分类号: <u>X705</u> 单位代码: <u>10335</u>

密 级: <u>公 开</u> 学 号: <u>11813057</u>

浙江大学

博士学位论文



英文论文题目: <u>Study on Enhancing Biohythane Production from Tofu</u>
(Soybean) <u>Processing Residue via Pretreatment and Anaerobic Digestion</u>

申请人姓名:	MAHMOUD MOHAMED AHMED HUSSEIN ALI
指导教师:	盛奎川 教授
学科(专业):	农业生物环境与能源工程
研究方向:	生物质能工程
所在学院:	生物系统工程与食品科学学院

论文提交日期 2022年6月

中国•杭州

ABSTRACT

Biofuel generation from biomass has received more attention as an alternative, renewable, sustainable, and clean energy source that replaces fossil fuel, alleviating energy demand and environmental concerns. Among biomass, tofu processing residue (TPR), which is a by-product of the tofu and soymilk production industry, is rich in carbohydrates (50-60%), proteins (20-30%), and fats (10-20%), as well as contain high moisture content ($\geq 85\%$), posing it as a suitable substrate for biofuels production through anaerobic digestion (AD) technology. TPR deteriorates rapidly and is thus mainly disposed of in landfills, causing environmental concerns. Therefore, how to efficiently treat TPR is a big concern for soybean processing plants. AD is the most sustainable and cost-effective method to treat organic wastes along with energy recovery as well as it reduces the emission of greenhouse gases, generated during the self-decomposition of biowastes. In this research, energy recovery from TPR was improved by the production of biohythane from one-stage and two-stage AD through controlling the operational conditions, pretreating TPR using ultrasonic pretreatment, dilute sulfuric acid (H₂SO₄) pretreatment, and the simultaneous combination of ultrasonic and dilute H₂SO₄ pretreatments, as well as adding molybdate (MoO₄²⁻) and ferric chloride (FeCl₃). The main results are as follows:

1) Two-stage AD of TPR was investigated considering the impacts of operational conditions on microbial community diversity and biohythane production. The results showed that the optimal conditions were dark fermentation (DF) operated at the substrate to inoculum ratio (SIR) of 8 and 37 °C for 36 h, followed by methanogenic fermentation (MF) performed at the SIR of 1 and 37 °C for 13 d, which produced 324.4 ml/g-VS_{fed} of biohythane along with 103 mmol/L acetic acid and 38 mmol/L propionic acid. Two-stage AD improved specific energy recovery by 41.5% compared to one-stage AD, producing a biogas yield of 189.6 ml/g-VS_{fed}. SIR and temperature affected microbial community diversity of DF system, where high SIR of 8 speciated hydrogen producers such as *Mobilitalea* and *Clostridium sensu stricto 1* at thermophilic and mesophilic temperatures, respectively, whereas low SIR of 0.5 stimulated methane generation by the speciation of *Methanoculleus thermophilus*. Likewise, hydrogenotrophic methanogens (*Methanomassiliicoccus*) enriched MF reactors operated at low SIR. Overall, this study demonstrated two-stage AD as an efficient technology for producing clean energy and value-added products using TPR.

- 2) TPR has received more attention as a source of bioenergy. However, their low solubility has hindered biohythane generation. Consequently, the ultrasonic and H₂SO₄ pretreatments were combined and compared for the first time to improve the hydrolysis of organic matter and carbohydrates as well as to increase free amino nitrogen generation from TPR. Besides, the impact of pretreatments on biohythane generation via one-stage AD was also investigated. Under the optimal conditions of 7.54% substrate level, 8% H₂SO₄ concentration, 80 °C, and 50 min, the coincident ultrasonic-H₂SO₄ pretreatment enriched the contents of soluble chemical oxygen demand, reducing sugar, and free amino nitrogen to 49675 mg/L, 26 g/L, and 1721 mg/L, respectively, greater than individual pretreatments. Also, Biohythane yield increased by 4.20-12.58% over control (389.39±23.8 ml/g-VS_{fed}). Furthermore, the highest hydrogen yields of 42.5±2.08 and 28.1±1.07 ml/g-VS_{fed} and the sulfate removal efficiencies of 93 and 92% were achieved with ultrasonic-H₂SO₄ and H₂SO₄ pretreatments, respectively, indicating the enhancement of acidogenic and sulfidogenic activity.
- 3) Biohythane production through one-stage AD of sulfate-rich hydrolyzed TPR has been hampered by high H₂S production. Herein, two-stage AD was investigated with the addition of MoO₄²⁻ (0.24-3.63 g/L) and FeCl₃ (0.025-5.4 g/L) to the DF stage to improve biohythane production. DF supplemented with 1.21 g/L MoO₄²⁻ increased hydrogen yield by 14.6% compared to the control (68.39 ml/g-VS_{fed}), while FeCl₃ had no effective effect. Furthermore, the maximum methane yields of MF were 524.75 ml/g-VS_{fed} with 3.63 g/L MoO₄²⁻ and 521.60 ml/g-VS_{fed} with 0.6 g/L FeCl₃ compared to 466.07 ml/g-VS_{fed} of the control. The maximum yields of biohythane and energy were 796.7 ml/g-VS_{fed} and 21.80 MJ/kg-VS_{fed} with 0.6 g/L FeCl₃ when the sulfate removal efficiency was 66.71%, and H₂S content was limited at 0.08%. Therefore, adding 0.6 g/L FeCl₃ is the most beneficial in improving energy recovery and sulfate removal with low H₂S content.

