio of 1.02 g cassava-TS/g sludge-VSS resulted in the highest solid removal efficiency and VFA/COD ratio, while starch removal efficiency was still near 100 percent, and acidification was relatively high. As a further benefit, the VFA distribution was more suitable for the subsequent methanogenic fermentation stage.

*Keywords: Cassava residue; Anaerobic fermentation; F/M ratio; Two stage*

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## INTRODUCTION

Nowadays, energy shortages have been drawing increasing attention around the world. Thus, the search for sustainable substitute energy has become a focus of study in recent years. To avoid conflicts relative to human sustenance, such as “competition between food and energy,” an ideal source of substitute energy would consist mainly of waste resources. For instance, hydrogen can be produced from cornstalk waste (Zhang et al. 2007), and methane can be produced from manure (Angelidaki and Ellegaard 2003).

Cassava is one of the most efficient crops in terms of carbohydrate production. In the subtropical region of southern China, cassava is the fifth largest crop in terms of production, after rice, sweet potato, sugar cane, and maize (Wang et al. 2010). In Guangxi Province, the cassava industry is booming. According to data from Guangxi Province Statistic Bureau of China, the cassava yield in 2010 was 1.73 million tons, accounting for 70 percent of the national output. Based on many studies, cassava can also be processed and converted into value-added components such as ethanol (Tran et al. 2010) and L-lactic acid (Ohkouchi and Inoue 2006), in addition to its being used as food. In Guangxi Province, cassava brewery has developed rapidly. However, cassava residue is generated in large amounts during the production of ethanol, and these wastes have potential to cause serious environmental problem if disposed of improperly. Cassava residue, mainly consisting of starch and also a small amount of cellulose, hemicelluloses, and lignin, is a very promising resource to produce renewable energy.

Two-stage anaerobic fermentation is an economical solution to the treatment of cassava residue. Such an approach deals not only with the problem of environmental pollution, but it also can result in the production of methane, a valuable liquid fuel. Paixão et al. (2000) investigated the anaerobic digestion of wastewater from cassava industry with acidogenic and methanogenic physical separation stages and observed that the separation of stages selected different bacterial groups in each digester. A novel full recycling process of ethanol production from cassava through two-stage anaerobic treatment was proposed by Zhang et al. (2010) to treat the distillery wastewater. It was expected to achieve zero wastewater discharge, in addition to requiring little consumption of energy (Zhang et al. 2010). Luo et al. (2010) researched simultaneous hydrogen and methane production through two-stage anaerobic fermentation of cassava stillage. Although the two-stage anaerobic treatment process is more stable than the conventional single-stage process, considerable attention still needs to be paid, since improper effluent carry-over from an acidogenic fermentation stage can cause the failure of a subsequent methanogenic fermentation stage (Ahring et al. 1995). The F/M ratio is an important operating parameter in anaerobic acidogenic fermentation (Pan et al. 2008), and if it can be controlled properly, the pH value will be maintained at an appropriate range without extra addition of alkalinity during the process. Moreover, variations in the F/M ratio will cause the different distributions of VFA in the acidogenic stage, thereby influencing the efficiency of subsequent methanogenic stage. Yang et al. (2007) researched the batch $H_2$ fermentation of stimulated cheese processing wastewater at four F/M ratios (0.5, 1.0, 1.5, and 2.0). The major VFA products observed at the F/M ratios of 1.0, 1.5, and 2.0 were butyric and acetic acids, with the amount of butyric acid approximately two-fold greater than acetic acid. However, at the F/M ratio of 0.5, acetic acid was generated in slightly larger amounts than butyric acid. In addition, ethanol was produced in all bioreactors, and small amounts of propionic acid and valeric acids were detected at the F/M ratios of 0.5 and 1.0. Thus for a two-stage anaerobic technology, it is critically important to optimize the F/M ratio during the anaerobic acidogenic fermentation process.

