Coupling process of simultaneous desulfurization–nitrification and microalgal cultivation for biogas and effluent from anaerobic digestion

脱硫–硝化同時処理槽および微細藻類生産槽の統合プロセスによる 嫌気消化槽バイオガスおよび消化液処理

18D5701　関根 睦実　　指導教授　戸田 龍樹

SYNOPSIS

有機性廃棄物のメタン発酵処理において、生成バイオガスの脱硫や消化液（メタン発酵排水）中の NH$_4^+$除去等の後段処理に伴う環境負荷や費用の削減は課題の 1 つである。そこで、脱硫-硝化および微細藻類培養を組み合わせた新規のバイオガス脱硫および消化液処理を考案する。本プロセスでは、硫黄酸化細菌によるバイオガス脱硫と硝化細菌による消化液の硝化を統合処理することで反応槽を削減し、硝化後の消化液は、NH$_4^+$が無害な NO$_3^-$になるため無希釈で微細藻類生産に利用し、栄養塩を除去できる可能性がある。微細藻類が生成した酸素を用いることで脱硫-硝化槽の機械的曝気を削減できる可能性がある。本研究では提案プロセスの確立に向け、バイオガス脱硫と消化液の硝化の同時処理法を開発し、また脱硫-硝化処理後の消化液の微細藻類培地としての有用性を評価した。最後に、全体プロセスを運転し処理安定性、微細藻類生産性ならびに物質収支を評価した。同時脱硫・硝化においては S^{2-}による硝化阻害が課題となるが、基質供給時間を延長した順次回分式反応槽を用いることで既報値より高い 128 mg-S L^{-1} d^{-1} の S^{2-}負荷速度下で 100%の両処理を達成した。基質の連続的供給と反応槽の高い汚泥保持能が細菌群集の馴養に寄与したと考えられる。そこで CO$_2$ を除く合成バイオガスと消化液を類似した特徴を持つ連続撹拌槽型膜反応槽を用いて同時処理し、同様に高い脱硫・硝化が得られた。また、酸素供給塔を別設することで脱硫ガスへの O$_2$ の混入を 0.5%以下に抑えられ、脱硫後のバイオガスの安全性が担保された。処理後の消化液を無希釈で用いた *Chlorella sorokiniana* NIES-2173 株の回分培養では、人工培地と同程度の高い増殖が得られ、処理後の消化液の培地としての有用性が示された。最後に、3 L 脱硫-硝化槽および 4.5 L 微細藻類槽（光連続照射）を同時に連続運転した結果、100%の脱硫-硝化、0.5 g L^{-1} d^{-1} の微細藻類生産性、および 23%の窒素除去能を維持した。屋外での 1 日 12 時間の明期を想定すると、各槽を 3:40 の体積比で設けることで排水基準値以下への窒素除去が見込め、本提案プロセスの実現可能性が示された。

Keywords: post anaerobic digestion treatment, aerobic bacterial consortium, sulfide inhibition, microbial acclimatization, nitrogen removal, microalgal mass cultivation, free ammonia inhibition, *Chlorella sorokiniana*

INTRODUCTION

Anaerobic digestion (AD) is an economical and environmentally friendly treatment method of organic wastes since anaerobic microbes generate biogas containing energetically usable CH$_4$ and CO$_2$ from organic carbon. However, environmental loads and costs for post-treatments of the generated biogas and effluent (ADE) are remaining unsolved issues for the further spread of the AD. Biogas also contains 0.05–0.3 % H$_2$S, which is highly toxic and corrosive. Bio-desulfurization by sulfur-oxidizing bacteria (SOB) has a lower environmental impact and lower cost than other physicochemical treatments, but it still consumes energy and cost mainly for the construction and mechanical aeration. Regarding ADE, contaminated nutrients, e.g., NH$_4^+$ and PO$_4^{3-}$ should be removed by such as nitrification-denitrification treatment with organic addition and aeration [1] after the liquid-solid separation. Also, these nutrients can be used as a liquid fertilizer or microalgal culture medium. However, there is a low demand for liquid fertilizer, especially in the urban area. Microalgal cultivation requires 2–50 times dilution of ADE with a large amount of freshwater to avoid NH$_3$ inhibition.

