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Methane recovery from cassava starch wastewater via anaerobic digestion: Effect of inoculum source and kinetic study

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Abstract

The effects of inoculum sources on anaerobic digestion (AD) of cassava starch wastewater (CSW) were examined. Four different inoculums were used in this study, including the inoculum derived from anaerobically digested cassava starch wastewater (ADCS), pig manure (ADPM), cow manure (ADCM), and fresh elephant manure (EM). ADCS presented the highest methane potential of 351.04 NmL/g-volatile solid $(VS)_{added}$. ADPM and ADCM derived from animal manures presented the lower methane potentials of 305.34 NmL/gVS_{added} and 329.95 NmL/ g-VS_{added}, respectively. EM showed the lowest methane potential of 239.43 NmL/gVS_{added}. Total chemical oxygen demand removal efficiencies showed the same trend with the methane potential and varied between 51.5% and 34.2%. The modified Gompertz equation well-fitted to the experimental data with R^2 more than 0.99. The methane potential of ADCS was 343.41 NmL/ g-VS_{added}, which is not significantly different from the data obtained from the experiment. ADCS also presented the shortest lag phase of 3.75 days. Thus, ADCS could be the ideal inoculum for AD of CSW because of the good acclimation of the microorganisms to the substrate. However, when the availability of inoculum sources is concerned, the ADPM and ADCS could be the appropriate alternative inocula.

Keywords: Anaerobic digestion, Cassava starch wastewater, Inoculum source, Modified Gompertz

1. Introduction

The conversion of waste to energy has been one of the promising concepts to simultaneously enhance national environment and energy security. Since Thailand's economy strongly relies on agricultural businesses, agro-industries have been one of the major sectors driving the nation's economy. Based on the aforementioned statements, refuses from agricultural product processing have a high potential to serve as organic feedstocks to produce renewable energy (i.e., biomethane) through anaerobic digestion (AD) to partially serve the national energy demand. AD is a popular bio-based waste-to-energy mechanism to convert a variety of organic materials to high-energy gaseous biofuels, that is, methane and hydrogen in the absence of oxygen. This technology could simultaneously produce renewable energy as well as mitigate environmental issues.

Thailand is also a cassava starch exporter; thus, starch production factories are one of the dominant agro-industries in Thailand [1]. Unfortunately, a significant amount of wastewater (i.e., 20% weight of the produced cassava starch) could be generated [2]. In 2015, approximately 30 million tons of cassava were produced in Thailand, resulting in the generation of 6 million m^3 of high-strength wastewater, that is, high organic material contamination [3]. Cassava starch wastewater (CSW) is typically in acidic condition and contains high organic substances with chemical oxygen demand (COD) of more than 18,000 mg/L [4]. Thus, more than 100,000 tons of COD were generated and could pose extreme anger to contaminated aqua environments and ecosystems. However, CSW has been considered as one of the potential substrates for AD due to its highly biodegradable organic materials (i.e., nonstructural carbohydrate, among others). And based on the high-soluble organic substances in this wastewater, unlike lignocellulosic or other high-solid material, hydrolysis might not limit the biochemical reactions. Thus, the reactor volume (or hydraulic retention time) could be decreased, resulting in the enhancement of the system's economic feasibility. Typically, after construction and equipment installation, anaerobic digesters need to be started by being inoculated with effective seed to accelerate the microorganisms' biochemical reactions. An inoculum from the appropriate source could reduce the start-up period and confirm the efficiency and stability of the AD system.

The effects of inoculum on AD of various substrates have been presented by many authors. Dhamodharan et al [5] conducted the AD of food waste in a batch mode using five different livestock dungs, namely, poultry dung, goat dung, cow dung, swine dung, and rhinoceros dung as inocula. The results indicated that cow dung was the appropriate inoculum with high methane production of 227 mL/g-volatile solid $(VS)_{removed}$ and volatile solids (VS) removal of 55%. The abundance of methanogens, self-inhibition, acclimatization, and adaptation could play a key role in the effectiveness of inoculum [6,7]. In addition, inoculum could be a source for many trace elements to serve to involve anaerobic microorganisms. Sufficient trace elements, that is, Ni and Mo in the seeded inoculum, could positively affect the AD of food waste, that is, increasing the methane production and hydrolysis rate as well as shortening the lag phase [7]. Based on the aforementioned statement, appropriate inoculum could enhance the performance of anaerobic processes. However, the limitation of local inoculum sources for being seeded to the full-scale anaerobic digesters could burden the success of AD systems. Selecting inoculum from appropriate available sources could optimize the performance of AD systems.

