

## Original Article

# Screening Halophytic Plant Species for Phytoremediation to Rehabilitate Salinised Farmlands in Uzbekistan

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## ABSTRACT

Possible salt-accumulating plant species were investigated for their potential in phytoremediation of the salinised farmland in the Karakalpakstan region, Uzbekistan, together with its methane fermentation for the post-treatment of biomass. The examined plant species of *Amaranthus retroflexus*, *Atriplex nitens*, *Bassia scoparia*, and *Karelinia caspia* yielded the highest biomass at the end of August, whilst high salt uptake was found during their young stage in spring. Based on the biomass weight per plant, the salt content, and the cultivation density, *A. nitens* was the most promising species, which might remove the salt from the soil with about 1.8 kg-NaCl equivalent/kg-dry soil in the peak period. The result suggested the consecutive cultivation of the plant species for 3–6 years would rehabilitate the farmland to allow growth of wheat, sunflower, and sorghum. The kinetic model modified from IWA-Anaerobic Digestion Model No.1 (ADM1) showed the above four plant species had a similar biodegradability to that of the ordinary energy crop (*Erianthus arundinaceus*). The model predicted 60–70% of the biomass chemical oxygen demand (COD) could be converted to methane at an solids retention time (SRT) of about 50–60 days in an anaerobic digester. When the SRT was extended to 200 days, the methane conversion efficiencies were expected to reach about 80%.

**Keywords:** ADM1, energy crop, hydrolysis, halophyte, phytoremediation

## INTRODUCTION

Soil salinisation is a common issue leading to the reduction of agricultural productivities over semi-arid–arid regions, particularly Central Asia [1,2]. Among the Central Asian countries, Uzbekistan is known as one of the most constrained regions by salt-induced soil degradation, where its western part of the agricultural areas (the Karakalpakstan region neighbouring the Aralkum desert) is especially being moderately–heavily salinised in improper irrigation methods as well as climate change [3]. Geographically, the groundwater of the region is somewhat saline (about 3.0–7.9

mS/cm = 1,600–4,800 mg-NaCl equivalent/L) with shallow water tables of about 0.5–2.5 meters depth from the land surface, which often results in anthropogenic soil salinisation in the farmlands [1]. When much surface water is wrongly irrigated over the farmlands, the water reaches the saline water tables. Consequently, the saline water is hydraulically lifted toward the soil surface by a capillary effect during the evapotranspiration, which eventually creates salt deposits on the soil surface. On the other hand, in case that the irrigation is wrongly squeezed, the crop harvest may decline accordingly. Theoretically, a drip irrigation system is a promising method to cope with the unwanted salt deposits, where a lim-

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ited amount of irrigation water is fed over the farmland using water pipeline networks equipped with pumps [4]. However, because of the huge capital cost for the system installation, this method is only acceptable in relatively small-scale farms (e.g., cultivation in a greenhouse) and/or high-income countries [5,6]. At present, physical soil leaching using surface water (soil washing) before sowing plants is thought to be the sole option for controlling the soil salinisation in the low-income countries. Nevertheless, since the effluent after the soil leaching contains salt to a certain extent, the effluent may negatively impact the downstream farming areas, which is especially true for landlocked regions, such as the Karakalpakstan region [1]. Furthermore, the availability of surface water for soil leaching treatment is generally in the spring season only, and this amount seems to be being lowered in frequent droughts due to a recent climate change occurring in Central Asia [7].

To remove the salts from the salinised farmlands without the soil-leaching treatment, phytoremediation might be an interesting alternative option, where salt-accumulating plants are intentionally cultivated at the farmlands. Since the plant species are capable of accumulating inorganic ions into their cell vacuoles [8], consecutive cultivation of the plant species in multiple years and/or mixed cultivation of the plants and crops may alleviate the soil salinisation, leading to sustainable income for the local farmers. Although the phytoremediation may generally require a longer remediation period than the soil-leaching treatment, because of its low-cost feature, the technology has been successfully demonstrated in the contaminated agricultural farms as well as soils of mines to reduce heavy metal ions (e.g., As and Cd) using selected inorganic accumulators (e.g., pteridophytes and *Noccaea caerulea*) [9,10]. Hence, phytoremediation using salt-accumulating plants might also work in reducing salts from the salinised farmlands. Among the salt-accumulating plant species, halophyte is known as a sodium-ion accumulator, which naturally grows in salinised lands, including deserts. About 1,560 species of halophytes have been identified in 550 genera and 117 families worldwide [11]. Nevertheless, it seems that the studies/projects to remove the salts from salinised soils using halophytes have not been explicitly conducted but have still been in a hypothetical stage speculated from the salt fractions of the plant species [12].

In the viewpoint of engineering for phytoremediation, since this method is merely to transfer the target substances from the soil to the plant biomass, the rehabilitation of salinised farmlands must be equipped with a post-treatment process for the plant biomass after its cultivation. Among

the possible post-treatment processes, biogas recovery with a methane fermentation system may be of interest because the system enables the separation of the salt fraction into the digestate, whilst the biodegradable organic fraction of the plant biomass can be utilised as a sort of energy crop to obtain biogas for the alternative fuel in the regions [13]. Therefore, the biogas may help to maintain the farmers' income to a certain extent during the phytoremediation projects. For the final disposal of the salts, the salt-containing digestate may be landfilled in a controlled disposal site, in which the depth from the land surface is slightly underneath the water table. In this way, the salt fraction of the digestate can be eventually returned to the saline groundwater by the hydraulic diffusion where no labour-intensive operation is needed.

With respect to the studies about halophytes in the literature, it seems that these are mostly conducted in the field of physiology to reveal the metabolism for the inorganic ion transfer in association with biomass growth and in the ecological investigation to analyse the life cycle of each plant species in the wild conditions (e.g., in deserts and during drought) [2,8]. Since the engineering information to select relevant halophyte species for the aforementioned phytoremediation of salinised soil is highly limited in literature, an experimental study to investigate the methane conversion efficiencies of halophyte biomass is desired besides the demonstration of agricultural cultivation for the wild halophyte species in the salinised farms. From this context, three species of tall halophytes (1.0–1.7 m height) and one tall salt-tolerant crop (about 0.8 m height) found in literature are worth investigating (halophytes: *Atriplex nitens* Schkuhr., *Bassia scoparia* (L.) A.J.Scott, and *Karelinia caspia* (Pall) Less, a salt-tolerant crop: *Amaranthus retroflexus* L.) [2, Fig. 1 and Table S1]. Although the plant species are somewhat shorter than ordinary energy crops used in industrial biogas production systems (e.g., *Erianthus arundinaceus*: about up to 2 m height) [14,15], these are the ones of the tallest annual plants available in the Karakalpakstan region, Uzbekistan. According to the aforementioned study [12], their investigated halophyte species having a high sodium fraction (*Halocnemum strobilaceum*, *Atriplex verruciferae*, *Salsola crassae*, and *Salicornia europaea*) were supposed to be a potential halophyte to recover salts into the biomass. However, since these plant heights were all short and had limited biomass per plant, the plant species were not considered for the agricultural demonstration in this study.

To evaluate the degree of methane recovery from the plant biomass, the conventional BMP test (Biochemical Methane Potential test in batch mode) is supposed to not be relevant.