This thesis proposes a novel coupling process of simultaneous desulfurization-nitrification (SDN) and microalgal cultivation for biogas desulfurization and digested effluent treatment after anaerobic digestion as post-treatment of AD (Figure 1). Both nitrifying bacteria for the ADE nitrification and SOB for the biogas desulfurization live in a similar aerobic environment under neutral pH and less than 30ºC [2, 3]. Therefore, these treatments may proceed in a single reactor. Also, because ADE treated by SDN contains nitrate (NO$_3^-$) which is harmless for living organisms including microalgae, the treated ADE could be used as a medium without dilution. Furthermore, the utilization of O$_2$ produced by microalgae might reduce mechanical aeration for SDN.

To establish the coupling process of SDN and microalgal cultivation, there are several challenges. First, sulfide (S^{2-}) inhibits nitrification by more than 50% with
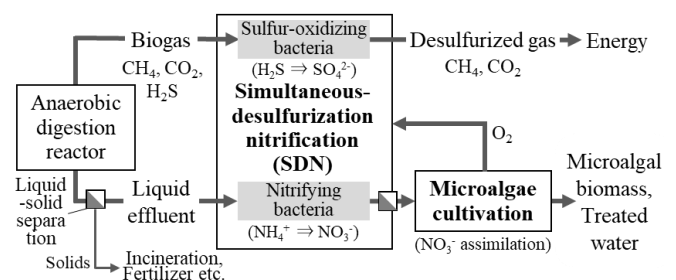

Figure 1. A coupling process of desulfurization-nitrification and microalgal cultivation for biogas desulfurization and digested effluent treatment (Japanese Patent Application No. 2019-35294)

a few milligrams per liter [4]. In the simultaneous treatment of S^{2-} and NH_4^+ using a continuous stirred tank reactor (CSTR), the acclimatization of microbes needed a minimum 2-week inhibition period to achieve 100% nitrification efficiency after a stepwise increase of S^{2-} loading rate to 74 mg-S L^{-1} d^{-1} [5]. A more stable nitrification treatment is required to combine with biogas desulfurization. Second, for the direct biogas supply into the SDN reactor, dissolved inorganic carbon (DIC) inhibitory effect on nitrifying bacteria and oxygen contamination into biogas should be investigated. That is because CO_2 which is about 40% in biogas would significantly increase the DIC concentration of the SDN reactor, and O_2 concentration in biogas is necessary to be suppressed to avoid the explosion. Third, regarding microalgal cultivation using ADE treated by SDN as a medium without dilution, high concentrations of digestate components may inhibit microalgal growth. Moreover, concentrations of some components associated with microalgal growth may change through SDN, causing inhibition or improvement of microalgal growth. Therefore, it is required to evaluate the microalgal productivity of the treated ADE.

To establish the environmentally friendly and low-cost treatment of biogas and ADE with microalgal production, the following studies in this thesis were conducted to develop the proposed coupling process: development of SDN process using aerobic bacterial consortium, integration of biogas desulfurization and ADE nitrification, evaluation of treated ADE usability for microalgal culture medium without dilution, and coupling of SDN and microalgal cultivation for biogas and ADE treatment.

MATERIALS AND METHODS

Through the present study, a centrifuged liquid ADE (Total ammonium nitrogen (TAN) approx. 900 mg-N L^{-1}) obtained from the Hokubu Sludge Treatment Center, Yokohama was used as substrate after filtration using a 0.45 μm pore size glass filter. Nitrifying sludge used was also supplied from the same center. A typical green microalgae *Chlorella sorokiniana* NIES-2173 supplied from the National Institute for Environmental Studies was used as a target species.

Simultaneous desulfurization-nitrification (SDN) using aerobic bacterial consortium

ADE with NaHS solution instead of H_2S gas in biogas was continuously treated using nitrifying sludge. 2.1-L sequential batch reactor (SBR) with a long fill period was used. The reactor was operated with 24 h cycle: 23.5 h for filling and reaction, 0.2 h for settling, 0.3 h for discharging. Thus, it has the same long filling operation and higher sludge retention time (SRT) compared with CSTR, which are preferable for acclimatization of the bacterial consortium [6]. Hydraulic retention time (HRT) was 3 days. Operation period was divided into five phases, and S^{2-} loading rate (SLR) was stepwise increased from 0 to 32, 64, 128, 256 mg-S L^{-1} d^{-1} by changing the NaHS concentration of the substrate. The TNA loading rate (NLR) was maintained at approx. 300 mg-N L^{-1} d^{-1}. Reactor pH and temperature were

adjusted to 7.5 and 30°C by adding 2N NaOH solution and using a water bath. During the filling and react period, dissolved oxygen concentration (DO) was maintained more than 3.0 mg L^{-1} by continuous aeration. At the end of each SLR phase, sulfide tolerance and sulfide removal rate of sludge were evaluated by batch bioassay using sludge taken from the reactor.