Keywords: Sulfuric acid pretreatment, Ultrasonic pretreatment, Anaerobic digestion, Biohythane, Ferric chloride, Molybdate

TABLE OF CONTENTS

ABSTR	ACT	xi
LIST O	F TABLES	XX
LIST O	F FIGURES	xxii
LIST O	F ABBREVIATIONS	xxvi
СНАРТ	TER 1. LITERATURE REVIEW	1
1.1.	Introduction	1
1.2.	Soybean residues	4
1.3.	Anaerobic digestion technology in China	7
1.4.	Anaerobic digestion overview	8
1.4.1.	One-stage anaerobic digestion	9
1.4.2.	Two-stage anaerobic digestion	9
1.5.	Factors that influence anaerobic digestion	17
1.5.1.	Substrate	17
1.5.2.	Inoculum	19
1.5.3.	Substrate to inoculum ratio	19
1.5.4.	Temperature	20
1.5.5.	pH	21
1.5.6.	Carbon to nitrogen ratio	22
1.5.7.	Retention time	22
1.5.8.	Trace elements	23
1.6.	Pretreatment	23
1.6.1.	Physical pretreatment	24

1.6.2.	Chemical pretreatment	28
1.6.3.	Biological pretreatment	29
1.6.4.	Choice of ultrasonic and acid pretreatments over other pretreatments	30
1.7.	Inhibitors of anaerobic digestion	30
1.7.1.	Ammonia	30
1.7.2.	Sulfur, sulfate, and sulfide	31
1.7.3.	Fatty acids	32
1.7.4.	Inhibitors formed during pretreatment	32
1.8.	The objectives	33
1.9.	Layout of the dissertation	34
СНАРТ	TER 2. MATERIAL AND METHODS	35
2.1.	Feedstocks and inoculum preparation	35
2.2.	Anaerobic digestion devices	35
2.2.1.	Conventional batch system	35
2.2.2.	Developed batch system	36
2.3.	Analytical methods	37
2.3.1.	Determination of physicochemical characteristics	37
2.3.2.	Determination of biogas production and composition	42
2.4.	Calculation methods	43
2.4.1.	Calculation of total solid and volatile solid	43
2.4.2.	Calculation of the hydrolysis yield of COD and carbohydrate	43
2.4.3.	Calculation of anaerobic biodegradability and sulfate removal	44
2.4.4.	Calculation of biogas component production, energy recovery, and kin	etic parameters
of mo	dified Gompertz model	44

2.5.	Statistical analysis	45
СНАРТ	ER 3. BIOHYTHANE PRODUCTION FROM TOFU PROCESSING RI	ESIDUE VIA
TWO-S	TAGE ANAEROBIC DIGESTION: OPERATIONAL CONDITI	ONS AND
MICRO	BIAL COMMUNITY DYNAMICS	46
3.1.	Introduction	46
3.2.	Experimental design and set-up	48
3.2.1.	Anaerobic digestion	48
3.2.2.	Microbial community analysis	48
3.3.	Results and discussion.	50
3.3.1.	Characteristics of feedstock and inoculum	50
3.3.2.	Fermentative hydrogen production	51
3.3.3.	Fermentative methane production	58
3.3.4.	Overall assessment of two-stage anaerobic digestion versus one-sta	ge anaerobic
digesti	ion of TPR	59
3.3.5.	Microbial community dynamics	61
3.4.	Summary	76
СНАРТ	ER 4. COMBINATION OF ULTRASONIC AND ACIDIC PRETREATM	MENTS FOR
ENHAN	ICING BIOHYTHANE PRODUCTION FROM TOFU PROCESSING R	ESIDUE VIA
ONE-ST	TAGE ANAEROBIC DIGESTION	77
4. 1. I	ntroduction	77
4.2.	Experimental design and set-up	79
4.2.1.	Pretreatment of tofu processing residue	79
4.2.2.	Anaerobic digestion	80
4.3.	Results and discussion	81
4.3.1.	Preliminary experiments	81

