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Research article

Energy recovery wastewater treatment plants through anaerobic digestion

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ABSTRACT

Anaerobic co-digestion (AcoD) helps improve the treatment of organic waste and the recovery of energy in wastewater treatment plants. The current work describes a complete assessment of the various kinetic modeling techniques and the effects of different feedstock compositions on the performance of AcoD based on extensive datasets and sophisticated computational modeling. Eighteen different biomethane potential (BMP) datasets were used to determine several key kinetic parameters, including first-order hydrolysis coefficients (k_{hyd}, d^{-1}; 0.08–0.70). The first-order kinetic model was shown to have overwhelmingly better predictive ability ($R^2 > 0.95$) and parameter identifiability with respect to the Monod-type models. The incorporation of the modified GISCOD framework with the inhibition function for long-chain fatty acids (LCFA) provided tools for highly accurate simulation of co-digestion dynamics and operational cost reduction of 10.2%. However, feedstock with protein content over 2.5 wt% resulted in significant ammonia inhibition (p-value<0.01) and a reduction of 18–22% of methanogenic activity. Multivariate sensitivity analysis showed protein and lipid fractions to be the predominant controls for process stability and methane yield. Quantitative descriptions were able to clarify the results.

KEYWORDS

Anaerobic co-digestion
Kinetic modeling
Wastewater treatment
Biogas production
GISCOD model

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1. Introduction

The problem of sustainable management of urban effluents is becoming increasingly urgent for modern societies, given the speed of urbanization and the increase in water usage. Wastewater treatment plants (WWTPs) not only protect the environment and public health but also provide considerable potential for recovering energy and resources [1].

Anaerobic digestion stands out as an effective treatment technology for the biological conversion of sewage sludge to biogas and the recovery of energy. During this process, sewage sludge undergoes the microbial breakdown of its organic constituents, generating methane and carbon dioxide in an oxygen-free environment. The practice of anaerobic digestion lessens the volume of excess sludge and produces renewable energy, which can be energy to WWTPs and other industries. Use of this technology decreases fossil fuel dependence, which helps achieve the sustainable development goals.

Anaerobic digestion has been studied worldwide as it recovers energy, which helps reduce energy use, cuts down excess sludge, and produces biogas [2]. According to studies, adjusting operational settings like temperature, carbon-to-nitrogen ratio (C/N), and some pretreatment processes boosts methane production significantly.

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Apart from generating energy, anaerobic digestion has other sustainability advantages like cutting down on GHGs, abating organic contaminants, and reducing odor problems. For example, anaerobic digestion case studies have proved in Danish WWTPs that it lowered operational costs and emissions of CO_2. In Iran, as Nasr et al. [3] have noted for instance, anaerobic digesters in Isfahan's WWTPs can generate over 30% of the energy required to operate these plants. However, the implementation of this system is still faced with numerous challenges, such as a lack of adequate funding, poor management, old equipment, and seasonal temperature changes [4]. Overcoming the barrier requires effective policy, financial incentives, and training. Isfahan (located at 32.65°N and 51.67°E) is one of the largest industrial cities in Iran. It faces the challenge of water scarcity and rapid population growth [5, 6]. These facts point to the urgent need for developing sustainable wastewater treatment technologies. This study seeks to analyze the benefits and challenges of implementing anaerobic digestion for energy recovery in Isfahan wastewater treatment plants. The study findings will, therefore, be used to develop policies and support initiatives for sustainable urban wastewater management.

2. Materials and Methods

2.1. Introduction to the study area: Isfahan Water and Wastewater Company

The Isfahan Water and Wastewater Company (IWWC) was established in 1965 after the Isfahan Water Organization, its predecessor, to provide water and wastewater services in Isfahan Province. In 1991, following a law passed by the Iranian Parliament that required the establishment of water and wastewater companies in the cities, the company changed its name to IWWC. Since then, IWWC has been establishing water and wastewater management units in various cities across the province [7].