(A) *Amaranthus retroflexus* L.(B) *Atriplex nitens* Schkuhr.(C) *Bassia scoparia* (L.) A.J.Scott(D) *Karelinia caspia* (Pall.) Less**Fig. 1** Examples of salt tolerant plants growing in the Karakalpakstan region, Uzbekistan.

This is because the inoculum often used for the BMP tests (a digestate collected from an anaerobic digester in a municipal wastewater treatment plant) is thought to not adapt quickly for the hydrolysis of lignocellulose-rich materials, which might result in an underestimation of the biological degradation kinetics. To cope with this issue, the experimental scheme may be modified in a fed-batch system where the materials are dynamically fed to the bioreactor at regular intervals, whilst the digestate is not withdrawn during the experiments except for sampling events. From this dynamic response of the incubation, the biological degradation kinetics of the plant biomass could be obtained based on a modified IWA Anaerobic Digestion Model #1 (ADM1) [16,17], which has been recently developed to model the anaerobic digestion of lignocellulosic compounds.

## MATERIALS AND METHODS

### Cultivation of selected plant species

The seeds of *A. nitens*, *B. scoparia*, and *K. caspia* were collected and sorted out from the plants growing naturally in the Karakata saline depression zones of the Kyzylkum desert, Uzbekistan, whilst the commercial seeds of *A. retroflexus* were also obtained from the International Center for Bio-saline Agriculture (ICBA, Dubai, UAE). The seeds of each plant species were planted in the experimental site of a total of 480 m<sup>2</sup> (20 m × 6 m per plant species) in the abandoned salinised farmland in the Karabuga district, the Karakalpakstan region, Uzbekistan. The preliminary soil analysis (8 samples in zero–0.25 m depth from the land surface) revealed that the salt equivalent to about 6.6 g-NaCl (standard deviation = 2.8) was present per 1 kg of dry-based soil (or per 0.82 L of land)

(**Fig. S1**). Based on the water content of the land (about 0.27 kg-water/kg-dry soil), the salt concentration of the water was estimated to be 25 g-NaCl equivalent/L-water, which was as high as 70% of sea water. After tilling the farm soil, the commercial chemical fertiliser ( $\text{N:P}_2\text{O}_5:\text{K}_2\text{O} = 180:80:60$  in  $\text{g/m}^2$ ) and a mulch (mostly composed of straw, about 2  $\text{kg/m}^2$ ) were spread over the experimental site together with the seeds in winter (December 2023) for *A. nitens*, and in early spring (March–April 2024) for the other three plant species, respectively. The irrigation using the surface water was conducted in April to stimulate the germination of these plants. After this treatment, the number of nurseries/young plants per unit area was squeezed and fixed to be 8 plants/ $\text{m}^2$  for *A. retroflexus*, 43 plants/ $\text{m}^2$  for *A. nitens*, 19 plants/ $\text{m}^2$  for *B. scoparia*, and 12 plants/ $\text{m}^2$  for *K. caspia*, respectively. These plant cultivation densities were based on the expected horizontal projected area of leaf per adult plant. In July, to cope with the very hot temperature in the region, a small amount of surface water was fed along with the furrows of the experimental site. Except for these quick irrigations, no surface water was fed in the experiment.

During the annual cultivations lasting until the end of September 2024, 25 plants of each plant species were randomly sampled over the experimental site at the end of every month, and each wet weight was measured at the site. Because of the small height of *A. retroflexus* in May, the biomass of the plant in May was not sampled for chemical analysis. Subsequently, each air-dried sample was weighed, and mechanically powdered using the milling machine (HC-800, Golden Wall, Hangzhou, China) to obtain the composites for the analysis of the chemical constituents. The ratios of air-dry weight to the wet weight were highly varied depending on the plant species and the seasons (*A. retroflexus*: 0.22 (July)–0.31 (August), *A. nitens*: 0.31 (June)–0.56 (August), *B. scoparia*: 0.26 (June)–0.78 (September), *K. caspia*: 0.34 (June)–0.48 (September)).

### Biochemical methane potential test

A limited number of plants were preliminarily cultivated in the experimental site between 2021 and 2022, and its dry samples were stored for the fed-batch tests. The samples collected in July 2021 were fed to the jar-fermenters between day 0 and day 120, whilst the samples collected in June 2022 and those collected in September 2022 were dosed between day 121 and day 250, and between day 251 and day 300, respectively. Due to the droughts in the years, the plant growth was somewhat inhibited compared to the aforementioned cultivation in 2024, which resulted in the low nitrogen

fractions of the plant samples (**Table S2**). To fix the effect of particle size on the microbial reactions, the powdered plant biomass samples were sieved between 0.35 mm (500 mesh) and 0.50 mm (355 mesh) and dried at 105 °C for 24 hours prior to the experiments. Since feeding the plant biomass to the reactors without dilution might result in an unwanted sodium inhibition for the methanogens during the experiments [18], each powdered plant biomass sample was mixed with tap water in such a way that the sodium concentration of the slurry was conditioned to be below 2.0 g-Na/L. The sodium concentrations in the fed-batch tests (below 2.0 g-Na/L) were much lower than the half-inhibition concentrations reviewed in the aforementioned literature (4.4–26.9 g-Na/L) [18], and was supposed to give a limited sodium inhibition in the experiments. From the conditioning, the total solid (TS), volatile solid (VS), and COD of the slurries for the influents were ranged in the concentrations of 21.4–28.4 g-TS/L, 13.6–18.9 g-VS/L, and 12.6–18.4 g-COD/L, respectively.

Using the anaerobic digestate collected from the municipal wastewater treatment plant (Hiagari, Kitakyushu, Japan) for the inoculum, the fed-batch tests were conducted for each plant species with four identical sets of the mesophilic jar-fermenters (working volume 1 L, inner diameter: 114 mm, working height: 100 mm, agitation speed: 200 rpm with a 60-mm turbine blade, 2 units of M-1000 (EYELA, Tokyo, Japan) and 2 units of MDL-1000 (BEM, Tokyo, Japan)). Each jar-fermenter was equipped with a water-sealed 250-mL glass vessel,  $\text{CO}_2$ -adsorbing caustic pellets of 250 mL (soda lime, Kishida Chemical, Tokyo, Japan), and  $\text{H}_2\text{S}$ -adsorbing ferric oxide pellets of 250 mL (Kishida Chemical) in series, followed by an automated precise gas meter (MGC-1 with Rigamo system, Ritter, Bochum, Germany) to monitor the methane gas production.

The fed-batch tests were performed in such a way that the slurry of each plant species was manually fed to the corresponding jar-fermenter for 4–6 days per week with a fixed hydraulic retention time of 30 days, and the corresponding volume of the digestate to the fed slurry was manually withdrawn from the jar-fermenters with a syringe. Using a high-speed centrifuge with 16,780 g for 15 minutes (CR22GII, Himac, Tokyo, Japan), the digestates were centrifuged, and its pellets were manually returned to each jar-fermenter. In this way, the particulate fraction of the digestate could be retained in the jar-fermenter, allowing a dynamic simulation for the fate of particulates. The solids of the jar-fermenters were not withdrawn except for the sampling events to measure VS concentrations of the system. The supernatant obtained from the centrifuge had negligibly low suspended

solid concentration (below 1 mg-SS/L) and was analysed for soluble COD, soluble lignin, and total soluble carbohydrate in every 10 days. During the operation for 300 days, the volumetric loading rate (VLR) was increased from 1.0 g-COD/L/d to about 2.2 g-COD/L/d in a stepwise manner. According to the preliminary analysis, the sodium present in the plant biomass powder was immediately dissolved in the liquid phase (within 1 hour). Therefore, the soluble salts were supposed to be correspondingly accumulated in the reactor in proportion to the COD fed.