The previous researchers tended to optimize the performance of AD of starch production wastewater using various reactor configurations, such as anaerobic pond [8], upflow anaerobic sludge blanket [9], anaerobic membrane reactor [10], and anaerobic filter [10]. However, to the best of the authors' knowledge, the study focusing on the effect of inoculum source on AD of CSW might still be limited. Thus, this study aims to investigate the effects of inoculum sources on the methane yield during the series of batch study of AD treating CSW. The kinetic model was also fitted to the experimental data to explain the phenomena. Additionally, the knowledge obtained from this study could be used as one of the guidelines to develop an appropriate start-up strategy and enhance system efficiency.

2. Materials and methods

2.1 Feedstock

The CSW was collected from the cassava-starch-producing factory located in Kamphaeng Phet province, Thailand. The wastewater was then stored in the temperature-controlled room at 4±2 ˚C. The stored wastewater was left at room temperature for a couple of hours before being used as the substrate. The characteristics of CSW are shown in Table 1.

2.2 Inoculum

The inocula used in this study were collected from four different sources in Chiang Mai province, Thailand. The sludge from the AD of anaerobically digested cassava starch wastewater (ADCS) was withdrawn from the bench-scale anaerobic bioreactor treating starch wastewater at the Energy Research and Development Institute-Nakornping, Chiang Mai University laboratory. The anaerobically digested pig manure (ADPM) and anaerobically digested cattle manure (ADCM) were obtained from the full-scale anaerobic digesters of the local farms treating pig manure and cow manure, respectively. The elephant manure (EM) was also collected from the private elephant camp. The inocula were stored at 4±2 ˚C and reactivated at 35±2 ˚C for several days before being transferred to the serum bottles. The characteristics of the inoculum used in this study are presented in Table 1.

2.1 Experimental setup

The biochemical methane potential (BMP) test was conducted according to the German Standard Procedure [11]. The 1-L serum bottles were used as the reactors for the batch experiments in this study (Figure 1). The substrate to inoculum ratios of all conditions was controlled at 0.5 (VS-based). Finally, distilled water was added to the bottles to adjust the bottle content's volumes to 400 mL. $NaHCO_3$ was added to adjust pH to around neutral and provided additional alkalinity. Nutrients and trace elements were supplied to facilitate the involved microorganisms as presented in the standard procedure [11]. Control sets were prepared in the same procedure as the experimental units except no CSW was added to represent baseline methane yields from the inocula. Subsequently, the bottles' headspaces were purged with nitrogen gas to create an anaerobic condition. Finally, the prepared bottles were incubated in mesophilic condition at the temperature of 35±2 ˚C in the temperature-controlled room. The experiments were terminated when the accumulated methane production reached a plateau (i.e., 45 days). In addition, all bottles were manually mixed once a day after quantifying gas production to increase the chances of contact between microorganisms and substrate. At the end of the experiment, the bottle contents were harvested and analyzed for pH, COD, total solid (TS), VS, volatile fatty acid (VFA), and alkalinity. The experiments were triplicated to ensure repeatability. The daily biogas production and biogas composition were quantified as described in section 2.4. The biogas production converted to at a standard temperature and pressure condition (0 ˚C and 1 atm). The schematic of the experimental setup is shown in Figure 1.