By 2019, the company had implemented a plan to integrate urban and rural water and wastewater services, which expanded its service coverage to 100 cities and 948 villages within Isfahan Province. In the wastewater segment, the company's infrastructure manages approximately 733,933 sewer connections and has installed 718,116 sewer service connections, serving 2 million people in 37 cities and 5 villages.

IWWC has also focused on wastewater treatment and reuse, investing more than 37 trillion rials in this area, and encouraging the private sector to participate in similar projects. Moreover, the treated wastewater is directed to the heavy industries such as Mobarakeh and Esfahan Steel Companies, minimizing their use of fresh water and promoting sustainable water resource management. Additionally, the company has played a significant role in reducing environmental pollution in the Zayandeh Rud River basin through the construction and upgrading of several wastewater treatment plants [8].

Among its wastewater treatment plants, South Isfahan Wastewater Treatment Plant (South Isfahan WWTP) is one of the key treatment plants that employs a conventional activated sludge process with a design capacity of about 145,000 cubic meters per day. This facility is vital for the treatment of municipal wastewater from the southern parts of Isfahan city.

The South Isfahan WWTP is situated at 32°3600 N latitude and 51°4130 E longitude (approximately 32.60°N, 51.69°E) at an altitude of approximately 1,570 meters above sea level. The climate of the region is semi-arid, which is typical of the central parts of Iran.

IWWC's wastewater collection and treatment system, as well as its reuse programs, significantly contribute to this goal by improving the quality of water and reducing pollutant loads in the Zayandeh Rud River. Treated wastewater is reused in industrial and agricultural applications, promoting sustainability in this water-scarce region [9].

2.2. Benchmark simulation modeling platform

The benchmark simulation model (BSM) platform was developed to facilitate model-based comparisons of wastewater treatment plants (WWTPs) operational strategies, especially automated control systems. It allows for the comparison of different process setups and control strategies (e.g., dissolved oxygen control) that are otherwise difficult to compare due to differences in load and plant design [10].

The BSM platform comprises six main components:

- A standard WWTP layout with fixed tank volumes,
- Process models representing all treatment stages,
- Predefined influent flows and loads characterized by steady-state and dynamic simulations,
- Sensor and actuator models for realistic process monitoring and control implementation,
- A predefined simulation protocol, and
- An evaluation framework including the Effluent Quality Index (EQI), Operational Cost Index (OCI), and Risk Index.

BSM1 is based on a simple activated sludge unit, while BSM2 represents a full-scale wastewater treatment plant (WWTP). The study uses BSM2, which is a standard WWTP designed to meet effluent quality standards typical of modern WWTPs in developing countries.

The water treatment line starts with a 900 m^3 primary clarifier followed by an activated sludge system consisting of five reactors in series. Reactors 1 and 2 have volumes of 1,500 m^3 each and are not aerated, while reactors 3 to 5 have a combined volume of 9,000 m^3 and are aerated. Internal recirculation (5 → 1) of the system allows flexible feed and recirculation flows. Air and carbon source can be added to all reactors. The 6,000 m^3 secondary clarifier has a surface area of 1,500 m^2 [11].

In the sludge treatment line, waste activated sludge is combined with primary sludge and fed into an anaerobic digester with a liquid volume of 3,400 m^3. The digested sludge is dewatered, and the supernatant is recycled to the water line through a storage tank. The sludge line also includes a cogeneration unit that produces electricity and heat from the biogas generated; the heat is used exclusively to maintain the temperature of the digester.

The initial layout of the plant was designed based on an empirical mass balance model by Otterpohl and Freund [12]. The activated sludge process was simulated using the activated sludge model No. 1 (ASM1), while the secondary clarifier was modeled using a one-dimensional, 10-layer model developed by Takács et al. [13]. The anaerobic digestion process was represented by the anaerobic digestion model No. 1 (ADM1), and dewatering units were represented by ideal separation.

According to the BSM2 protocol, the plant was simulated for 609 days with a dynamic influent that varied every minute to reflect daily and weekend patterns as well as long-term changes due to temperature and holidays. The first 245 days were utilized for plant stabilization and control setting adjustments, while the remaining 364 days were used for performance evaluation.