Integration of biogas desulfurization and ADE nitrification

To demonstrate the stable operation of the SDN reactor under biogas supply, firstly, the batch bioassay was conducted using ADE in which CO_2 is dissolved to estimate CO_2 inhibitory effect on nitrification. Then, treatment stability and O_2 contamination into desulfurized gas were evaluated by continuous treatment of synthetic biogas and ADE.

<u>Evaluation of CO_2 inhibitory effect on nitrification</u>

5, 20, and 40% of CO_2 gas (N_2 base) were supplied 10 min into a serum bottle containing 100-mL 20 times diluted ADE with 11.9 g L^{-1} HEPES (Initial pH7.5). After the addition of nitrifying bacteria, it was cultivated for 72 h under 30°C temperature and 160-rpm shaking.

<u>Simultaneous biogas desulfurization and ADE nitrification</u>

ADE and synthetic biogas without CO_2 (0, 0.125, 0.25, and 0.5 % H_2S, N_2 base instead of CH_4) were treated by a continuous stirred tank membrane reactor (CSTMR, Figure 2 (A)) containing nitrifying sludge, consisted of 3.0-L CSTR, an external membrane cartridge filter (0.22 μm pore size) for discharge, and 50-mL O_2 bubbling column. pH, temperature, and HRT were the same as previous experiment using the SBR. The biogas flow rate was 30 NmL L^{-1}. Thus, NLR was approx. 300 mg-N L^{-1} d^{-1} and SLR was stepwise increased from 0 to 25, 50, 100 mg-S [Phase 1–4]. DO was adjusted to 3.0 mg-O_2 L^{-1}.

Evaluation of treated ADE usability for microalgal culture medium without dilution

After evaluating different nitrogen utilization characteristics, productivity of treated ADE was compared with untreated ADE and synthetic medium by batch experiment.

<u>Usability of NO_3^- and NH_3 tolerance of microalgae</u>

The usability of NO_3^- of *C. sorokiniana* NIES-2173 was evaluated using C-medium with different nitrogen source and concentration (NO_3, TAN and NO_2^-; 33, 100, 500, and 1000 mg-N L^{-1}), and 4.8 g L^{-1} HEPES. The initial pH was 7.5. Glass tubes were used with 5-mL effective volume.

NH_3 tolerance was evaluated using C-medium with different NO_3/TAN ratio (NH_3 concentration: 0, 0.9, 1.8, 2.7, and 3.6 mM), 4.2-g L^{-1} $NaHCO_3$ and 4.8 g L^{-1} HEPES. The initial pH was 8.0. Twelve-well microplates were used with 2-mL effective volume.

Light intensity and temperature were maintained at 150 μmol photons m^{-2} s^{-1} and 25 °C, respectively.

<u>Usability of treated ADE without dilution for the microalgal cultivation</u>

The usability of treated ADE without dilution for the microalgal cultivation was evaluated by comparison with C-medium, untreated ADE with 1, 3, 6, and 10

times dilution. Untreated/treated ADE medium was used after supplementing with the same concentration of Mg and other trace metals as C-medium. 4.2-g L^{-1} $NaHCO_3$ was added in all conditions. Initial pH was adjusted to 7.5 in all conditions. The 10-mL glass tube was used as a container with 5-mL effective volume and cultivation was conducted under 150-μmol-photons m^{-2} s^{-1} light intensity and 25°C temperature.