The present study researched the effect of F/M ratio on the anaerobic acidogenic fermentation of cassava residue from brewery under mesophilic conditions. The main objective was to explore optimal F/M ratio in order to obtain better cassava usage efficiency and VFA distributions in the anaerobic acidogenic stage, thereby facilitating the subsequent stage for methane production.

## EXPERIMENTAL

### Materials

*Seed sludge*

The seed sludge was obtained from a municipal sewage treatment plant located in Tianjin, China. The characteristics of the sludge were as follows: pH 7.52, total solids (TS) 13.22 g/L, volatile solids (VS) 10.56 g/L, total suspended solids (TSS) 13.19 g/L, volatile suspended solids (VSS) 10.14 g/L, total phosphorus (TP) 1.24 mg P/L, total nitrogen (TN) 6.15 mg N/L, $NH_4^+$-N 1.56 mg N/L, and chemical oxygen demand (COD) 186.56 mg/L.

*Feedstock*

The cassava residue was collected from a cassava brewery located in Guangxi, China. It was dried in an oven and milled to powder before using as the feedstock. The main characteristics of the cassava powder are summarized in Table 1.

**Table 1.** Composition of the Cassava Powder Used as Feedstock

| Parameters | Units | Average Values |
|---|---|---|
| VS | g/g TS | 0.97 |
| Ash | g/g TS | 0.03 |
| Starch | g/g TS | 0.60 |
| Cellulose | g/g TS | 0.18 |
| Hemicellulose | g/g TS | 0.05 |
| Lignin | g/g TS | 0.04 |
| Total Kjeldahl nitrogen (TKN) | g/g TS | 5.45 |
| COD of cassava hydrolysate[a] | mg/g TS | 1012.36 |

[a] COD of cassava powder hydrolysate was obtained by the methods described in analytical methods. It was determined to quantify the overall COD released per unit cassava powder when cassava was hydrolyzed, thereby assessing the acidification efficiency at different F/M ratios in the experiments.

## Methods

*Inoculation and start-up*

The experiments were carried out in six CSTR reactors with volumes of 1000 mL. At the beginning of experiments, each reactor was loaded with 900 mL seed sludge and placed in a water bath at a controlled mesophilic temperature of 35°C. During the start-up stage, the experiments were conducted in semi-continuous mode, in which 0.5 g starch and 15 mL nutrient solution were added to each reactor periodically. The composition and concentration of the nutrient elements solution were 13.333 g/L $NH_4Cl$, 2.667 g/L $KH_2PO_4$, 1.111 g/L $Na_2SO_4$, 0.478 g/L $MgCl_2$, 0.008 g/L $CaCl_2$, and 0.108 g/L $FeCl_3 \cdot 6H_2O$. After acclimatizing the acidogenic bacteria for a period of time, the substrates in the six reactors were mixed together and stored at 4°C. Table 2 shows the composition of the cultured seed sludge.

*Batch fermentation of cassava powder*

The acidogenic fermentation of cassava powder was performed in 1000 mL CSTR reactors in batch tests. The reactors were filled with 700 mL of cultured seed sludge and cassava powder of 1, 3, 5, 10, 15 and 20 g, respectively. The pH of each reactor was adjusted to 6.0 using NaOH solution. The temperature of the acidogenic fermentation was controlled at 35°C. In all experiments, the mixed liquor in the reactor was first purged with nitrogen for 5 to 10 minutes to ensure an anaerobic condition prior to anaerobic acidogenic fermentation of starch and cassava powder. Then, the reactors were sealed to maintain an anaerobic environment during the process. During the course of experiments, the pH and VFA values were monitored at designated time intervals. Once acidogenic fermentation was completed, COD, TS, VS, TSS, VSS, pH, TN, TP, starch, and VFA were determined. All experiments were performed in triplication.