	Impact of acid concentration and solid loading ratio on pretreated tofu ue characteristics	
	Impact of temperature and retention time on pretreated tofu process cteristics	_
	Effects of pretreatment method on the microscopic surface morpholossing residue	
4.3.5.	Biohythane production	92
4.3.6.	A kinetic study using the Gompertz model	99
4.3.7.	Correlations between physicochemical characteristics and biohythane	e production
4.4.	Summary	. 101
СНАРТ	ER 5. IMPACTS OF MOLYBDATE AND FERRIC CHLORIDE ON BIO	HYTHANE
PRODU	CTION THROUGH TWO-STAGE ANAEROBIC DIGESTION OF SULI	FATE-RICH
HYDRC	DLYZED TOFU PROCESSING RESIDUE	. 102
5.1.	Introduction	. 102
5.2.	Experimental design and set-up	. 104
	Impacts of molybdate and ferric ions on dark fermentation of sulfate-rich processing residue	, ,
5.2.2.	Methanogenic fermentation of dark fermentation effluent	. 105
5.3.	Results and discussion	. 105
5.3.1.	Characteristics of feedstock and inoculum	. 105
	Impacts of molybdate and ferric ions supplementation on dark f	
	Impacts of molybdate and ferric ions supplementation on methanogenic f s performance	
5.3.4.	Biohythane production via two-stage anaerobic digestion	. 127

5.4.	Summary	130
СНАРТ	TER 6. GENERAL CONCLUSION	131
6.1.	Conclusions	131
6.2.	Novelty aspects	135
6.3	Recommendations	135
REFER	ENCES	136

LIST OF TABLES

Table No	Title	Page
Table 1. 1	The reduction CO ₂ emission (kg) of different transport means	3
	running by biogas (Yousuf et al., 2016).	
Table 1. 2	Gaseous biofuels generated from AD of organic matters	11
	(Rawoof et al., 2021).	
Table 1.3	Summary of metabolic reaction and functional microorganisms	13
	during acidogenic fermentation (Luo et al., 2019).	
Table 1.4	Methanogenic reactions that occur in methanogenic	17
	fermentation (Luo et al., 2019).	
Table 1.5	Biohythane generation from different biowastes.	18
Table 3. 1	Experimental setup details.	49
Table 3. 2	Physicochemical characteristics of TPR and inocula.	51
Table 3. 3	The results of the DF process with TPR at different operational	57
	conditions.	
Table 3. 4	The results of MF of DF effluent and one-stage AD of TPR for	62
	13 d.	
Table 3. 5	Statistical indices of alpha diversity analysis of the microbial	66
Table 3. 3	community.	00
	·	
Table 3. 6	Beta diversity indices based on bray Curtis.	66
Table 4. 1	Experimental setup details.	80
Table 4. 2	Physicochemical characteristics of inoculum and feedstocks.	81
Table 4. 3	Performance of ultrasonic-H ₂ SO ₄ pretreatment at different	85
	SLRs and acid concentrations.	

Table 4. 4	Tests of Between-Subjects Effects.	86
Table 4. 5	Results of Batch one-stage AD of pretreated TPR for 21 d under the mesophilic condition.	98
Table 5. 1	The physicochemical characteristics of feedstock and inoculum.	107
Table 5. 2	Results of DF operated with hydrolyzed TPR and MoO_4^{2-} addition for 90 h under mesophilic conditions.	115
Table 5. 3	Results of DF operated with hydrolyzed TPR and ferric ion addition for 90 h under mesophilic conditions.	116
Table 5. 4	Results of MF of M1-M7 and control operated under mesophilic conditions for 144 h.	125
Table 5. 5	Results of MF of F1-F8 operated under mesophilic conditions for 144 h.	126
Table 5. 6	Effects of molybdate addition on biohythane yield and energy recovery.	129
Table 5. 7	Effects of ferric chloride addition on biohythane yield and energy recovery.	129