The evaluation framework will use the Effluent Quality Index (EQI) that combines weighted averages of chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammonia, nitrate, and total suspended solids (TSS). The Operational Cost Index (OCI) is a relative measure of operational costs, which mainly considers energy requirements for mixing, aeration, pumping, and carbon addition [14].

The BSM platform is continuously updated to support performance and control assessments in new areas. For all modeling purposes in this study, Matlab/Simulink software (MATLAB versions 7.1 to 8.4, The MathWorks, Inc) was used to analyze data collected from 150 respondents [15].

2.3. Modeling of anaerobic co-digestion

This study focuses on the energy recovery in wastewater treatment plants (WWTPs) through anaerobic digestion. Anaerobic co-digestion (AcoD) refers to the simultaneous digestion of sewage sludge and other organic substrates. Integration of AcoD into modeling tools is necessary for design and optimization. This section will focus on the application of anaerobic co-digestion in the benchmark simulation model No. 2 (BSM2). This includes the estimation of substrate-dependent parameters and the characterization of substrates using model state variables.

2.4. Energy recovery through anaerobic digestion

Improving the energy balance in WWTPs is not only a matter of optimizing energy-intensive processes but also of maximizing energy recovery from the influent wastewater. The energy contained in the wastewater can be classified into three types: thermal, organic, and inorganic. Among them, the energy recovery from internal sludges (primary and waste-activated) through anaerobic digestion (AD) is the most feasible and commonly employed [16].

Numerous studies have demonstrated that the biogas produced in wastewater treatment plants (WWTPs) is rich in biomethane and can be used to power the plants, thus achieving energy neutrality. Therefore, there is a need to enhance the digestion process to achieve maximum biogas generation.

When the digester capacity allows, gas production can be enhanced by adding external or internal organic substrates (co-substrates) directly into the digester. During AD, the organic matter is partially degraded to biogas, while the nutrients are accumulated and returned to the system in the AD supernatant. Typically, the internal nutrient load accounts for 10–20% of the total nitrogen load to a WWTP. In plants, the lack of chemical precipitation, e.g., in the case of biological treatment (bio-P plants), the organic portion of phosphorus is mineralized, and the total amount of phosphorus is recycled.

According to Vahlberg et al. [17], a successful anaerobic co-digestion process requires the selection of appropriate substrates and effective feeding strategies. Mata-Alvarez et al provide a comprehensive review of substrate characteristics and applications. Ideally, the co-substrates should have a high potential for methane generation, low tendency to increase the volume of the resultant solids, and a nutrient composition that is compatible with that of the host WWTP. Generally, they differ from WWTP sludge in the way they decompose and the resultant biogas they generate. Many co-substrates can be used, but the choice is limited to those locally available because transportation costs are prohibitive [18].

2.5. Optimization of digestion in WWTPs

Many wastewater treatment plants' anaerobic digestion facilities operate below capacity. Typically, the organic loading rate (OLR) is much lower than feasible, and the resultant dilute sludge feed occupies excessive reactor volume. However, the digester stability and efficiency can be improved through operational optimization based on the measures that have been proven to work, such as sludge thickening, better mixing, and stable heating. In most cases, these strategies lead to an increase in hydraulic retention time (HRT) in the reactor [19].

When HRT exceeds approximately 25 days, as is common in mesophilic AD, the digester is said to have excess capacity. Low OLR and excess volume facilities can take advantage of this by implementing anaerobic co-digestion. Co-digestion means that co-substrates are digested together with the primary sludge. Co-substrate sources may be internal or external. Internal sources include the fats and oils that accumulate in the grease traps [20].

2.6. Modeling digestion in BSM2

In the BSM2 simulation model, the anaerobic digester is represented as a continuously stirred tank reactor (CSTR) with a total volume of 3,500 m^3 (3,400 m^3 liquid volume). The system is designed to operate at a hydraulic retention time (HRT) of 19 days. Temperature compensation of model parameters was done as described by Batstone et al. [21], which allowed the digester model to run at any temperature within the mesophilic range without recalculating the kinetic parameters. The default temperature is 35 degrees Celsius, but the user can change it to model thermophilic digestion.