Coupling of SDN and microalgal cultivation for biogas and ADE treatment

Biogas desulfurization and ADE nitrification by SDN and microalgal cultivation using treated ADE were performed simultaneously (Figure 2 (A)(B)). The operation of the SDN reactor (CSTMR) was the same as the previous experiment. *C. sorokiniana* NIES-2173 was semi-continuously cultivated in the 4.5-L airlift reactor. ADE treated by SDN was used as a culture medium after supplementing Mg and other trace metals with concentrations of 5 times higher than those of C-medium. The reactor was operated at 5-d HRT and 2-d SRT under pH7.5, 25°C temperature, and 500-μmol photons m^{-2} s^{-1} light intensity. Air was continuously supplied and pH was adjusted by periodic CO_2 supply.
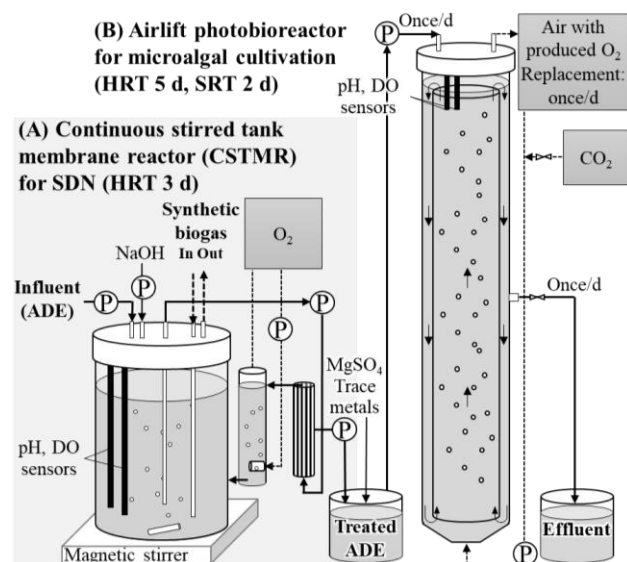

Figure 2. Schematic diagram of reactors set up for SDN of biogas and ADE and continuous microalgal cultivation.

RESULTS AND DISCUSSION

Simultaneous desulfurization–nitrification (SDN) using aerobic bacterial consortium

In Phase 1–4, the supplied TAN was completely removed and oxidized to NO_3^- (Figure 3). S^{2-} inhibitory effect on nitrification was not shown. Stable nitrification was achieved at up to 128 mg-S L^{-1} d^{-1} SLR which is high enough to treat H_2S in actual biogas. In Phase 5, TAN and NO_2^- were accumulated in the reactor. NO_2^- and NO_3^- conversion efficiencies of supplied TAN were 77% and 20%, respectively, indicating severer inhibition on NO_2^- oxidizing bacteria than on NH_4^+ oxidizing bacteria. Supplied S^{2-} was completely removed throughout the experimental period. SO_4^{2-} conversion efficiency of S^{2-} was 23–84% in Phase 2–5. Since H_2S in the exhaust gas was not detected through the operation period, it is possible that S^0 which is one of the sulfur
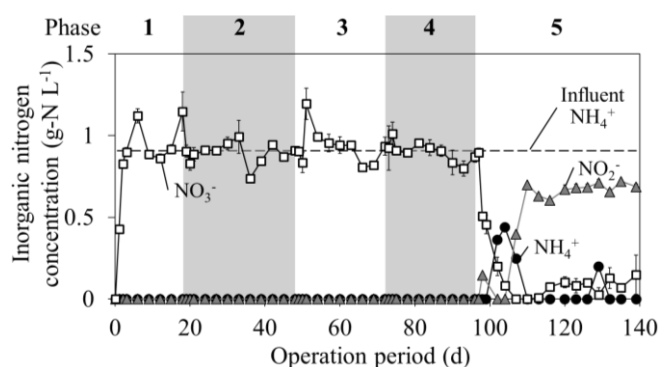

Figure 3. Inorganic nitrogen composition of effluent.

intermediate oxidation states, was produced and accumulated in the SBR, but even so, S^0 is harmless to microbes [7].

As a result of batch bioassay, 50% inhibitory S^{2-} concentration (S^{2-}-IC_{50}) on TAN removal efficiency of the reactor sludge increased from 1.43 to 3.65, 2.72, 5.86 mg-S L^{-1} through Phase 1–4. The maximum S^{2-} removal rate of the sludge tended to decrease from 0.31 to 0.25, 0.14, 0.17 g-VSS^{-1} h^{-1}. Also, it was revealed by amplicon sequencing that the compositional shift of nitrifying bacteria to *Nitrosomonas nitrosa* and *Nitrobacter* spp. during the experiment. The effective acclimatization of the bacterial consortium might have increased sulfide tolerance of the reactor not by the increasing sulfide oxidizing bacteria but rather by the shift of dominant nitrifying bacteria.