### Chemical analysis for plant biomass constituents

Using the commercial fibre analysis instrument (Fibretherm FBS6, Gerhardt GmbH, Königswinter, Germany), the organics of plant biomass samples were chemically fractionated into (i) readily biodegradable carbohydrate (e.g., pectin), (ii) hemicellulose, (iii) cellulose, and (iv) lignin, according to the Van Soest method, which was the standard method of the Association of Official Analytical Collaboration International (AOAC) [19]. The protein fractions were also approximated from the Kjeldahl nitrogen of the plant biomass with a factor of 6.25 g-protein/g-N [20]. The lipid fractions of the plant biomass were measured using the automated Soxhlet extraction instrument (B-811, Büchi, Labortechnik AG, Flawil, Switzerland) with 0.5–2 g of dry samples. From the ash fractions of the plant biomass samples, phosphorus, sodium, potassium, magnesium, calcium, and sulphur were measured using ICP-atomic emission spectrophotometer (ICP-AES) (ICPE-9820, Shimadzu, Kyoto, Japan). Specifically, the ash samples were dissolved in the mixture of nitric acid and hydrochloric acid (water 20 mL + HNO<sub>3</sub> 2 mL + HCL 8 mL) for 1 hour on a hot plate at 200°C. Subsequently, its filtrate was further diluted with 1 + 10 HNO<sub>3</sub> solution for the ICP-AES analysis. The particulate and soluble COD concentrations were measured with a commercial COD analytical kit (TNT823, HACH, Düsseldorf, Germany), which was equivalent to the standard method of the US-APHA Standard Method (#5220 D) [21]. For measuring the COD of powdered plant biomass samples, the samples were weighed and carefully homogenised in deionised water to create a slurry suspension using an ultrasonic disrupter (UD-200, Tomy, Tokyo, Japan). Subsequently, the slurry suspension of 0.3 mL was placed in the COD analytical kit. The soluble total carbohydrate concentrations were analysed based on the phenol-sulphuric acid method [22] with a glucose standard (Kishida Chemical). The soluble lignin present in the digestate was measured by adopting the Lowry-Folin method [23] with an alkali-extracted lignin standard (Tokyo Chemi-

cal Industry, Tokyo, Japan), which had high detectivity for phenolic compounds as well as protein. The VS concentrations were measured according to #2540 of the US-APHA Standard Method [21].

### Kinetic analysis of methane fermentation

As described in the results and discussion section, since the organics other than carbohydrates were limited in the plant biomass samples, it was decided to simplify the model structure of ADM1 for analysing the BMP test results, and the modified ADM1 model for lignocellulosic compounds developed in the previous study [17] was adopted accordingly (Table S3). Specifically, the released soluble biodegradable compound ( $X_{CB}$ ) from the lignocellulosic compound (COD-based plant biomass,  $X_C$ ) at process #1 (enzymatic decomposition of lignocellulose) was assumed to be a composite of carbohydrates, proteins, and lipids. The composite compound was subsequently hydrolysed to a composite monomer substrate ( $S_B$ ) by the composite acidogenic microorganism ( $X_{APO}$ ) (process #2), which was the substrate for the composite acidogen. To express the production of unbiodegradable soluble lignin ( $S_{LG}$ ) associated with a release of inert carbohydrate particles coated by a lignin film ( $S_{CH\_U}$ ), the physical fragmentation of lignin was defined in process #3. Since only acetate ( $S_{AC}$ ) was detected in volatile fatty acids during the fed-batch tests, an acetate fermentation pathway (production of acetate and hydrogen ( $S_{H_2}$ )) was also assumed for the acidogenic reaction (process #4), in association with the corresponding growth of acetoclastic methanogen ( $X_{MPO\_AC}$ ) and hydrogenotrophic methanogen ( $X_{MPO\_H_2}$ ). As the fed-batch tests were to focus on the decomposition of the lignocellulosic compound governed by processes #1 and #3, the above simplification would not reduce the analytical quality. Based on this context, other less-affective parameters on processes #1 and #3 (the maximum specific growth rates, the half-saturation coefficients for growth of microorganisms (in processes #4–6), and the specific decay rates (in processes #7–9)), and the biochemical stoichiometries relating the processes #4–9 were all retained in those of the ADM1 model. The lignin fraction ( $f_1$ ) of the COD-based plant biomass ( $X_C$ ) was slightly varied depending on the plant biomass samples as tabulated in Table S4 (*A. retroflexus*:  $f_1 = 0.03$ – $0.04$ , *A. nitens*:  $f_1 = 0.04$ – $0.05$ , *B. scoparia*:  $f_1 = 0.05$ – $0.08$ , *K. caspia*:  $f_1 = 0.07$ – $0.09$ ). The production of  $S_{LG}$  in process #3 was assumed to be proportional to  $f_1$ , whilst 1% of carbohydrate present in  $X_C$  was assumed to be the source of  $S_{CH\_U}$  [17]. For instance, in the case of  $f_1 = 0.03$ , the plant biomass might ultimately yield a  $S_{CH\_U}$  of 3.97% ( $= 0.03 + (1 - 0.03) \cdot 1\%$ ), and

a  $S_{LG}$  of 3% on the basis of  $X_C$  in the methane fermentation process, respectively. The parameter estimation for the dynamic incubations was conducted using the maximum likelihood method equipped with the commercial process simulator, GPS-X ver. 8.5 (Hydromantis Environmental Software Solutions Inc., Hamilton, Canada).