The original anaerobic digestion model No. 1 (ADM1) considers particulate substrates as composite particulate matter (X_c). Disintegration is the first process, and it models the breakdown of the cell components into carbohydrates (X_{ch}), proteins (X_{pr}), and lipids (X_{li}). The third step considers the degradation of complex molecules outside the cell, which is not biological. Disintegration follows first-order kinetics and results in the formation of three fractions of X_c (approximately 30% each) and 10% of inert material (X_I).

Hydrolysis converts X_{ch}, X_{pr}, and X_{li} into their soluble counterparts: monosaccharides (Ssu), amino acids (Saa), and long-chain fatty acids (LCFA, Sfa), respectively. This process is also described by first-order kinetics.

This also means that, when modeling the conversion of particulate substrates into soluble products, their stoichiometric composition in terms of X_{ch}, X_{pr}, X_{li}, and X_I is assumed to be the same. Because the two steps occur in sequence, the slower step controls the overall rate of protein degradation [22].

2.7. Substrate characterization

Accurate substrate characterization is fundamental to the success of modeling. Kinetic parameters dependent on the substrate, as well as organic and nitrogen content must be found and converted into the 26 ADM1 state variables. Ever since the release of ADM1 in 2002, various fractionation techniques have been proposed for total nitrogen (TN) and chemical oxygen demand (COD) in substrates. Though certain methods yield full characterization of feed, their complexity and reliance on sophisticated analysis make them unsuitable to be employed in the majority of AD studies [23].

A recent overview highlights that input characterization remains a major issue, especially for mixed digesters. Simplicity, transparency, economy, and required precision determine the practicability of the characterization methods. In the current contribution, a realistic but precise method for characterization of the substrate is proposed. The input model is constructed in two stages to allow anaerobic co-digestion modeling in BSM2 and at the WWTP scale:

- Estimation of the biodegradable fraction of COD, which is strongly correlated to the maximum biomethane potential (B_0), and substrate-specific model parameters such as hydrolysis rates for suspended solids.
- Separation of TN and COD into ADM1 state variable categories in accordance with the approximated COD and basic physicochemical substrate data.

3. Results and discussion

3.1. Estimation of substrate-dependent parameters in anaerobic co-digestion modeling

This paper evaluated and compared three different models/methods used to estimate the highest biomethane potential (B_0, ml CH_4/g volatile solids) and hydrolysis rate coefficients (k_{hyd}, d^{-1}).

A common approach is to estimate hydrolysis rates from continuous digester tests or biomethane potential (BMP) tests. In BMP tests, substrate is hydrolyzed with excess inoculum present, and the volume of biogas and biomethane is measured over time. The cumulative biogas vs. time curve is fitted to equations to estimate B_0, estimated biodegradable portion (f_d as biodegradable fraction), and k_{hyd} by simultaneous optimization routines (Angelidaki and Sanders [24]). The first-order kinetic function is expressed as follows:

$$B(t) = B_0(1 - e^{-k_{hyd}.t}) \tag{1}$$

This model was compared with a Monod-type function proposed by Nghiem et al. [25]:

$$B(t) = 1 + k_{hyd}.tF_0.G.k_{hyd}.t \tag{2}$$

where F_0.G represents the degradable particulate matter at the start of the experiment, converted to methane volume (ml CH_4/g volatile solids). This value can be interpreted as the ultimate methane yield of the substrate, but differs numerically from B_0 in the first-order model. As can be observed in Fig. 1, the two models have contrasting dynamics. The first-order model goes asymptotically to the maximum gas production, and the hydrolysis rate coefficient is the same as the time constant—i.e., the time taken for 63% of B_0. The Monod-type function, however, increases steadily and never plateaus within the time frame of the BMP test; $1/k_{hyd}^{-1}$ is the half-saturation time, or the time taken to get to 50% of F_0.G. The application of ADM1 in BSM2 mostly follows the initial model description by Batstone et al. [21], with one major exception of particulate substrate degradation. An interface should be applied to convert ASM1 state variables into ADM1 form, as detailed by Nopens et al. [26]. In this model, all of the feed chemical oxygen demand (COD) is instantaneously divided between X_{ch}, X_{pr}, X_{li}, and inert particulate material (X_I), without passage through the composite particulate material (X_c) compartment. Only the dead biomass has disintegration held in place.