Integration of biogas desulfurization and ADE nitrification

Evaluation of CO_2 inhibitory effect on nitrification

After 10 min babbling of 5, 20, and 40% CO_2 gas, dissolved inorganic concentration (DIC) of 20 times diluted ADE substrate was changed from 27 mg-C L^{-1} to 50, 219, 371 mg-C L^{-1} in each condition. Under these DIC concentrations, NO_3^- production rate was 5.1, 3.3, and 2.0 mg-N g-VSS^{-1} h^{-1} in each condition and it decreased 61% in the 40% CO_2 babbling condition. Therefore, CO_2 should be removed from the biogas before biogas desulfurization with ADE nitrification. Developing membrane technologies are probably appropriate for the CO_2 separation because the CO_2 molecular size (3.3 Å) is smaller than H_2S (3.6 Å) and CH_4 (3.8 Å) [8].

Simultaneous biogas desulfurization and ADE nitrification

In all phases, both H_2S of the desulfurized gas and S^{2-} of the effluent were not detected. Unlike a previous experiment using the SBR, almost all the supplied H_2S was oxidized to SO_4^{2-}. TAN was also completely removed and oxidized to NO_3^-. O_2 concentration of desulfurized gas was maintained at 0.38±0.11% less than 0.5% which is typically the upper limit to introduce biogas into the natural gas grid [3].

Batch bioassay using the reactor sludge at the end of each phase revealed that S^{2-}-IC_{50} for the TAN removal efficiency increased from 0.84 mg-S L^{-1} to 15.8 mg-S L^{-1} which is higher than the maximum values in the previous experiment using the SBR. S^{2-} removal rate was also more than three times faster than that in the previous experiment, and SOB *Thiobacillus* increased with SLR. Therefore, faster S^{2-} removal following SOB

growth probably increased S^{2-} IC_{50} in the CSTMR, suggesting the CSTMR is more suitable to acclimatize nitrifying sludge to S^{2-}.

Evaluation of treated ADE usability for microalgal culture medium without dilution

Usability of NO_3^- and NH_3 tolerance of microalgae

Specific growth rate under using NO_3^-, TAN, and NO_2^- at different concentrations, 33, 100, 500, 1000 mg-N L^{-1} were 0.24–0.38 d^{-1}, 0.32–0.44 d^{-1}, and 0–0.30 d^{-1}, respectively, and tended to decrease at high concentration. The highest specific growth rate was obtained in conditions using TAN probably because the assimilation of NO_3^- and NO_2^- requires energy for the uptake of these into cells and reduction to NH_4^+ [9]. In the condition using NO_2^- at 500, 1000 mg-N L^{-1}, specific growth rate markedly reduced maybe because NO_2^- severely inhibited Photosystem II [10].

Specific growth rate under different NH_3 concentration, 0, 13, 25, 38, and 50 mg-N L^{-1} was 1.85, 1.32, 0.77, -0.14, -0.16 d^{-1}, respectively. Accordingly, 50% effective concentration of NH_3 for *C. sorokiniana* NIES-2173 was calculated as 22.4 mg-N L^{-1}.

Therefore, NH_4^+ is suitable as a nitrogen source to obtain the highest specific growth rate. However, especially in mass cultivation using a large scale reactor and ADE containing a high concentration of NH_4^+, NO_3^- or low concentration of NO_2^- is suitable as a nitrogen source to avoid NH_3 inhibition because complete pH adjustment is difficult. Besides, NO_2^- produced by partial nitrification should not occur in the SDN reactor.

Usability of treated ADE without dilution for the microalgal cultivation

The specific growth rate until day 2 was almost the same in all conditions except condition using untreated ADE without dilution (Table 1), indicating that the difference of nitrogen source (NO_3^- or NH_4^+), and other contaminants in untreated/treated ADE did not affect specific growth rate. However, under using untreated ADE without dilution, high NH_3 concentration (12 mg-N L^{-1}) probably inhibited microalgal growth. In addition, the maximum optical density at 750 nm (OD_{750}) was clearly changed by the difference of nitrogen source. High OD_{750} was obtained under using treated ADE and C-medium containing NO_3^-. By contrast, OD_{750} was lower by more than 60% in every condition using untreated ADE compared to a condition using C-medium. During the cultivation period, pH increased until in the range of 8.9 to 11.2 in every condition. This increase led to an increase of NH_3 concentration in the range of 91 to 329 mg-N L^{-1}, and

NH_3 inhibition on microalgal growth in condition using untreated ADE. Therefore, undiluted treated ADE has similar microalgal productivity with C-medium, and is appropriate to use for the mass cultivation of microalgae without NH_3 inhibition without dilution.