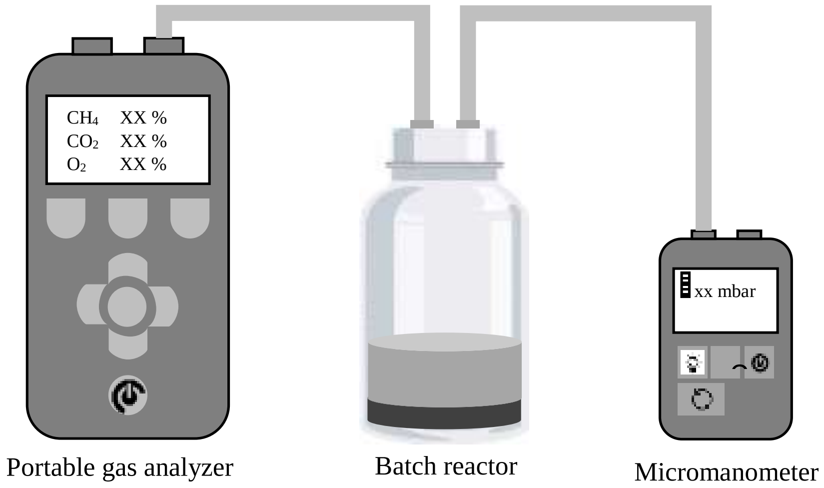

Figure 1 Experimental setup of the BMP test.

2.4 Analytical methods

pH was determined using a pH meter (Mettler Toledo [S220], Columbus, OH, USA); total COD (TCOD), filtered COD (FCOD), TS, and VS were analyzed following the standard methods [12]. VFA and alkalinity were examined following the titration method as described by Anderson and Yang (1992) [13]. Daily biogas production was measured by a micromanometer (MP 112; KIMO Instrument, France). The biogas compositions were analyzed using a portable gas analyzer (GFM416; Gas Data Limited, United Kingdom). Methane potential was calculated as $NmLCH_4/g\text{-}VS_{added}$ (at 0 ˚C and 1 atm). All parameters were analyzed in duplicate except biogas production.

2.5 Kinetic study

The modified Gompertz equation (as presented in equation 1) was applied to fit the experimental data (i.e., cumulative methane yields) from all inocula in this study [14].

$$Y = Mexp\left\{-exp\left[\frac{Rm\,e}{M}(\lambda - t) + 1\right]\right\} \quad (1)$$

Where Y represents the accumulated methane volume ($NmL/g\text{-}VS_{added}$); t is experimental time (d); M stands for methane production potential ($NmL/g\text{-}VS_{added}$); R_m is the maximum methane production rate ($NmL/g\text{-}VS_{added}/d$); λ is the lag phase (d); and e is an Euler's number (2.718). The calculation of the variables was performed using Microsoft Office Excel with the solver function.

2.6 Statistical data analysis

The experimental data were statistically analyzed by Statistical Package for the Social Sciences, IBM, USA using an analysis of variance with a significance level (α) of 0.05 followed by a post hoc Tukey's test.

3. Results and discussion

3.1 Substrate and inoculum characteristics

CSW was prepared as mentioned in section 2.1 and used as the sole substrate in this study. The pH of wastewater is in an acidic condition that might be from high VFA (i.e., more than 5,000 mg/L) as presented in

Table 1. A similar pH of this specific wastewater was also reported by Fettig et al. [15]. TCOD, representing contaminated organic matters in wastewater, is high, and almost half portion is in soluble form as presented as FCOD/TCOD in Table 1. Based on this character, hydrolysis might not be the limiting stage during AD of CSW. In addition, VFA/FCOD was 26% that could represent the high potential of methane production during AD because of high-specific substrate for acetoclastic methanogens. However, the reactor might be at risk from VFA accumulation resulting in reactor failure. The ratio of VS and TS is also high (i.e., more than 0.9) that confirms high organic contents in this wastewater. In addition, the ratio between TCOD, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus (TP) could indicate a balance of organic substance and macronutrients of the substrate. The TCOD/TKN/TP ratio of CSW used in this study is 100/1.8/0.3 that should be able to serve AD without the addition of external nutrients [16]. However, regarding low pH and high VFA, an anaerobic system of CSW could suffer from organic overloading when the system is not operated and controlled properly. The appropriate high-nutrient inoculum could be an additional source of nutrients and enhancing system efficiency and stability [7].

Inocula had a ratio of VS and TS in the range of 0.68-0.75 (Table 1) that is appropriate for the AD process and can potentially result in high methane production through AD.