**Table 2.** Composition of the Cultured Seed Sludge

| Parameters | Units | Average Values |
|---|---|---|
| pH | — | 5.00 |
| TS | g/L | 11.01 |
| VS | g/L | 8.18 |
| TSS | g/L | 8.54 |
| VSS | g/L | 6.59 |
| TP | mg P/L | 82.56 |
| TN | mg N/L | 316.30 |
| COD | mg/L | 1433.30 |
| Acetic acid | mg/L | 894.41 |
| Propionic acid | mg/L | 226.62 |
| Butyric acid | mg/L | 232.23 |
| Starch | g/g TS | — |

*Analytical methods*

The composition of cassava powder, including cellulose, hemicelluloses, and lignin, was analyzed according to the National Renewable Energy Laboratory method (Sluiter et al. 2008). The COD of cassava powder hydrolysate produced by this laboratory's method was determined by standard method (Wei 1998).

The concentrations of VFA were determined by an HPLC (Labliance, USA) equipped with a refractive detector, using a column (BioRad Aminex HPX-87H, 300×7.8mm) at 65°C and 5 mmol/L $H_2SO_4$ as eluent at a flow rate of 0.6 mL/min.

The content of starch was analyzed with a two-step hydrolysis method. The first hydrolysis step was conducted by using thermostable α-amylase, and the second step was performed by adding HCl and refluxing in a boiling water bath for 1 hour. Then, the hydrolysate was filtrated and analyzed for glucose by means of HPLC.

The samples from acidogenic fermentation process were centrifuged at 8000 rpm for 5 minutes, and filtrated through 0.45 μm filters to determine COD, TN, TP, and VFA. COD, TN, TP, TS, VS, TSS, VSS, and TKN were analyzed according to the China standard methods for the examination of water and wastewater (Wei 1998). The pH was determined by using a pH-meter (HORIBA, D-51, Japan).

## RESULTS AND DISCUSSION

### Effect of F/M Ratio on pH Values

It has been established that running the acid-stage reactor in the pH range of 5.7 to 6.0 offers a stable and highly favorable substrate for the methane reactor (Zoetemeyer et al. 1982). Therefore, in this study, the initial pH of the reactors at different F/M ratios was adjusted to 6.0 by addition of NaOH solution. As can be seen from Fig. 1, the pH values at different F/M ratios all showed a similar trend of declining rapidly in the first 10 hours, and gradually decreasing to a stable value during the residual fermentation period. It also can be seen that the pH values at the final state decreased with the increase of F/M ratios, whereas at higher F/M ratios, the pH values were performed closely. Initially, the pH values decreased fast because the cassava residue was metabolized by the acidogens and VFA were produced and accumulated. Afterwards, as a result of the buffering capability of acetic acid and acetate solution, the pH values stayed mainly in the range of 4.4 to 4.7, which was near the biggest buffering capacity of acetic acid and acetate solution, and it remained stable even at relatively high F/M ratios. The other important buffer solution in anaerobic fermentation was bicarbonate and carbonic acid solution, with the highest buffering capacity at around pH 6.4, which was just above the initial pH of the reactors. Therefore, the bicarbonate and carbonic acid solution did not play an important role of buffering in the experiments.