However, because the rate of disintegration in ADM1 is constrained by default parameters, hydrolysis rate coefficients must be reduced in order to model realistic degradation kinetics. This reduction is not represented in the default BSM2 parameters, and this generates an unrealistically efficient digestion process that overestimates digester capacity and biogas production. Hydrolysis rate coefficients were estimated in the current research from numerous continuous experiments of various sludge types and substrates. It also provides a recommendation to modify the default hydrolysis rate coefficient for mixed sludge.

Limited research has been conducted where anaerobic co-digestion (AcoD) has been included in WWTP models. Flexibility in simulating common substrates and feeding strategies, the ability to evaluate energy recovery and plant-wide effects, and compatibility with operation descriptions are some of the most important characteristics of such models.

3.2. Parameter optimization and model evaluation

The optimization routine defined optimal values for the rate of hydrolysis coefficient (k_{hyd}) and maximum biomethane potential (B_0) or fraction of degradable substrate (F_0.G) by minimizing a pre-defined cost function. In the original studies, each model was linearized to simplify the computational process so that optimization linear programming components could be used. However, linearization often increased errors in the lower part of the dataset, which could negatively affect the parameter values.

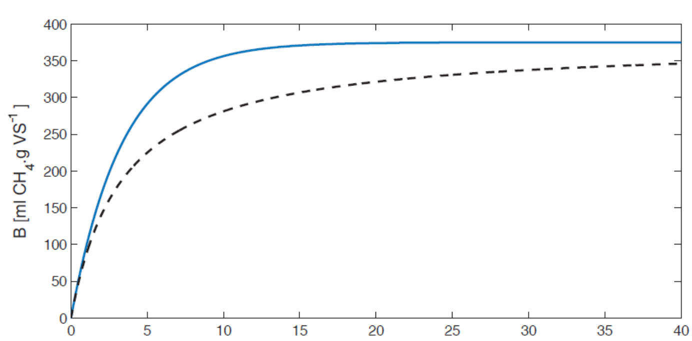

Fig. 1. Examples of model outputs for the first-order function in Eq. 1 (blue line) and the Monod-type function in Eq. 2 (black dashed line).

In the study presented here, the optimization was performed in MATLAB using the fminsearch function, which uses the Nelder-Mead simplex algorithm. The optimization requires a cost function that estimates the error between the output of the model f(x) and the observed data points y (for n data points). The most commonly used cost function is the sum of squared errors (SSE), defined as:

$$SSE = \sum_{i=1}^{n} \left(y(i) - f(x(i)) \right)^2 \tag{3}$$

Cost functions have also been examined. For example, Nghiem et al. [25] chose the sum of absolute errors (SAE) because the methodology is better at handling outliers, which can diminish SSE estimates due to squaring. Outliers in particular pose a considerable challenge in the linearized Monod-type model in the lower data range, as the reciprocal terms (t^{-1} and B_0^{-1}) magnify small values. However, the optimization in SAE may provide more unstable estimates of parameters relative to the SSE. As a result, the two models were qualified using both SSE and SAE cost functions and their results compared.

Uncertainty analysis was conducted using a frequentist approach based on maximum likelihood estimation, where the uncertainty analysis provided covariance matrices of the estimates and confidence intervals to improve the robustness of the estimated parameters.

In order to validate the models, datasets from 18 different biomethane potential (BMP) tests conducted at the Isfahan WWTP were examined. Types of substrate analyzed included secondary sludge, a mixture of sludges, food waste, plant waste, and fats, oils, and grease (FOG). The estimated parameters are shown in Fig. 2. The hydrolysis rate coefficient (k_{hyd}) estimated through the first-order kinetic model (Eq. 1) ranged from 0.08 d^{-1} to 0.70 d^{-1}, and values for B_0 were from 315 to 1010 ml CH_4/g volatile solids, which agreed well with values reported in the literature [27].