Table 1 The characteristics of cassava wastewater and inocula

Parameters	Unit	CSW	ADCS	ADPM	ADCM	EM
pH	–	3.90±0.02	7.85±0.13	7.46±0.11	7.39±0.09	7.43±0.13
VFA	mg/L	5,373±93	288±138	188±20	1,770±23	1,240±11
TCOD	mg/L	45,764±3,470	12,289±23	12,228±60	17,972±164	13,339±122
FCOD	mg/L	20,801±639	457±47	947±30	2,530±431	3,720±50
FCOD/TCOD	–	0.45	0.04	0.08	0.14	0.28
TS	mg/L	35,452±1,687	24,250±1,700	95,445±283	43,588±619	32,158±414
VS	mg/L	32,399±1,718	18,250±283	67,355±3,833	30,570±207	21,765±1,527
VS/TS	–	0.91	0.75	0.71	0.70	0.68
TKN	mg/L	803±26	N/A	N/A	N/A	N/A
TP	mg/L	153±12	N/A	N/A	N/A	N/A
TCOD/TKN/TP	–	100/1.8/0.3	N/A	N/A	N/A	N/A

N/A: Not applicable.

3.2 Methane production and yield

Typically, the inocula used in this study could be categorized into two groups: (a) the inocula derived from an anaerobic digester treating organic wastes, that is, ADCS, ADPM, and ADCM and (b) fresh animal manure, that is, EM. The daily methane yield has been illustrated in Figure 2.

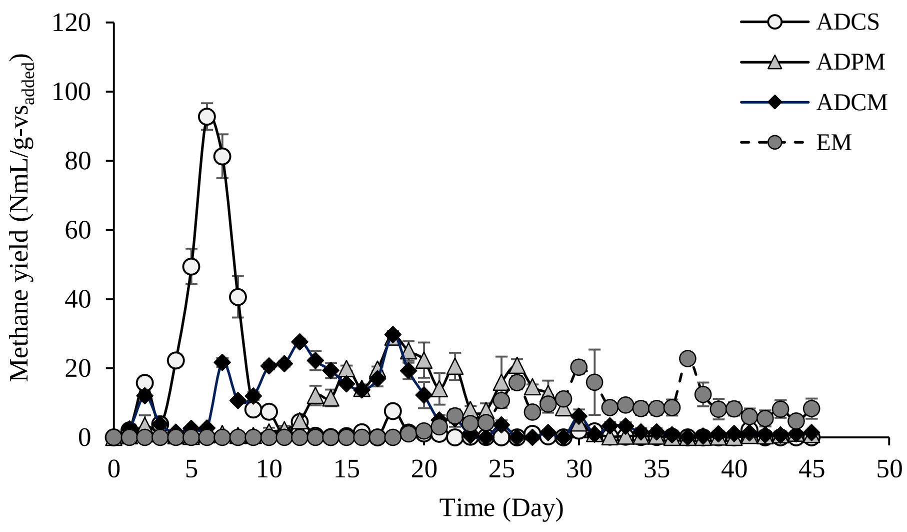

Figure 2 Daily methane yields during anaerobic digestion of cassava starch wastewater with different inocula.

For ADCS and ADCM, methane started showing up at the early stage of the experiments (i.e., day 2) with the methane yields of 15.74±0.9 and 12.08±0.3 NmL/g-VS_{added}, respectively. However, only 3.42±2.96 NmL/g-VS_{added} of methane was observed from ADPM, and no methane was observed from EM at the same experimental period. The maximum daily methane yields of ADCS, ADPM, ADCM, and EM were observed on day 6, 18, 18, and 37 with the maximum values of 92.8±6.36, 29.0±7.44, 29.8±3.80, and 22.8±9.43 NmL/g-VS $_{added}$, respectively. The significantly lower daily methane yield ($\alpha = 0.05$) of the second category of the inoculum sources derived from the EM was investigated compared with anaerobic bioreactors inoculum (ADCS, ADPM, and ADCM).