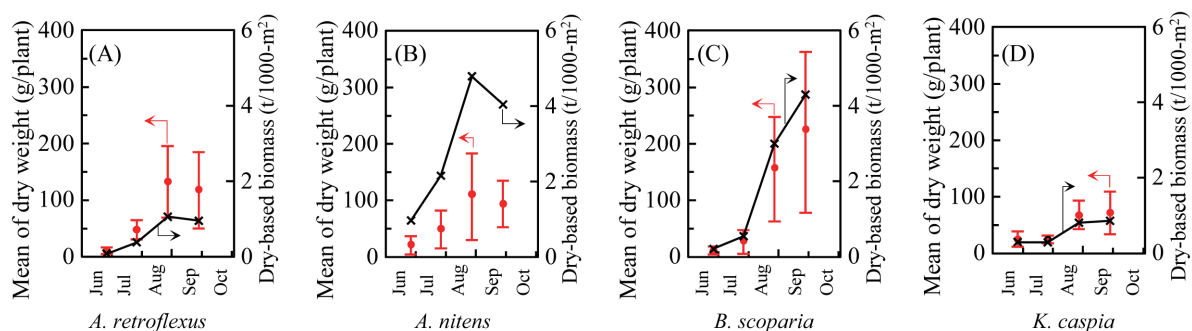
## RESULTS AND DISCUSSIONS

### Plant cultivation

Based on the dataset for the dry weight of plants in each month, the measured dry weights of the four plant species were linearly plotted on each linearised log-normal distribution (Fig. S2(A)–(D)). From the regression of the data plots, the mean dry weight and its standard deviation for each plant species were expressed along with the cultivation period. As shown in Fig. 2(A), the mean dry weight of *A. retroflexus* was calculated to be about 11 g-dry weight/plant in the initial phase (at the end of June), which peaked at the end of August and eventually reached about 133 g-dry weight/plant. At the end of September (crop maturation stage), the mean dry weight per plant decreased slightly (97 g-dry weight/plant). The range of dry weight covering 68.2% of the population ( $\log(\text{mean}) \pm \log(\text{standard deviation})$ ) at the end of August was between 69 and 195 g-dry weight/plant, indicating that the biomass of individual plants slightly varied. As calculated in the Y2-axis of Fig. 2(A), the cultivation yielded about 1.06 t of the dry-based plant biomass per 1,000 m<sup>2</sup> in the peak period. Because of the necessity to harvest edible crops with acceptable quality, dense cultivation of more than 8 plants/m<sup>2</sup> seemed not attractive for the plant species. Hence, the 1.06 t of the biomass harvest per 1,000 m<sup>2</sup> was thought to be practically the utmost, which was equivalent to about 1/5 of the energy crop cultivation using *E. arundinaceus* (about 5 t/1,000-m<sup>2</sup> on the basis of dry weight) [15,24].

As shown in Fig. 2(B), the biomass of *A. nitens* was also developed sharply until the end of August, and the mean dry weight peaked in the month at about 111 g-dry weight/plant. The range of dry weight covering 68.2% of the population for the plant species was somewhat wider than that of *A. retroflexus*. This might be due to the scattered seed qualities collected from the natural environment, unlike the commercial *Amaranthus* seeds. Because of the higher cultivation density of *A. nitens* (43 plants/m<sup>2</sup>) than *A. retroflexus* (8 plants/m<sup>2</sup>), the cultivation could attain a biomass production of 4.79 t/1,000-m<sup>2</sup> on the basis of dry weight. It should be noted that the biomass production was close to that of the aforementioned energy crop cultivation using *E. arundinaceus* [15,24]. This experimental result suggested that *A. nitens* could be a resource for alternative energy production in the Karakalpakstan region as well as the tool for the salt removal from the soil.

As shown in Fig. 2(C), the growth of *B. scoparia* was slightly behind *A. nitens* until the end of August. The plant growth peaked at the end of September, where its fruit maturation was recognised. In the period, the mean dry weight of the plant species reached about 226 g-dry weight/plant (mean  $\pm$  standard deviation = 79–363 g-dry weight/plant), which was about twice as much as *A. retroflexus* and *A. nitens*. However, due to the low cultivation density (19 plants/m<sup>2</sup>), the attainable biomass harvest of the plant species was limited to 4.30 t/1,000-m<sup>2</sup> on the basis of dry weight, and almost comparable to that of *A. nitens*. Therefore, an experimental investigation with high cultivation density of the plant species could be reserved in future studies for the two kinds of halophytes. As shown in Fig. 2(D), the biomass growth of *K. caspia* was found to be the lowest among the four examined plant species. The dry weight-based biomass was only about 72 g-dry weight/plant even in the peak period of the end of September (mean  $\pm$  standard deviation = 34–109 g-dry



**Fig. 2** Plant growth dynamics in terms of plant dry weight in the salinised farmland, Karabuga, Karakalpakstan, Uzbekistan ((A) *A. retroflexus*, (B) *A. nitens*, (C) *B. scoparia*, (D) *K. caspia*).

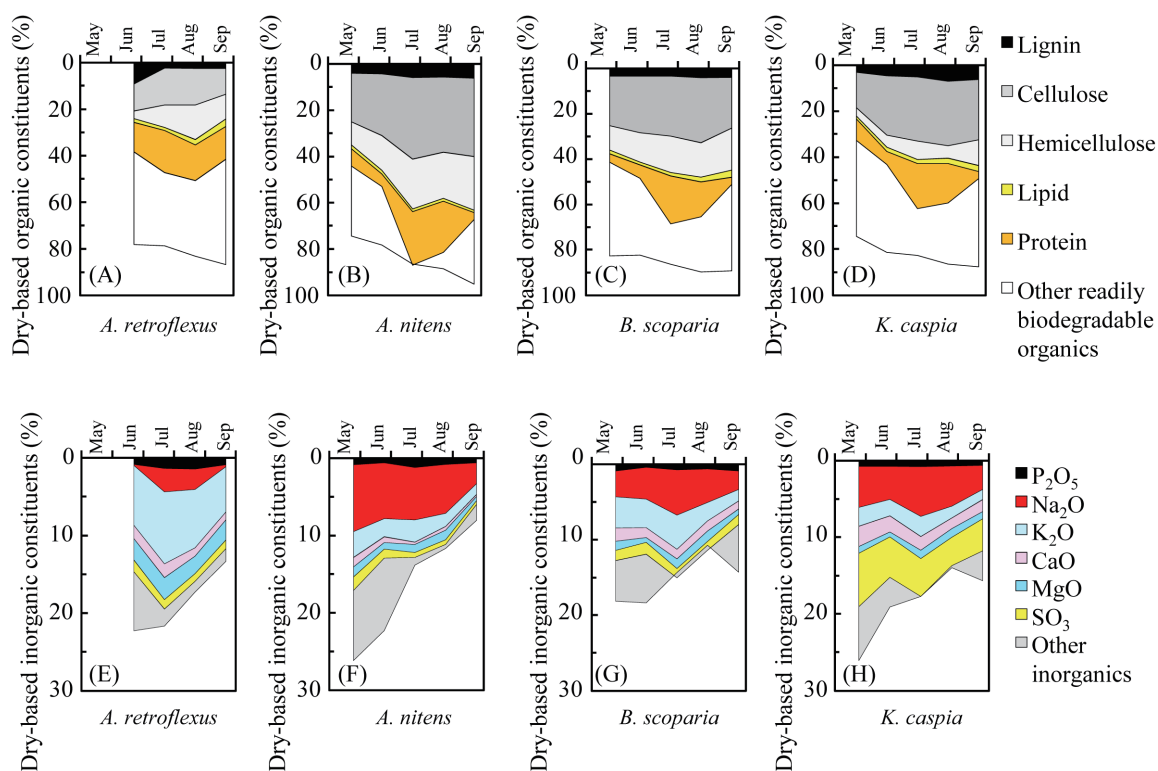
weight/plant) and about as low as 30–60% of the other plant species. Since the biomass of *K. caspia* yielded only 0.87 t/1,000 m<sup>2</sup> on the basis of dry weight, this plant species was thought not relevant for biomass production purposes even in more dense cultivation than that conducted in this study (19 plants/m<sup>2</sup>).