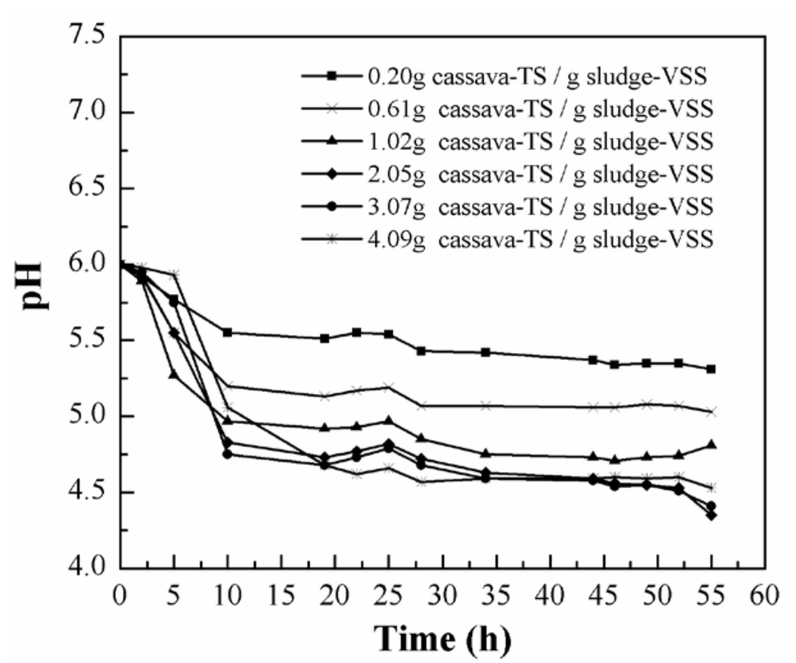


**Fig. 1.** Time-course profiles of pH during the anaerobic acidogenic fermentation of cassava at different F/M ratios

## Effect of F/M ratio on VFA Concentrations and Distributions

The trend of pH values was in accordance with the performance of the VFA shown in Fig. 2, which was produced fast in the first 24 hours. The results in Fig. 2 showed that the VFA concentrations at the final state increased with the increase of F/M ratios. However, the increased rate of VFA at the F/M ratio of 4.09 g cassava-TS/g sludge-VSS was lower than that at the F/M ratios of 2.05 and 3.07 g cassava-TS/g sludge-VSS in the first 22 hours, which was also consistent with the trend of pH values. This was tentatively attributed to the inhibition of high concentrations substrate and the requirement of relatively long time for acclimation of the acidogens. Therefore, after 22 hours of acclimation, the VFA concentrations at the F/M ratio of 4.09 g cassava-TS/g sludge-VSS exceeded the others and achieved the highest production.

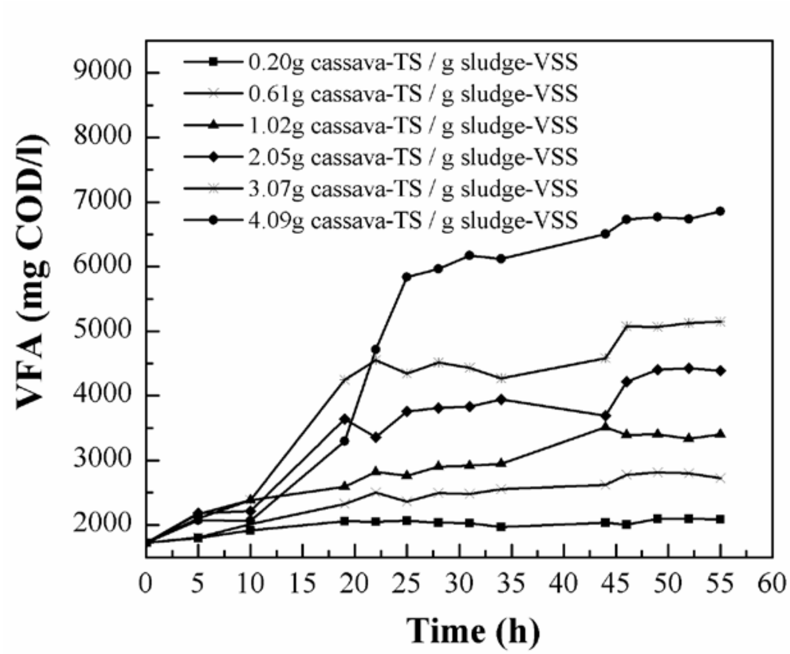


**Fig. 2.** Time-course profiles of VFA production during the anaerobic acidogenic fermentation of cassava at different F/M ratios