Cumulative methane yield is a parameter reflecting the methane potential of a specific substrate in a batch experiment. The cumulative methane yields of ADCS, ADPM, ADCM, and EM as presented in Figure 3 were 351.04±12.25, 305.34±13.32, 329.95±4.41, and 239.43±27.76 NmL/g-VS_{added}, respectively. It is clear that ADCS presented the highest cumulative methane yield compared with other inocula. However, the value was not significantly different from that of ADCM. However, EM presented a significantly lowest methane yield compared with other inoculums. Typically, the involved anaerobic microorganisms during the AD process require a certain amount of time during the start-up period to adapt to the new substrate and environment. For the lag phase, ADCS also showed the shortest lag phase of only a day followed by ADCM and ADPM. EM presented the longest lag phase of almost 21 days. This phenomenon might be from the better acclimation to anaerobic condition of the inoculum derived from anaerobic digesters. In addition, ADCS was collected from the reactor fed with starch wastewater that is similar to the substrate of this experiment (i.e., CSW). Thus, the best adaptability of the microorganism in this inoculum could be observed and resulted in the highest methane yield compared with those from other inocula [17]. Moreover, EM was the only inoculum presented the first methane yield after 19 days of the experiment that is almost half of the experimental period. It could be because EM contains high-lignocellulosic materials that could hinder anaerobic biodegradation regarding the recalcitrant structures [18]. Thus, using EM as an inoculum could result in low methane yield and a long lag phase that can decrease the efficiency and stability of anaerobic systems [19]. The effectiveness of an inoculum derived from an anaerobic digester was also presented by De Vrieze et al. [6]. The authors successfully applied four inocula from full-scale AD plants to in the BMP of molasses without any significant effect on the methane yields.

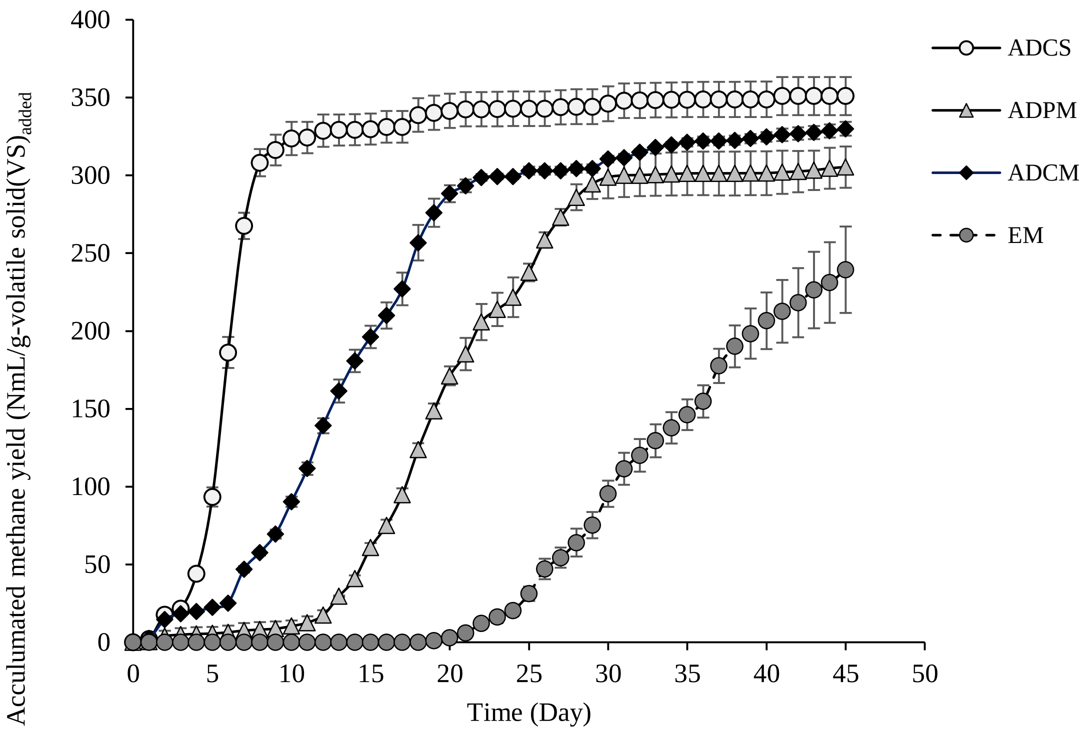

Figure 3 Cumulative methane yields during anaerobic digestion of cassava starch wastewater with different inocula.

3.3 Methane content

The final methane contents of ADCS, ADPM, ADCM, and EM were 61.1±0.10%, 65.4±0.68%, 62.8±0.71%, and 58.50±0.61%, respectively (Fig. 4). The results found in this study were similar to those reported by other researchers, that is, 50-70% and 54% presented by Lu et al. [9], and Araujo et al. [20], respectively. Normally, based on the characteristics of substrates, AD of carbohydrate-rich wastewater might result in lower methane contents compared with those from lipid- and protein-rich wastewater [21]. Thus, the inoculum derived from the AD of carbohydrate-rich wastewater (i.e., ADCS) resulted in the lower methane content compared with those originated from animal manures (i.e., ADPM and ADCM) that contain higher protein contents. EM presented the lowest methane content that confirms the low activity of involved microorganisms.