### Plant biomass constituents

As shown in **Fig. 3(A)–(D)**, the organic fractions of the plant biomass on the basis of dry weight were slightly dynamic during the cultivation period. Over the plant species, the dry weight-based organic fractions at the end of September were somewhat high compared to those in the initial phase (74–82% in May–June vs. 85–92% at the end of September). Overall, the lignin contents of the plant biomass accounted for about 2.4–9.0% of the dry matter, and no noticeable difference was recognised among the plant species and during the cultivation periods. In average, the cellulosic contents (cellulose + hemicellulose) of each plant species accounted for about 23% (*A. retroflexus*, **Fig. 3(A)**), 48% (*A. nitens*, **Fig. 3(B)**), 40% (*B. scoparia*, **Fig. 3(C)**), and 32% (*Ksarelina caspia*, **Fig. 3(D)**) of the dry matter, respectively. The low cellulosic content of *A. retroflexus* was attributed to the high leaf fraction compared to the other three plant

species. It seemed that the cellulosic contents were almost proportional to the organic fraction over the cultivation periods. This consistency might lead to a consistent specific enzymatic decomposition rate of the plant species in the methane fermentation. The lipid contents of the plant biomass were highly limited and accounted for only 1.0–5.0% of the dry matter. In contrast, the protein contents peaked at the end of July. In the peak period, the protein contents on the basis of dry weight were 15% for *A. retroflexus*, 23% for *A. nitens*, 21% for *B. scoparia*, and 20% for *K. caspia*, respectively. Since the protein contents of about 20% were close to that of alfalfa hay biomass (11.3–25.3%) [25,26], the plant biomass harvested in summer might be utilised for an alternative forage if the animals had a preference.

With respect to the inorganic fraction of the plant biomass, as shown in **Fig. 3(E)–(H)**, the highest sodium content (as Na<sub>2</sub>O on the basis of dry weight) was found in the plant biomass of *A. nitens* collected at the end of May (8.5%, **Fig. 3(F)**). In the period, *K. caspia* also had their highest sodium content over the cultivation periods (5.2%, **Fig. 3(H)**), whilst the sodium contents of *A. retroflexus* (3.0%, **Fig. 3(E)**) and *B. scoparia* (5.9%, **Fig. 3(G)**) peaked at the end of July. The sodium contents were all lowered towards the maturation stage (the end of summer). This dynamic re-



**Fig. 3** Fractionation of plant biomass ((A, E) *A. retroflexus*, (B, F) *A. nitens*, (C, G) *B. scoparia*, (D, H) *K. caspia*).

sponse suggested that the plant species might perform high evapotranspiration per biomass during the rapid growing stage (spring–early summer), leading to the high sodium accumulation in their biomass. Similar to the fate of sodium, the phosphate fractions of the plant biomass were slightly decreased along with time. The highest phosphate contents (as  $P_2O_5$  on the basis of dry weight) were found in the plant biomass samples collected in summer (from the end of June till the end of August) (*A. retroflexus*: 1.4%, *A. nitens*: 1.2%, *B. scoparia*: 0.72%, and *K. caspia*: 0.77%), whilst the  $P_2O_5$ -based phosphate was lowered to 70–80% of the peak at the end of September. High potassium was also detected in the plant biomass samples during the rapid growing stage, and was the highest at the end of May (*A. retroflexus*: 7.6%, *A. nitens*: 3.2%, *B. scoparia*: 4.0%, and *K. caspia*: 2.4% on the basis of  $K_2O$ ). Overall, *A. retroflexus* seemed to require more potassium to maintain their growth compared to the three halophyte species. At the end of September, the potassium contents were also lowered by about 50%. A small amount of calcium and magnesium were also present in the plant biomass samples with a range of 0.30–2.6% (as  $CaO$ ) and that of 0.60–2.7% (as  $MgO$ ), respectively. The samples of *K. caspia* collected in May and June had a relatively high sulphur fraction (5.2–6.8% as  $SO_3$ ), which was more than 4 times of the other three plant species (0.60–1.7% as  $SO_3$ ). This selective uptake of sulphate might be due to the inherent physiological nature of *K. caspia*. Nevertheless, since the anion accumulation was not the main topic of the study, further discussion was not conducted.

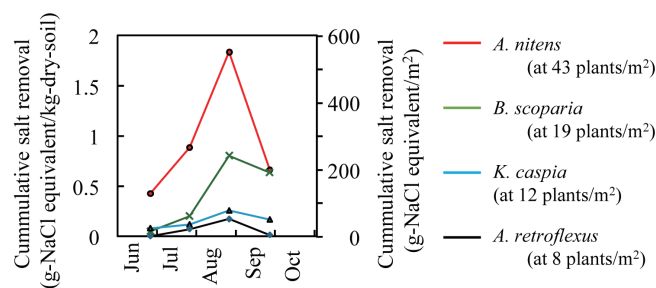
### Theoretical prediction for salt removal performance of halophytes

From the above experimental results, the salt removal performances of each plant species were theoretically calculated assuming the root working depth of 0.25 m [27,28] and adopting the apparent soil density of the experimental site (1,210 kg-dry soil/m<sup>3</sup>-land). As shown in Fig. 4, it appeared that *A. nitens* could remove the salt of 1.83 g-NaCl equivalent from 1 kg of dry-based soil per batch, when the plant biomass was collected from the salinised farmland at the end of August. *B. scoparia* was an alternative plant species if the plant biomass allowed a higher cultivation density than that conducted in this study. The salt removal performances of *K. caspia* and *A. retroflexus* were lower than 20% of that of *A. nitens*. Hence, these plant species were thought not relevant for the purpose of biological salt removal.

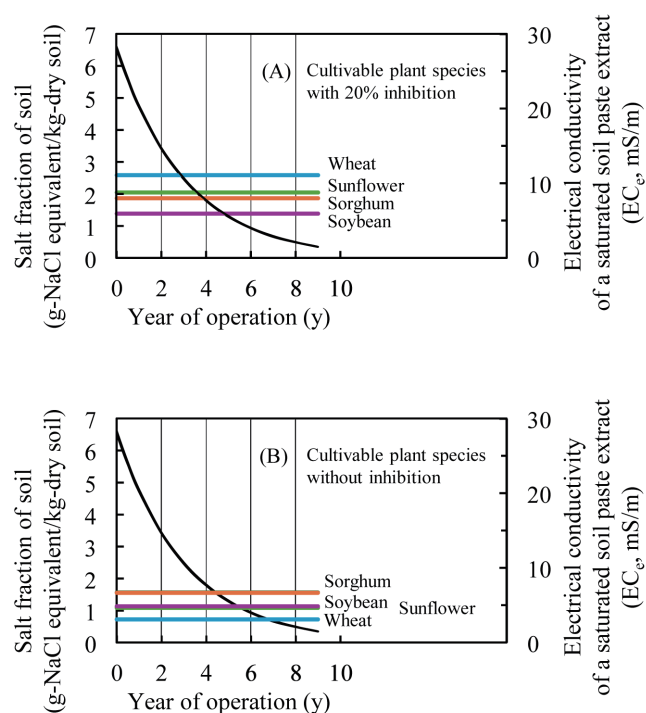
Next, assuming that the salt removal of the plant species was proportional to the salt fraction of the soil (a first-order

reaction) and no salt was physically transported to/from the land during the cultivation period, the salt fraction of soil at any time of  $t$ ,  $C_{salt(t)}$  might be expressed as  $C_{salt(t)} = C_{salt(0)} \exp(-kt)$ , where  $C_{salt(0)}$ : initial salt fraction of the soil = 6.6 g-NaCl equivalent/kg-dry soil),  $k$ : specific rate = 0.32 1/y ( $= -\ln(1 - 1.83/6.6)$ ). As shown in Fig. 5(A), the dynamic salt fraction of the soil was obtained by the integration of  $r_{salt}$  with time and the initial salt fraction of the soil. Furthermore, by adopting the threshold concept of plant growth in the salinised farmland [29], the examples of possible cultivable plant species were also listed in the graph, at which each plant species might yield their crops with a reduction of 20% from the environment without the salt inhibition [30]. The cultivation of wheat might be possible in the land after 3 years of the consecutive cultivation (consecutive phytoremediation) of *A. nitens*. After 4 years of the consecutive phytoremediation, more profitable crops (sunflower and sorghum) might be targeted in the cultivation. To realise the cultivation of soybean, the consecutive phytoremediation should be continued for more than 5–6 years. When the phytoremediation was continued for 4–7 years, the above agricultural plant species were expected to grow without the salt inhibition (Fig. 5(B)). To maximise the crop production after the phytoremediation, the cultivation of sorghum could be initiated first, followed by the cultivations of soybean, sunflower and wheat.