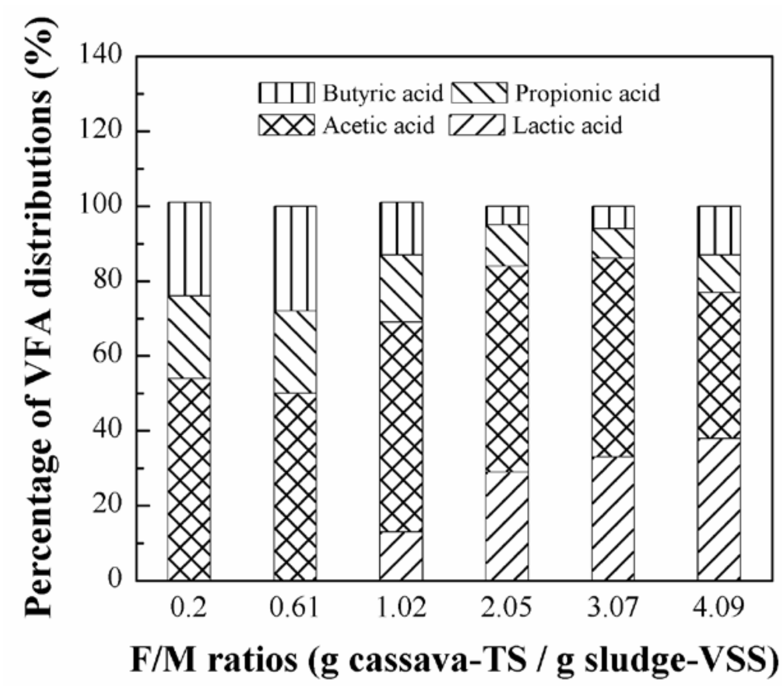


**Fig. 3.** Distributions of VFA at the final state of different F/M ratios

The distributions of VFA at the final state with different F/M ratios are shown in Fig. 3. The results showed that the VFAs were mainly comprised of acetic, propionic, and butyric acids at the F/M ratios of 0.2 and 0.61 g cassava-TS/g sludge-VSS, while they consisted of lactic, acetic, propionic, and butyric acids at the other F/M ratios in the experiments. The compositions of VFA at different F/M ratios were marked by the presence of acetic acid as a major product ranging from 39 percent to 56 percent of the total VFAs. At the lower F/M ratios, lactic acid can not be determined, while it increased dramatically with the increase of F/M ratios at the higher F/M ratios. Especially at the highest F/M ratio of 4.09 g cassava-TS/g sludge-VSS, lactic acid accounted for 38 percent of VFA, which almost equaled the amount of acetic acid (39 percent of VFA). Reversely, propionic and butyric acids experienced a downward trend with the F/M ratio increase. The lowest points of propionic acid was 8 percent when the F/M ratio was fixed at 3.07 g cassava-TS/g sludge-VSS, while for the butyric acid, the lowest point was 5 percent when the F/M ratio was fixed at 2.05 g cassava-TS/g sludge-VSS.

**Table 3.** VFA Concentrations at the Final State of Different F/M Ratios

| Parameters | Units | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| F/M ratio | g cassava-TS/g sludge-VSS | 0.20 | 0.61 | 1.02 | 2.05 | 3.07 | 4.09 |
| VFA | g COD/L | 2.09 | 2.78 | 3.38 | 4.41 | 5.11 | 6.79 |
| Lactic acid | g COD/L | — | — | 0.43 | 1.26 | 1.70 | 2.61 |
| Acetic acid | g COD/L | 1.12 | 1.39 | 1.89 | 2.42 | 2.73 | 2.61 |
| Propionic acid | g COD/L | 0.46 | 0.62 | 0.60 | 0.50 | 0.40 | 0.65 |
| Butyric acid | g COD/L | 0.51 | 0.77 | 0.47 | 0.23 | 0.28 | 0.91 |