LIST OF FIGURES

Figure No.	Title	Page
Fig. 1. 1	The main fluctuation in energy carriers from 2017 to 2040 (Tabatabaei et al., 2020).	2
Fig. 1. 2	Biofuels' production technologies.	2
Fig. 1. 3	The common resources of H ₂ production.	3
Fig. 1. 4	The Chinese production of soybean in 2015-2021 (provided by Statista platform).	5
Fig. 1. 5	Schematic diagram of tofu processing residue production	6
Fig. 1. 6	Biogas production proportion from different digesters in 2008 and 2017 (Lu & Gao, 2021).	8
Fig. 1. 7	Biochemical reaction occurred in anaerobic digestion (adopted from Luo et al. (2019)).	10
Fig. 1. 8	Schematic of the pretreatment categories of biowastes (Argun et al., 2017).	25
Fig. 2. 1	Schematic diagram of conventional batch system.	36
Fig. 2. 2	Photo of conventional batch system.	36
Fig. 2. 3	Photo of the mixing and reactor module (A) and gas flow meter module (B).	37
Fig. 2. 4	Photo of TGD-22MC centrifuge.	38
Fig. 2. 5	Photo of electrical oven (A) and furnace muffle (B).	38
Fig. 2. 6	Photo of pH meter (A) and elemental analyzer (B).	39
Fig. 2. 7	Photo of LH-TX6 digester (A) and LH-T725 analyzer (B).	39
Fig. 2. 8	Photo of Cary 60 UV-Vis spectrophotometer.	40
Fig. 2. 9	Photo of CIC-D120 Ion chromatograph.	41

Fig. 2. 10	Photo of a gas chromatograph (GC 2014, Shimadzu).	41
Fig. 2. 11	Photo of scanning electron microscopy.	42
Fig. 2. 12	Photo of Gasboard-3200Plus.	43
Fig. 3. 1	The effects of SIR on thermophilic DF. Biogas components yield (a), H ₂ production (b), and CH ₄ production (c).	53
Fig. 3. 2	The cumulative yields of biogas (a) and H_2 (b) of the DF process for 120 h at the mesophilic and thermophilic conditions. Biogas and H_2 yields of mesophilic DF for 36 h (c).	55
Fig. 3. 3	The yields of biogas components of MF process operated at mesophilic and thermophilic using different SIRs (a). CH ₄ yields of MF process for 13 d (b) where solid lines depicted mesophilic temperature and dash lines represented thermophilic condition. CH ₄ yield of one-stage AD of TPR at mesophilic for 13 d (c). The yields of biogas components of two-stage AD compared to those of one-stage AD at the optimal conditions (d).	60
Fig. 3. 4	Rarefaction curves of bacterial communities of samples A, B, C, D, E, F, and G, which are defined in section 3.2.2.	64
Fig. 3. 5	Rarefaction curves of archaeal communities of samples A, B, C, D, E, F, and G, which are defined in section 3.2.2.	65
Fig. 3. 6	The similarity of microbial communities under different operational conditions. bacterial communities are presented in charts a and c, while archaeal communities are presented in charts b and d. The microbial communities of A, B, C, D, E, F, and G are defined in section 3.2.2. Communities closely ordinated are likely more similar than distant one.	67
Fig. 3. 7	Taxonomy profile of bacterial and archaeal communities at the phylum level (a-b) and genus level (c-d) within the communities of A, B, C, and D which are defined in section 3.2.2.	70
Fig. 3. 8	Taxonomy profile of bacterial and archaeal communities at the phylum level (a-b) and genus level (c-d) within the communities of A, E, F, and	75