It should be noted that the true salinity of soil was basically governed by the operational soil humidity (water content) of the farmland as well as the salt fraction of soil. Therefore, the above forecast might be somewhat optimistic in the regions having frequent drought unless the soil humidity was properly maintained by the farmers. Nevertheless, this theoretical study encouraged to demonstrate the consecutive phytoremediation lasting for years in the experimental site.



**Fig. 4** Calculated performance of phytoremediation in terms of NaCl-based salt removal from the salinised farmland, Karabuga, Karakalpakstan, Uzbekistan (assumptions for Y1-axis: apparent soil density = 1,210 kg-dry soil/m<sup>3</sup>-land, root working depth = 0.25 m).



**Fig. 5** Expected phytoremediation for the salinised farmland, Karabuga, Karakalpakstan, Uzbekistan with a consecutive cultivation of *A. nitens* ((A): cultivable plant species with 20% growth inhibition, (B): cultivable plant species without growth inhibition)

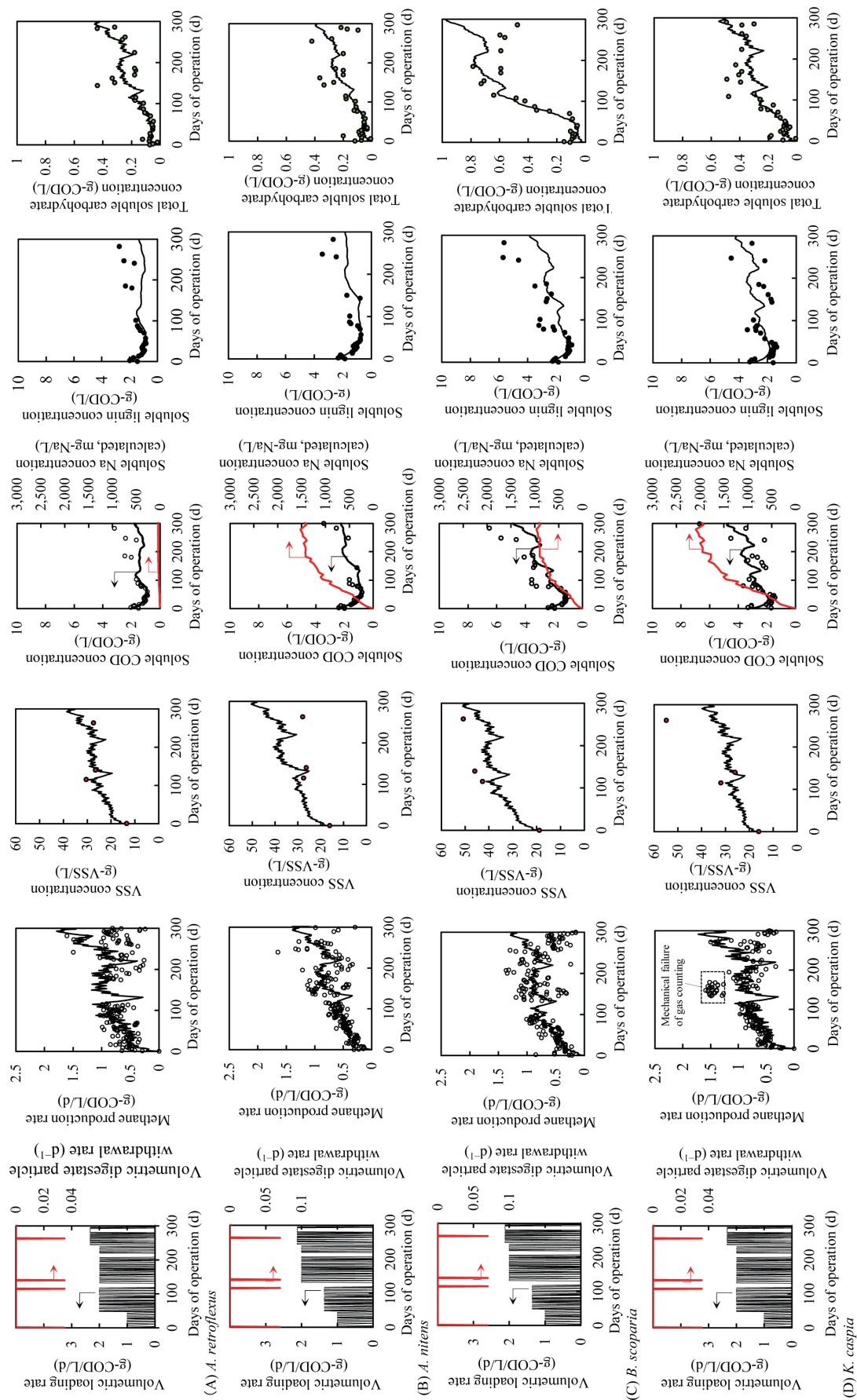
(Assumptions: specific salt uptake rate of *Atriplex nitens*: 0.32 1/y, cultivation density = 43 plant/m<sup>2</sup>, apparent soil density = 1,210 kg-dry soil/m<sup>3</sup>-land, root working depth = 0.25 m, cultivable plant species: adopted from [31] and [32]).

### Methane fermentation of plant biomass

For the system fed with *A. retroflexus* (**Fig. 6(A)**), about 0.7 g-COD/L/d of methane was generated in the VLR of 1.0 g-COD/L/d. The methane production approached about 1.5 g-COD/L/d in the later phase, where 2.2 g-COD/L/d of the biomass was loaded. During the experiment, a slight accumulation of VSS was recognised (from 15 g/L at day zero to 30 g/L at day 100), which was attributed to the increased VLR and the accumulation of the lignocellulosic compound in the reactor. The soluble COD of the digestate was ranged in 1.5–3.7 g-COD/L and mostly composed of soluble lignin. The soluble carbohydrate concentration was kept low throughout the experiment and ranged between 0.1 and 0.4 g-COD/L. Only a small amount of acetate (below 18 mg-COD/L) was detected in the volatile fatty acid species (data not shown). The data plots of methane production and the concentrations of soluble compounds were reasonably simulated using the

modified ADM1 model and the parameters tabulated in **Table 1**. In the model, the specific enzymatic decomposition rate of lignocellulose (process #1) was calculated to be 0.10 1/d with a 95%-confidence interval of  $3.7 \times 10^{-8}$ , which limited the methane production of the system. The production of soluble lignin was expressed with the elevating specific fragmentation rate of lignocellulose (process #3) where a maximal specific rate of  $0.00067 \text{ 1/d} \pm 5.5 \times 10^{-10}$  took place after about day 90. Compared to the anaerobic degradation of mature mung bean biomass (*Vigna Radiata*) [17], the specific rate of process #1 was about twice and that of process #3 was about 1/3, respectively. This suggested that the *A. retroflexus* biomass had a higher biodegradable nature with limited soluble lignin production than the mature mung bean biomass.