As can be seen from Table 3, at lower F/M ratios acetic acid contributed to the increase of most VFA. However, with the increase of F/M ratios, lactic acid appeared to be more important for VFA accumulation. Some researchers have reported that the lactic acid is easily degraded into propionic acid (Ren et al. 1997). Zhang et al. (2007) evaluated the influence of lactic acid on the mathanogenesis and found that propionic acid accumulation occurred in the methanogenesis reactor due to the high concentration of lactic acid fed. It has been widely studied that the methanogenesis of propionic acid is slow and the accumulation of propionic acid would cause process failure, so lactic acid and propionic acid are both considered to be undesirable intermediate products in the methanogenic process. In this study, lactic acid accumulation occurred when the F/M ratio was increased. At lower F/M ratio, the substrate may be limited for the utilization of all kinds of microbes; therefore, only predominant microbes can make good use of the substrate. But, with adequate substrate, the production of lactic acid increased with the increase of F/M ratio. According to the previous studies, the optimal conditions for the production of lactic acid from starchy substrate was pH 5.0 to 5.5 under mesophilic temperature. With pH of 4.0 or 6.0, the production of lactic acid was slightly lower than the optimal pH (Ohkouchi and Inoue 2006; Yokota et al. 1995; Calderon Santoyo et al. 2003). Upon that, it can be seen that the proper pH range for the production of lactic acid was wide. Thus, pH was not an appropriate parameter to control lactic acid production from a starchy substrate. However F/M ratio can play a crucial role in eliminating lactic acid accumulation in the acidogenic fermentation of starchy substrate, such as cassava residue. According to Fig. 3 and Table 3, the F/M ratio should be controlled so that it is not too high, such that excessive accumulation of lactic acid may be avoided.

## Solid Removal Efficiency

The TS, VS, TSS, and VSS removal efficiencies at the final state of different F/M ratios are shown in Fig. 4. The solid concentration represents the mixture concentration of cassava solid and seed sludge. With better solid removal efficiency, the solid waste produced during the process will be less, which is very important in large-scale engineering. Besides, if the sludge concentration is hypothesized to be constant due to short time fermentation, the solid removal efficiency can roughly reflect the cassava degradation efficiency. As can be seen, the TS and VS removal efficiencies reached the highest point of 16 and 24 percent at the F/M ratio of 1.02 g cassava-TS/g sludge-VSS, respectively, and showed relatively poor results at both the lowest and highest F/M ratios. The removal efficiencies of TSS and VSS showed a similar trend with the performance of TS and VS. The difference was the TSS and VSS reached their highest removal efficiency of 21 and 24 percent at the F/M ratio of 2.05 g cassava-TS/g sludge-VSS, respectively. So it can be seen that by controlling the F/M ratios in an appropriate range, better solid removal efficiency can be achieved, and then the solid waste produced will be less.