Due to this detailed comparison of both kinetic models using SSE and SAE cost functions, as well as incorporating full uncertainty analysis, the first-order kinetic model with SSE outperformed the Monod-type model and SAE in almost all instances across the datasets.

The maximum biomethane potential (B_0) and hydrolysis rate coefficient were also estimated using biomethane potential (BMP) test data modeled using the complete anaerobic digestion model No. 1 (ADM1). Using the complete ADM1 for parameter estimation provides a more realistic representation of the dynamic responses exhibited in BMP tests, particularly when the model is used in subsequent simulations.

One of the key advantages of using ADM1 is its ability to model inhibitory effects that some substrates provide during single-substrate BMP tests. In this instance, a novel inhibition function (I_{fa}) was modeled to represent the inhibition of acetate uptake (S_{ac}) by long-chain fatty acids (LCFA, S_{fa}), which are known to accumulate during lipid degradation and adversely affect digestion performance. The inhibition function is defined as follows:

$$I_{fa} = \begin{cases} e^{-2.77259 (K_{I,fa,high} - K_{I,fa,low} (S_{fa} - K_{I,fa,low})^2} & \text{for } S_{fa} > K_{I,fa,low} \\ 1 & \text{for } S_{fa} \le K_{I,fa,low} \end{cases} \tag{4}$$

where $K_{I,fa,low}$ and $K_{I,fa,high}$ are inhibition constants representing low and high concentration thresholds of LCFA, respectively. These terms must be derived specific to lipid-rich substrates to describe inhibition dynamics accurately.

Parameter estimation was done in MATLAB with the function that implements the Levenberg-Marquardt optimization algorithm. The estimation was performed on three different types of slaughterhouse waste substrates with unique compositions: rumen sludge (high carbohydrate), blood (protein), and dissolved air flotation (DAF) sludge (fat).

Table 1 contains a summary of the estimated parameters. The updated treatment of LCFA inhibition allowed the ADM1 model to represent the key inhibitory effects of LCFA on the digestion. This more mechanistic representation supports the use of a more detailed ADM1 model for parameter estimations when dealing with lipid-rich substrates.

3.3. Substrate fractionation

At the first step of substrate characterization—which includes estimating kinetic parameters, chemical oxygen demand (COD), and total nitrogen (TN)—the substrate must be fractionated into the state variables defined in ADM1. In order to identify the most impactful state variables, a sensitivity analysis was performed, and only selected output parameters that were relevant (i.e., biogas production, volatile solids degradation, and digestate composition) were summarized. The

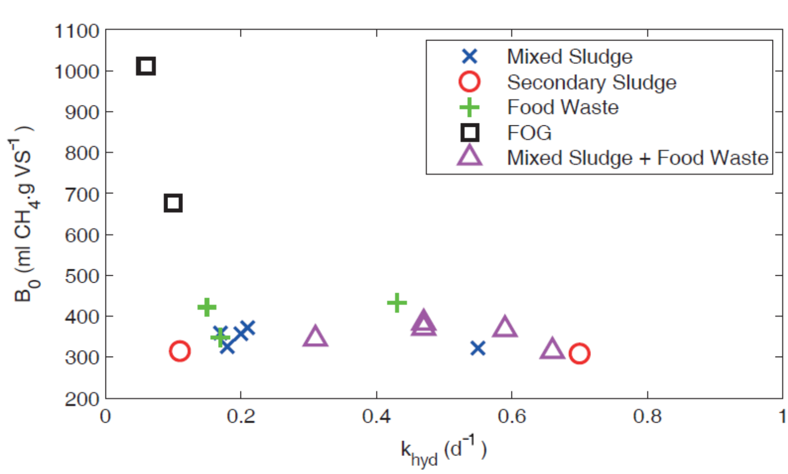

Fig. 2. Output of B_0 and k_{hyd} for 18 datasets using the first-order function model (Eq. 1).