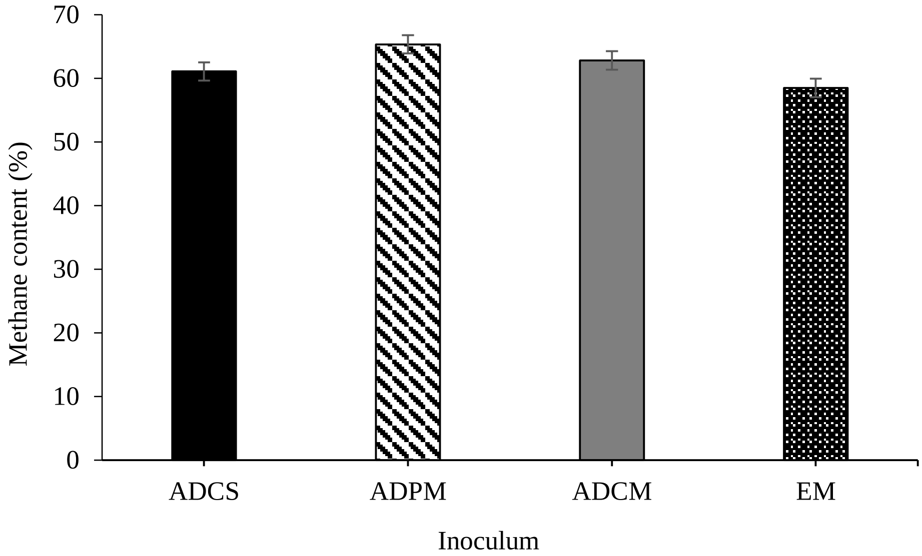

Figure 4 Methane contents during anaerobic digestion of cassava starch wastewater with different inocula.

3.4 Efficiency of BMP test

Efficiencies of BMP tests are shown in Table 2. pHs of the bottle contents of all experimental sets were maintained around neutral (i.e., 7.16-7.18) that is in the optimization range for AD [22]. VFA concentrations of the bottle contents were between 172±42 and 422±30 mg/L as CH_3COOH for the inoculum derived from anaerobic digesters (i.e., ADCS, ADPM, and ADCM). However, VFA concentration was significantly higher of 1,028±468 mg/L as CH_3COOH for fresh manure, such as EM. The VFA/alkalinity ratios of all experimental conditions were lower than the recommended value for AD, that is, less than 0.4 [22] that could ensure system stability. Based on the characteristics of the reactor contents presented in Table 2, all experiments in this study might not suffer from organic acid accumulation, which is one of the important phenomena causing system failure during AD [23].

TCOD removal strongly correlates with methane yield during AD. In this study, TCOD removals of ADCS, ADPM, ADCM, and EM were 51.48±1.79%, 42.67±1.69%, 43.93±1.65%, and 34.03±4.26%, respectively. ADCS presented a significantly higher TCOD removal compared with other inocula. This result could confirm the effective and fast acclimation of this inoculum to the substrate (CSW) and the digestion environments. TCOD removals of ADPM and ADCM that are both anaerobically digested animal manures presenting similar TCOD removals. Reversely, EM showed the lowest TCOD removal that also agreed with the methane yield. TCOD removal of ADCS in this study is similar to that reported by Racho and Pongampornnara [24], that is, 60%, when modified tapioca starch wastewater and the bottom sludge from equalizing tank of the starch factory were used as the substrate and inoculum, respectively. However, TCOD removal in this study is lower than that of an anaerobic reactor fed with starch wastewater and operated in continuous mode [24]. VFA removal from the system during the effluent withdrawal during continuous reactor operation could maintain the balance of the system and prevent VFA accumulation.

Table 2 Characteristics of the bottle contents before and after BMP tests.