With respect to the anaerobic degradation of the *A. nitens* biomass, as shown in **Fig. 6(B)**, the methane production was slightly lower than that from the *A. retroflexus* biomass. The soluble COD concentration of the system was maintained between 1.0–3.9 g-COD/L during the experimental period, and almost comparable to the soluble lignin concentration. Similar to the experimental result using *A. retroflexus*, the accumulation of soluble carbohydrate was highly limited (below 0.4 g-COD/L). The specific enzymatic decomposition rate of the lignocellulose was calibrated to be  $0.047 \text{ 1/d} \pm 3.7 \times 10^{-8}$ , which was as low as 50% of the *A. retroflexus* biomass (**Table 1**), whilst the maximal specific fragmentation rate of the lignocellulose ( $0.00035 \text{ 1/d} \pm 5.3 \times 10^{-11}$ ) was about 1/2 of the *A. retroflexus* biomass. As shown in **Fig. 6(C)** and **Table 1**, it seemed that *B. scoparia* had a very similar kinetic value for the specific enzymatic decomposition rate of lignocellulose to that of *A. nitens* ( $0.047 \text{ 1/d} \pm 1.2 \times 10^{-8}$ ). This kinetic property indicated that the plant species could yield comparable methane production to that from *A. nitens*. On the other hand, due to the high specific fragmentation rate of lignocellulose ( $0.0013 \text{ 1/d} \pm 4.0 \times 10^{-10}$ ), the *B. scoparia* biomass produced a noticeable soluble lignin in the methane fermentation process. In the experiment, the highest soluble COD was seen among the examined four kinds of plant species. At the end of the experiment (day 300), the soluble COD reached about 6 g-COD/L, which was mostly composed of lignin. Similarly, the soluble carbohydrate concentration was elevated along with time and reached as high as more than twice of the experiments using *A. retroflexus* and *A. nitens*. The accumulation of soluble carbohydrate was explained with the release of unbiodegradable carbohydrate which was assumed to be a sub-micron particle coated by a thin-lignin layer. As shown in **Fig. 6(D)** and tabulated in **Table 1**, the



**Fig. 6** BMP experimental results and its dynamic simulations ((A) *A. retroflexus* L., (B) *A. nitens*, (C) *B. scoparia*, (D) *K. caspia*).

**Table 1** Kinetics for anaerobic degradation of plant biomass.

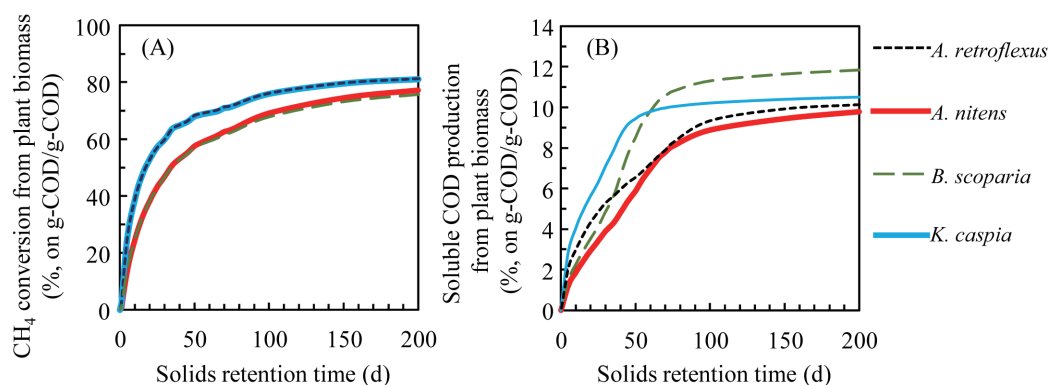
| Pro-<br>cess | Parameter                                                                        | Symbol                | <i>A. retroflexus</i>             | <i>A. nitens</i>                  | <i>B. scoparia</i>               | <i>K. caspia</i>                  |
|--------------|----------------------------------------------------------------------------------|-----------------------|-----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|
| #1           | Specific enzymatic decomposition rate of lignocellulose (1/d)                    | $k_C$                 | $0.10 \pm 3.7 \times 10^{-8}$     | $0.047 \pm 3.7 \times 10^{-8}$    | $0.047 \pm 1.2 \times 10^{-8}$   | $0.10 \pm 2.1 \times 10^{-8}$     |
| #2           | Specific hydrolysis rate of released biodegradable compounds (1/d)               | $k_H$                 |                                   |                                   | 10 <sup>♣</sup>                  |                                   |
| #3           | Specific fragmentation rate of lignocellulose (1/d)                              | $k_F$                 | $0.00067 \pm 5.5 \times 10^{-10}$ | $0.00035 \pm 5.3 \times 10^{-11}$ | $0.0013 \pm 4.0 \times 10^{-10}$ | $0.00095 \pm 2.4 \times 10^{-10}$ |
|              | Power coefficient for fragmentation (-) <sup>♠</sup>                             | n                     | 7                                 | 5                                 | 6                                | 7                                 |
|              | Half-saturation coefficient (d)                                                  | $K_T$                 | $89 \pm 4.1 \times 10^{-5}$       | $66 \pm 5.4 \times 10^{-5}$       | $55 \pm 1.4 \times 10^{-5}$      | $39 \pm 1.5 \times 10^{-5}$       |
| #4           | Maximum specific growth rate of acidogen (1/d)                                   | $\mu_{APO, \max}$     |                                   |                                   | 3.0 <sup>♣</sup>                 |                                   |
|              | Half-saturation coefficient of growth from monomer composite substrate (g-COD/L) | $K_{SB}$              |                                   |                                   | 0.5 <sup>♣</sup>                 |                                   |
|              | Maximum specific growth rate of acetoclastic methanogen (1/d)                    | $\mu_{MPO\_AC, \max}$ |                                   |                                   | 0.4 <sup>♣</sup>                 |                                   |
| #5           | Half-saturation coefficient of growth from acetate (g-COD/L)                     | $K_{AC}$              |                                   |                                   | 0.5 <sup>♣</sup>                 |                                   |
| #6           | Maximum specific growth rate of hydrogenotrophic methanogen (1/d)                | $\mu_{MPO\_AC, \max}$ |                                   |                                   | 2.1 <sup>♣</sup>                 |                                   |
|              | Half-saturation coefficient of growth from hydrogen (g-COD/L)                    | $K_{H2}$              |                                   |                                   | $7 \cdot 10^{-6}$ <sup>♣</sup>   |                                   |
| #7           | Decay of acidogen (1/d)                                                          | $b_{APO}$             |                                   |                                   | 0.02 <sup>♣</sup>                |                                   |
| #8           | Decay of acetoclastic methanogen (1/d)                                           | $b_{MPO\_AC}$         |                                   |                                   | 0.02 <sup>♣</sup>                |                                   |
| #9           | Decay of hydrogenotrophic methanogen (1/d)                                       | $b_{MPO\_H2}$         |                                   |                                   | 0.02 <sup>♣</sup>                |                                   |

♣: ADM1 default value, ♠: fixed in a try-error approach, ±: 95%-confidence interval