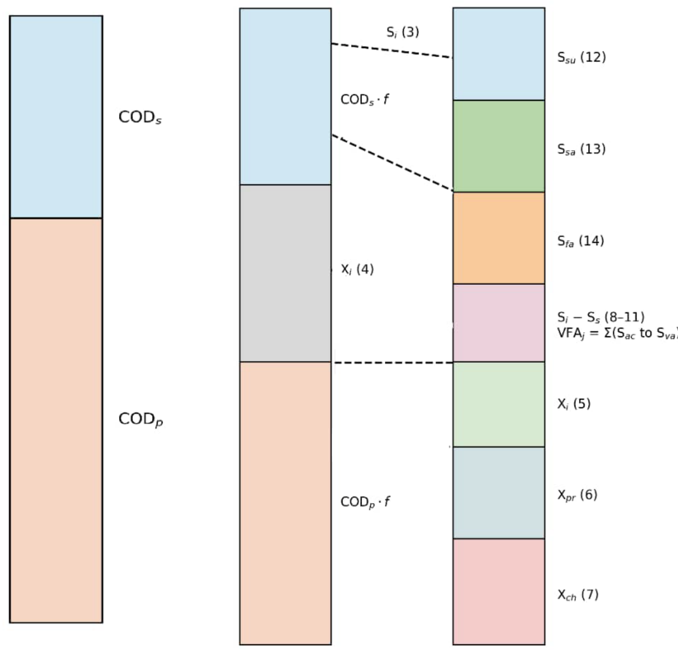

Fig. 3. Proposed COD fractionation scheme. Inspired by Ref. [28].

fractionation step is presented in a schematic form in Fig. 3. The particulate COD and soluble COD fractions are calculated first based on filtered COD and the estimated biodegradable portion (f_d). In relation to the biodegradable particulate COD, proteins and lipids can be measured and converted from their respective analyzed concentrations into COD equivalents, and then, assigned to X_{pr} and X_{li}, respectively. The remaining biodegradable particulate COD fraction can then be attributed to the carbohydrates (X_{ch}). The approach we took was similar to that proposed by others as both proteins and lipids are likely to be analyzed with a higher degree of accuracy in solid substrates, and the protein and lipid fractions are able to provide sufficient degrees of freedom for mass closure.

Concerning state variables of the soluble type, volumetric fatty acids (VFA) can be measured directly. Since soluble COD typically represents a small proportion of total COD (usually less than 10%), the soluble state variables - monosaccharides (Ssu), amino acids (Saa), and long-chain fatty acids (Sfa) are partitioned similarly to their particulate state variables.

3.4. Data requirements for substrate fractionation

The recommended substrate fractionation methodology entails comprehensive and complete data on various physicochemical parameters to adequately characterize model input. The most fundamental parameters include total solids (TS), volatile solids (VS), total chemical oxygen demand (COD), filtered COD, volatile fatty acids (VFAs; acetate, propionate, butyrate, and valerate), proteins, lipids, total ammonia nitrogen (TAN), and biomethane potential (BMP) testing results. The fractionation method was applied to three types of slaughterhouse waste substrates (rumen sludge, blood, and dissolved air flotation (DAF) sludge), which varied biochemically from each other. Simulations based on BMP tests suggested that the fractionation method was satisfactory and also characterized the expected composition of the substrate, with a prediction error less than the usually acceptable range. These results reaffirmed the consistency and reliability of the fractionation method.

3.5. Sensitivity analysis

Earlier research (Solon et al. [29]; Galí et al. [30]) determined that not all 26 ADM1 state variables are equally important to the model output variables. In order to identify the state variables that are most pertinent to the objectives of this research, a sensitivity analysis was completed.

The Monte Carlo simulations of the anaerobic digestion (AD) block of the BSM2 framework incorporated a broad range of feed compositions when conducting the sensitivity analysis. The model output variables identified for review were: total gas flow rate (Q_{gas}), methane flow rate, soluble inorganic nitrogen (SIN), pH, and volatile fatty acids (VFAs). These parameters are representative of some of the most important indicators for examining anaerobic co-digestion (AcoD) performance from the wastewater treatment plant perspective.