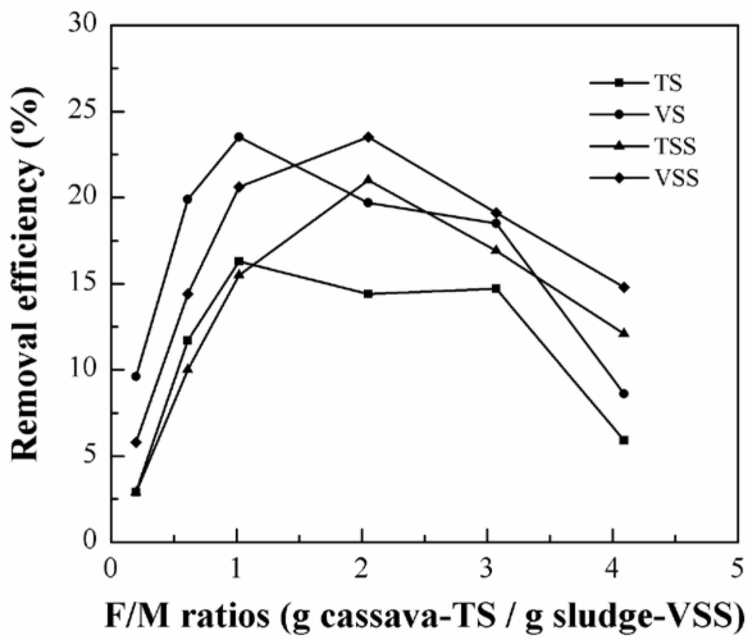


**Fig. 4.** Solid removal efficiency at the final state of different F/M ratios

## Optimal F/M Ratio of Acidogenic Fermentation of Cassava Powder

The performance of other parameters during cassava acidogenic fermentation at steady state for different F/M ratios is summarized in Table 4. Based on the values of COD, TN, and TP at the final state of the experiments, it was found that the nitrogen and phosphorus nutrients were adequate for the bacteria to use, with the COD:N:P ratio of 38-115:4:1. Compared to the TN and TP at the final state with those of initial values (shown in Table 2), it can be seen clearly that both TN and TP changed very little, which proved that anaerobic treatment was not suitable for the nitrogen and phosphorus removal.

**Table 4.** Final State Performance of Acidogenic Fermentation at Different F/M Ratios

| Parameters | Units | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| F/M ratio | g cassava-TS/g sludge-VSS | 0.20 | 0.61 | 1.02 | 2.05 | 3.07 | 4.09 |
| pH | — | 5.31 | 5.03 | 4.81 | 4.35 | 4.41 | 4.53 |
| TS | g/L | 12.00 | 13.30 | 14.87 | 20.97 | 26.64 | 35.74 |
| VS | g/L | 8.58 | 9.70 | 11.28 | 17.11 | 22.70 | 31.45 |
| TSS | g/L | 9.61 | 11.34 | 12.92 | 17.41 | 23.92 | 31.22 |
| VSS | g/L | 7.44 | 9.01 | 10.44 | 15.08 | 21.24 | 27.96 |
| TP | mg P/L | 71.69 | 71.52 | 73.04 | 78.64 | 84.00 | 94.79 |
| TN | mg N/L | 294.28 | 297.47 | 296.68 | 295.33 | 339.52 | 343.57 |
| COD | g/L | 2.71 | 4.56 | 3.42 | 7.90 | 9.68 | 9.46 |
| Acidification[a] | % | 27 | 26 | 24 | 20 | 17 | 19 |
| Starch removal efficiency | % | 100 | 100 | 100 | 88 | 70 | 53 |
| VFA/COD | % | 77 | 61 | 99 | 56 | 53 | 72 |

[a] Acidification was quantified by using the percentage of the initial cassava concentration converted to VFA. The initial cassava concentration was converted to the theoretical equivalent in g COD/L by using the value of hydrolysate COD in Table 2.

As can be seen from Table 4, the values of acidification decreased with the increase of F/M ratios, except for the F/M ratio of 4.09 g cassava-TS/g sludge-VSS. The calculated results of VFA production per unit cassava powder were similar to the performance of acidification, which were 0.27, 0.26, 0.25, 0.20, 0.17, and 0.19 g COD/g cassava-TS at the six F/M ratios, respectively. It can be seen clearly that the values of acidification and VFA production per unit cassava powder decreased significantly at F/M ratios higher than 1.02 g cassava-TS/g sludge-VSS. Potential biogas production in the methane fermentation stage at 35°C was 0.4 $m^3$/kg COD, and the potential methane content of the biogas was 65 percent according to Tchobanoglous et al. (2002). Based on these values, it can be calculated that the potential biogas production per ton cassava powder in the six acidogenic fermentation conditions were 168, 161, 151, 123, 103, and 116 $m^3$/ton cassava-TS, respectively. Obviously, the biogas produced at the F/M ratio of 2.05 g cassava-TS/g sludge-VSS was 28 $m^3$/ton cassava-TS less than that of 1.02 g cassava-TS/g sludge-VSS. Thus the F/M ratio should be controlled so that it is not higher than 1.02 g cassava-TS/g sludge-VSS.