*K. caspia* biomass had a similar biological degradability to the *A. retroflexus* biomass. The calibrated specific enzymatic decomposition rate of lignocellulose was  $0.10 \text{ 1/d} \pm 2.1 \times 10^{-8}$  and almost identical to that of *A. retroflexus*. Both accumulations of soluble lignin and soluble COD were slightly higher than those measured in the experiments using *A. retroflexus* and *A. nitens*. The kinetic parameter value for the specific fragmentation rate of lignocellulose was  $0.00095 \text{ 1/d} \pm 2.4 \times 10^{-10}$ , which was closed to that of the *B. scoparia* biomass. As shown in Fig. S3, the dynamic simulations yielded the accumulation of microbial biomass in the reactors. Overall, the acidogen concentrations reached 5.9–6.7 g-COD/L in each jar fermenter on day 300, whilst the acetoclastic methanogen and hydrogenotrophic methanogen also accumulated to be 1.8–2.0 g-COD/L and 1.1–1.2 g-COD/L, respectively. When the elemental constituents of the microbial biomass were assumed to be  $C_5H_7O_2N$ , the microbial biomass accounted for 13–17% of VSS at day 300. Overall, the lignocellulosic compounds accounted for about 64–76% of the COD-based

particulates (*A. retroflexus*: lignin = 3.3%, carbohydrates = 60.3%; *A. nitens*: lignin = 3.6%, carbohydrates = 73.6%; *B. scoparia*: lignin = 3.1%, carbohydrates = 72.9%; *K. caspia*: lignin = 4.5%, carbohydrates = 59.6%) (simulation results not shown).

From the above-calibrated kinetic parameters, the performance of methane fermentation in a continuous stirred-tank reactor was theoretically calculated in a steady-state mode and compared among the four plant species. As shown in Fig. 7(A), it appeared that the methane conversion efficiency of the *A. retroflexus* biomass and that of *K. caspia* were almost identical to each other, whilst the *A. nitens* biomass yielded very similar methane production to that from the *B. scoparia* biomass. The high similarities of the methane conversions were due to the high similarities for the specific enzymatic decomposition rate of lignocellulose (process #1) (*A. retroflexus* and *K. caspia*: about 0.10 1/d; *A. nitens* and *B. scoparia*: about 0.047 1/d). Overall, both the *A. nitens* biomass and *B. scoparia* biomass yielded methane with a 60–70% of



**Fig. 7** Model-based process performance in a continuous stirred-tank reactor ((A): methane conversion efficiencies of plant biomass, (B): soluble COD production from plant biomass).

methane conversion efficiency when the systems were operated with SRT of 50–60 days. The methane conversion was slightly improved by about 10% when the SRT was extended to 100 days, and by 15% with SRT of 200 days, respectively. Since the simulations indicated that very long SRT operation gave a limited improvement of methane conversion, the 60–70% of methane conversion at a SRT of 50–60 days was supposed to be practically utmost over the four plant species. As shown in **Fig. 7(B)**, the soluble COD fractions of the digestates at SRT of 50–60 days were estimated to be about 6–10% on the basis of fed plant biomass COD. *B. scoparia* and *K. caspia* seemed to generate higher soluble COD compared to *A. retroflexus* and *A. nitens* (about 10% vs. 7%). These features were due to the specific fragmentation rate of lignocellulose (process #3) (0.00095–0.00126 1/d vs. 0.00035–0.00067 1/d). The soluble COD fraction ( $\approx$  soluble lignin) might reach 10–12% on the basis of plant biomass COD when the system was operated in a very long SRT of beyond 60 days.

For practical application of the methane fermentation to the post-treatment of the cultivated halophyte biomass, the collected plant biomass after the phytoremediation should be diluted with water (e.g., up to about 30 g-TS/L) prior to the methane fermentation to alleviate the possible sodium inhibition. For the reactor configuration, a simple continuously-stirred tank reactor (CSTR) and a CSTR equipped with a solid/liquid separation module having a solid returning stream to the reactor were supposed to be attractive. The latter might be able to reduce the reactor volume and maximise the attainable volumetric loading rate [17].

The calculated COD-based methane conversion efficiencies of the *A. nitens* biomass and the *B. scoparia* biomass at a very long SRT of 200 days (ca. 80%) were close to the

maximal BMP using the available energy crop of *E. arundinaceus* (65–74% on the basis of COD) [15]. At an SRT of 50–60 days, which was somewhat longer than conventional biogas plants receiving readily biodegradable organics [31], 60–70% of methane conversion was expected in each plant biomass, which was similar to those of the conventional biogas plants. Hence these plant species were thought to be utilised as an alternative fuel for the region during the phytoremediation of the salinised farmlands. For the *A. nitens* biomass, recalling that 4.79 t of dry-based biomass was harvested per 1,000 m<sup>2</sup> of land at the peak period of the end August, the recoverable methane from the system was expected to be about 800 m<sup>3</sup> ( $\approx$  29 GJ on LMV (lower heating value basis)) per 1,000 m<sup>2</sup> of land per batch. This energy production might compensate for the annual fuel gas consumption of about 10 population equivalents [32]. In case of starting the operation of the methane fermentation reactor within autumn, the biogas production might meet the high demand season of fuels in winter. Since usable routes of the biogas were supposed to be generally region-specific, the attractive routes of the alternative fuel (e.g., kitchen and supplementary fuel for central heating) should be explored based on the exiting regional infrastructure.

## CONCLUSIONS

Using the plant biomass samples collected from the salinised abandoned farmland at the Karabuga district in the Karakalpakstan region of Uzbekistan, the statistical analysis of the plant growth, the fractionation of the biomass constituents, and the methane fermentation tests were conducted, and the following results were obtained.

1. Among the examined plant species of *A. retroflexus*, *A.*

*nitens*, *B. scoparia*, and *K. caspia*, a cultivation of *A. nitens* was the most promising to remove the salt from the salinised farmland due to its high salt accumulation into their biomass.

2. In the cultivation density of 43 plants/m<sup>2</sup>, a consecutive cultivation of *A. nitens* lasting for 3–6 years might ameliorate the soil salinity to allow a growth of ordinal crops (e.g., wheat and sorghum).
3. For *A. nitens* and *B. scoparia*, the modified ADM1 model indicated that about 60–70% of the biomass COD could be decomposed to methane at an SRT of 50–60 days in the methane fermentation process. The biomass of *A. retroflexus* and *K. caspia* had a slightly higher digestibility than the plant species. The methane production efficiencies of the four plant species seemed to be close to that of the ordinary energy crop of *E. arundinaceus*.

## AUTHOR CONTRIBUTIONS STATEMENT

All authors have made substantial contributions to this work. Hidenari Yasui conceptualized and designed the study. Rutrawee Sangcharoen conducted data analysis, and drafted the manuscript. Tolibaev Erlan, Qurbanov Alisher and Jolibekov Berdiyev contributed to data collection. Thanawat Kitithammarong performed the experiments. Kristina Toderich provided critical revisions. Meng Sun assisted in the interpretation of results. Ryosuke Endo, Kazuki Sugawara and Mitsuharu Terashima and contributed to the final manuscript revision. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

## CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest related to this study.

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## SUPPLEMENTARY MATERIALS

Supplementary Materials file for this article is available at the link below.

[https://www.jstage.jst.go.jp/article/jwet/23/3/23\\_25-004/supplement/\\_download/23\\_25-004\\_1.pdf](https://www.jstage.jst.go.jp/article/jwet/23/3/23_25-004/supplement/_download/23_25-004_1.pdf)

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