Coupling of SDN and microalgal cultivation for biogas and ADE treatment

Biogas desulfurization and ADE nitrification efficiency were maintained at 100% in the SDN reactor. O_2 consumption for these treatments was 1.36±0.06 NL L^{-1} d^{-1}. The microalgal productivity was 0.48±0.03 g-dry weight L^{-1} d^{-1} after day 5. NO_3^- removal efficiency in the algal reactor was 23%. O_2 production was 0.75±0.16 L L^{-1} d^{-1}. It was considered that simultaneous biogas desulfurization and complete nutrient removal from ADE could be achieved by the construction of an SDN reactor and a microalgal reactor with a volume ratio of 3:40 under 12 h per day of the light period.

The results of economic estimation of the developed process based on the mass balance data showed that though costs for CO_2 removal from biogas and microalgal cultivation is high, net cost in the coupling process is 12-times higher compared with conventional process (biodesulfurization of biogas, heat/power generation, and nitrification–denitrification of ADE) because of the potential to produce a large revenue from biomethane and microalgal biomass sales.

SUMMARY

To develop the proposed process, first, simultaneous desulfurization and nitrification for biogas and ADE treatment was established by effective bacterial acclimatization to S^{2-}. Second, O_2 contamination into treated biogas was suppressed less than 0.5% in the SDN treatment using MSCTR. On the other hand, CO_2 contained in biogas inhibited nitrification, therefore, a CO_2 removal system is necessary before the SDN process. Third, the high productivity of *C. sorokiniana* was obtained by using undiluted ADE treated by SDN, indicating that the consumption of a large amount of water for dilution can be suppressed. Then, stable operation of both SDN and microalgal reactors was demonstrated, and the appropriate volume ratio of SDN and the algal reactor was estimated as 3 to 40. Therefore, effective biogas desulfurization and nutrient removal from ADE can be achieved by the SDN and microalgal cultivation.

Table 1. The specific growth rate (μ) at first 2 days and the final optical density at 750 (OD_{750}) under different medium.

| Medium | C-medium | Untreated anaerobic digestion effluent (ADE) | | | | Treated ADE |
		Without dilution	3-fold dilution	6-fold dilution	10-fold dilution	
μ (d^{-1})	0.57 ±0.16	0.32 ±0.04 [a]	0.43 ±0.05	0.45 ±0.11	0.47 ±0.04	0.54 ±0.09
Final OD_{750}	0.71 ±0.01	0.08 ±0.01 [b]	0.23 ±0.02 [b]	0.26 ±0.03 [b]	0.43 ±0.03 [b]	0.71 ±0.02

Dunnett's test was conducted on specific growth rate and final OD_{750} (n=3) assuming C-medium condition was the control. a: significantly different at the 5% level, b: significantly different at the 1% level.

LITERATURES CITED

[1] Fux & Siegrist, *Water Sci. Technol.* 50, 19–26 (2004). [2] Antoniou et al., *Water Res.* 24, 97-101 (1990). [3] Muñoz et al., *Rev. Environ. Sci. Biotechnol.* 14, 727-259 (2015). [4] Bejarano-Ortiz et al., *J. Chem. Technol. Bio-technol.* 88, 1344-1349 (2013). [5] Beristain-Cardoso et al., *Bioresour. Technol.* 102, 3623-3625 (2011). [6] Nakhla et al., *Water Environ. Res.* 69, 6-13 (1997). [7] Nguyen, *Asian J. Org. Chem.* 6, 477-491 (2017). [8] Shah et al., *Chem. Rev.* 117, 9755-9803 (2017). [9] Collos & Harrison, *Mar. Pollut. Bull.* 80, 8-23 (2014). [10] Zhang et al., *Appl. Environ. Microbiol.* 83, 1-16 (2017).