Fig. 5. 1

106

	G, which are defined in section 3.2.2.	
Fig. 4. 1	Effects of (A) the acid type and (B) H ₂ SO ₄ concentration through	82
	coincident acid and ultrasonic pretreatment on FAN concentration, the	
	hydrolysis yield.	
Fig. 4. 2	Cumulative biogas yield of (A) AD without ventilation and pH	84
	adjustment, (B) AD with pH adjustment and no ventilation, (C) AD with	
	ventilation and pH adjustment, and (D) pH value on Day 3 and Day 9 for	
	treated and untreated TPR.	
Fig. 4. 3	Effects of (A) pretreatment method at ambient temperature for 30 min,	88
	(B) initial temperature, (C) pretreatment duration, and (D) pretreatment	
	method at 80 °C for 50 min on FAN concentration, the hydrolysis yield	
	of total COD, and carbohydrates.	
Fig. 4. 4	Linear correlation of temperature with (A) Reducing sugar, (B) FAN, and	90
	(C) SCOD. (D) The linear relationship between pretreatment duration	
	and reducing sugar. (E) Correlation matrix among reducing sugar, FAN,	
	and SCOD.	
Fig. 4. 5	SEM image of TPR particles (a) untreated, (b) pretreated with ultrasonic,	91
	(c) pretreated with H ₂ SO ₄ , and (d) pretreated with ultrasonic-H ₂ SO ₄ .	
Fig. 4. 6	Effects of pretreatment methods of TPR on (A) cumulative biogas	94
	production, (B) H ₂ concentration, (C) cumulative H ₂ yield, (D) CH ₄	
	concentration, (E) cumulative CH ₄ yield, and (F) sulfate concentration	
	and sulfate reduction efficiency.	
Fig. 4. 7	Effects of pretreatment methods of TPR on (A) daily biogas production,	95
	(B) biohythane components yield at different periods, (C) CH ₄ yield, (D)	
	H ₂ yield, (E) H ₂ S concentration and yield.	
Fig. 4. 8	Linear correlation among (A) Bio-H ₂ ; (B) Bio-CH ₄ and reducing sugar,	100
	FAN, SCOD, and COD/SO ₄ ²⁻ ratio. (C) Correlation matrix among	
	reducing sugar, FAN, SCOD, influent SO ₄ ²⁻ , COD/SO ₄ ²⁻ ratio, SO ₄ ²⁻	
	removal, biogas yield, H ₂ yield, H ₂ S yield, and CH ₄ yield.	

The schematic diagram of the experimental plan.

- Fig. 5. 2 Effects of MoO₄²⁻ and FeCl₃ doses on (A and D) biogas production, (B and E) H₂ concentration, and (C and F) cumulative H₂ yield from hydrolyzed TPR.
- Fig. 5. 3 Effects of MoO₄²⁻ and FeCl₃ concentrations on chemical characteristics 113 of DF reactor contents (A and E) VFAs production, (B and F) SCOD concentration, (C and G) SO₄²⁻ content and H₂S yield, and (D and H) CH₂O content.
- Fig. 5. 4 Effects of MoO₄²⁻ and FeCl₃ doses on (A and D) cumulative biogas production, (B and E) CH₄ concentration, and (C and F) cumulative CH₄ yield from MF operated with different substrates.
- Fig. 5. 5 Effects of MoO₄²⁻ and FeCl₃ concentrations on chemical characteristics 122 of MF reactor contents (A and E) VFAs content, (B and F) SCOD concentration, (C and G) SO₄²⁻ concentration and H₂S yield, and (D and H) CH₂O content.

LIST OF ABBREVIATIONS

AD: Anaerobic digestion SO₄²⁻: Sulfate

ACE: Abundance coverage-based estimator SO_3^{2-} : Sulfite

CH₄: Methane $S_2O_3^{2-}$: Thiosulfate

CO₂: Carbon dioxide SEM: Scanning electron microscopy

C/N: Carbon to nitrogen TPR: Tofu processing residue

COD: Chemical oxygen demand TS: total solid

CH₂O: Formaldehyde VFAs: Volatile fatty acids

Chao1: Species richness estimator VS: Volatile solid

COA: Coenzyme A 5-HMF: 5-hydroxymethylfurfural

DF: Dark fermentation

FAN: Free amino nitrogen

FeCl₃: Ferric chloride

GHGs: Greenhouse gases

H₂: Hydrogen

H₂S: Hydrogen sulfide

H₂SO₄: Sulfuric acid

HCl: Hydrochloric acid

H₃PO₄: Phosphoric acid

KOH: Potassium hydroxide

MF: Methanogenic fermentation

MoO₄²-: Molybdate

NH₄-N: Ammonium nitrogen

NaOH: Sodium hydroxide

NMDS: Non-metric multidimensional

scaling analysis

POME: Palm oil mill effluent

SIR: Substrate to inoculum ratio

SRB: Sulfate-reducing bacteria

SLR: Solid loading ratios

SCOD: Soluble chemical oxygen demand