Parameter	Condition	Unit	ADCS	ADPM	ADCM	EM
pH	Before	–	7.01±0.03	7.17±0.01	7.16±0.02	7.17±0.02
	After	–	7.18±0.01	7.17±0.02	7.16±0.03	7.10±0.01
VFA	Before	mg/L	2,290±10	1,508±20	1,466±13	1,369±46
	After	mg/L	172±42	348±46	422±30	1,028±46
Alkalinity	Before	mg/L	9,134±10	10,817±40	9,969±13	16,866±26
	After	mg/L	8,920±22	10,496±28	9,333±46	16,362±26
TCOD	Before	mg/L	39,592±1,682	45,427±597	34,023±1,846	30,989±1,683
	After	mg/L	19,195±105	26,045±230	19,056±473	20,406±221
FCOD	Before	mg/L	2,053±84	2,262±168	4,532±172	4,584±86
	After	mg/L	992±58	1,250±47	2,240±84	2,842±90
TS	Before	mg/L	36,535±163	40,040±1,471	36,840±427	42,800±339
	After	mg/L	16,325±148	28,425±278	26,475±293	33,240±368
VS	Before	mg/L	18,205±50	22,425±247	18,550±297	20,840±156
	After	mg/L	12,730±205	13,225±216	12,875±191	15,355±219
TCOD removal	–	%	51.5±1.79	42.7±1.69	43.9±1.65	34.2±4.24
FCOD removal	–	%	51.7±1.61	44.6±2.19	46.2±1.24	37.9±1.98
TS removal	–	%	27.9±1.72	30.0±2.82	28.1±1.83	22.3±1.62
VS removal	–	%	46.6±1.27	42.0±1.22	36.0±2.52	30.6±2.23

3.5 Kinetic study

The information obtained from the kinetic study could be used for analyzing and explaining the anaerobic process. Several models could be applied to fit the experimental data, including but not limited to first-order, logistic, and Gaussian equations (25-27). However, in this study, the modified Gompertz model was adopted to examine the kinetic parameters. The modified Gompertz model has been widely used to fit the experimental data from the batch study of AD [26]. The lag phase during acclimatization of microorganisms in inocula was included in this model; thus, it could effectively describe the AD process [28]. The kinetic parameters for AD of cassava wastewater using different inocula are presented in Table 3.

Table 3 Kinetic parameters for AD of cassava wastewater using different inocula.

Inoculum	M (NmL/g-VS_{added})	R_m (NmL/g-VS_{added})	λ (d)	R^2
ADCS	343.41	85.94	3.75	0.9943
ADPM	309.83	23.02	12.62	0.9976
ADCM	325.50	22.66	5.76	0.9962
EM	281.48	13.12	23.12	0.9988

The modified Gompertz model fitted well with the experimental data of all inocula with a high R^2 between 0.9943 and 0.9988. From Table 3, it is clear that ADCS presented the highest methane production potential of 343.41 NmL/g-VS_{added} followed by ADCM and ADPM. EM also showed the lowest methane production potential of 281.48 NmL/g-VS_{added}. The maximum methane production rates also showed the same trend with methane production potential. However, the maximum methane production rate of ADCM was slightly higher than that of ADPM. The lag phase is one of the important indicators for selecting inoculum for the AD process. Typically, the lag phase of AD of the carbohydrate-rich substrate could be from VFA inhibition during the early stage of the AD process [29]. The short lag phase during the start-up period of anaerobic digester could enhance the benefit of AD systems and enhance the efficiency of biogas production [29]. The lag phase of ADCS was significantly lower than those of other inocula. ADPM showed quite a long lag phase of 12.62 days, and EM presented the longest lag phase of more than 20 days, which is almost half of the experiment period.

4. Conclusion

Using appropriate inoculum is one of the key successes for optimizing the benefit of AD processes. By inoculating the system with the effective inoculum, the start-up period could be cut down as well as enhance biogas production. In this study, ADCS, originated from an anaerobic digester treating starch wastewater, showed the highest methane yield and TCOD removal efficiency of 351.04 NmL/g-VS_{added} and 51.5% respectively. The kinetic study also confirmed the experimental results. The shortest lag phase was also obtained from ADCS, of 3.75 days. It could be concluded that the inocula derived from anaerobic digesters presented a significantly higher methane yield and lower lag phase compared to that from fresh manure. However, the availability of the local inoculum sources in the area should also be considered.

5. Acknowledgements

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