VFA distribution is a crucial criterion for determining the optimal F/M ratio. According to the previous results, only a few kinds of simple energy and carbon compounds, such as $H_2/CO_2$, formic acid, and acetic acid, can be used by methanogens, and about 70 percent methane came from acetic acid in anaerobic fermentation (Harper and Pohland 1986; McCarty and Smith 1986). Therefore the criteria for selecting the fermentation products of acidogenic stage that are considered optimal for the methanogenic stage are: (1) the fermentation products must be directly utilizable by methanogens, with acetic acid being a prime example; (2) they must be readily convertible into methanogenic substrates by hydrogen-producing acetogens; and (3) they must contain as little as possible propionic acid and have no possibility for conversion into propionic acid (Ren et al. 1997). As shown by the results in Fig. 3, the percentage of acetic acid in total VFAs reached the highest point of 56 percent at the F/M ratio of 1.02 g cassava-TS/g sludge-VSS. Propionic acid is known to be relatively slow and hard to transform into acetic acid, and the accumulation of propionic acid will cause the failure of the operation. Besides, lactic acid will cause propionic acid accumulation, as discussed earlier. Hence, less production of propionic acid and lactic acid in the acidogenic stage will be better. Moreover, as shown in Table 4, the VFA/COD reached its highest point of 99 percent at the F/M ratio of 1.02 g cassava-TS/g sludge-VSS. The higher content of VFA in the ultimate COD of acidogenic fermentation stage is more beneficial to the methanogenic fermentation stage. Therefore, the acidogenic fermentation products at the F/M ratio of 1.02 g cassava-TS/g sludge-VSS were more favorable for the subsequent methanogenic stage than the other F/M ratios.

Starch removal efficiency is another important criterion to evaluate optimal F/M ratio. As can be seen in Table 2, the starch was the major component of cassava powder. Hence, it is necessary to know the utilization rate of starch. According to Table 4, the values of starch removal efficiency all reached 100 percent when the F/M ratios were not higher than 1.02 g cassava-TS/g sludge-VSS. However, with the increase of F/M ratios, the starch removal efficiency declined significantly, with only 53 percent at the highest F/M ratios considered in the experiment. If there is too much cassava starch remaining from the acidogenic fermentation stage, the starch will be continuously fermented to produce VFAs in the methanogenic fermentation stage, and this will easily cause the transformation of favorable VFA distributions of methanogens and the failure of the methanogenic fermentation stage, due to the too low alkalinity and pH values. Besides, too much cassava starch remaining also means the poor performance of acidogens and the failure of the acidogenic fermentation stage.

In summary, an F/M ratio of 1.02 g cassava-TS/g sludge-VSS may be considered as the optimum for the acidogenic fermentation of cassava powder, while producing an appropriate substrate for the methanogenic fermentation stage.

## CONCLUSIONS

1. The results of the present study demonstrated that F/M ratio is a very important parameter during the acidogenic fermentation of cassava residue because it can control the lactic acid production better than pH and also it can affect the pH, solid removal efficiency, acidification efficiency, and VFA distributions, thereby influencing the methanogenic fermentation.
2. VFA distribution is a crucial criterion for determining the optimal F/M ratio. Under the optimal F/M ratio of anaerobic acidogenic fermentation of cassava powder 1.02 g cassava-TS/g sludge-VSS, the percentage of acetic acid in total VFAs reached the highest point of 56 percent and the VFA distribution was more suitable for the subsequent methanogenic fermentation stage.
3. Starch removal efficiency is another important criterion by which to evaluate the optimal F/M ratio. The value of starch removal efficiency was 100% under the optimal F/M ratio, and this was beneficial to the subsequent methanogenic fermentation stage.

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