Table 1. Estimated substrate-dependent parameters for anaerobic co-digestion modeling of three types of slaughterhouse waste.

Substrate / Parameter	Paunch	Blood	DAF
B_0 (ml CH_4/g volatile solids)	299	520	1044
k_{hyd} (d^{-1})	0.125	0.310	0.103
$K_{I,fa,low}$ (kg $COD.m^{-3}$)	–	–	0.406
$K_{I,fa,high}$ (kg $COD.m^{-3}$)	–	–	0.714

The Monte Carlo simulations were carried out in accordance with the Batstone [31] methodology, generating 3,000 influent profiles varying in feed composition. The baseline sludge's COD loading of BSM2 at 2.4 kg.m^{-3}.d^{-1} received an additional 8.4 kg.m^{-3}.d^{-1} of co-substrate. Co-substrate distributions were produced under differing fractions of biodegradable particulate COD components—namely carbohydrates (f_{ch}), proteins (f_{pr}) and lipids (f_{li})—using inverse normalised random sampling. The distributions of these fractions are shown in Fig. 4.

3.6. Simulation results and principal component analysis (PCA)

The simulations were carried out for a duration of 130 days with constant influent conditions, simulating a steady-state scenario without the feedback from the wastewater treatment plant (WWTP). Simulation results were interpreted using principal component analysis (PCA) as depicted in Fig. 5.

The first two principal components explained 71.5% (component 1) and 25.2% (component 2) of the total variance in simulation results. Component 1 was positively correlated with biogas and methane flow rates and negatively correlated with volatile fatty acids (VFAs).

Conversely, component 2 was primarily influenced by the concentration of soluble inorganic nitrogen in the digestate.

Based on the PCA results (Fig. 5), three distinct regions of substrate compositions were identified:

- **Region I:** The substrate composition with positive results in component 1 and negative results in component 2 indicates well-working digesters with low VFA levels and higher methane production.
- **Region II:** With an increased protein content (X_{pr}), substrate compositions are transitioning from region I toward region II, which showed negative results in component 1 paired with positive results in component 2, located in the upper-left quadrant. This zone is associated with digester stress or complete failure due to ammonia inhibition (e.g., increased VFAs with less methane).
- **Region III:** Samples that score negative in both component 1 and component 2 describe high lipid content (X_{li}), suggesting digester failure due to long-chain fatty acid (LCFA) inhibition. When in this state, methane production has completely stopped, there are significantly high levels of VFAs, while overall pH has dropped, leading to process inhibition. The transition from region I to region III is rapid due to the threshold nature of LCFA inhibition, particularly if 58% of the co-substrate COD load (8.4 kg.m^{-3}.d^{-1}) is lipids (X_{li}).

3.7. Key findings from sensitivity analysis and model parameters

The analysis suggests that the two main input parameters that affect digester stability during anaerobic co-digestion, in addition to the biodegradable fraction, are the fractions of proteins (X_{pr}) and lipids (X_{li}). It is these components of the substrate that are important for the performance and stability of the process. The data shown in Fig. 7 indicates that carbohydrates (X_{ch}) are the main contributor to total biogas generation: as a greater fraction of X_{ch}, more carbon dioxide (CO_2) is produced, consistent with findings from Solon et al. [29].

Additionally, Figs. 6 & 7 show that high carbohydrate loads can also induce inhibition based on the pH caused by the accumulation of volatile fatty acids (VFAs): a consequence of this acidification is a reduction in digester performance.

Moreover, Fig. 6 demonstrates that the estimated parameter values representing inhibition for long-chain fatty acids (LCFA) (see Table 1) introduce a unique on-off behavior of the LCFA inhibition function (I_{fa}). In each of the simulation scenarios, Ifa assumes values of 1 (no inhibition) or 0 (complete inhibition) most of the time. This data provides insight into the total separation of region III (Fig. 7) (largely LCFA-induced failures) from regions I and II.

3.8. Implementation of anaerobic co-digestion in BSM2

Anaerobic co-digestion (AcoD) not only increases biomethane yield, but also affects several relevant features of the digestion process, such as biogas proportions, digester stability, biosolid generation, and

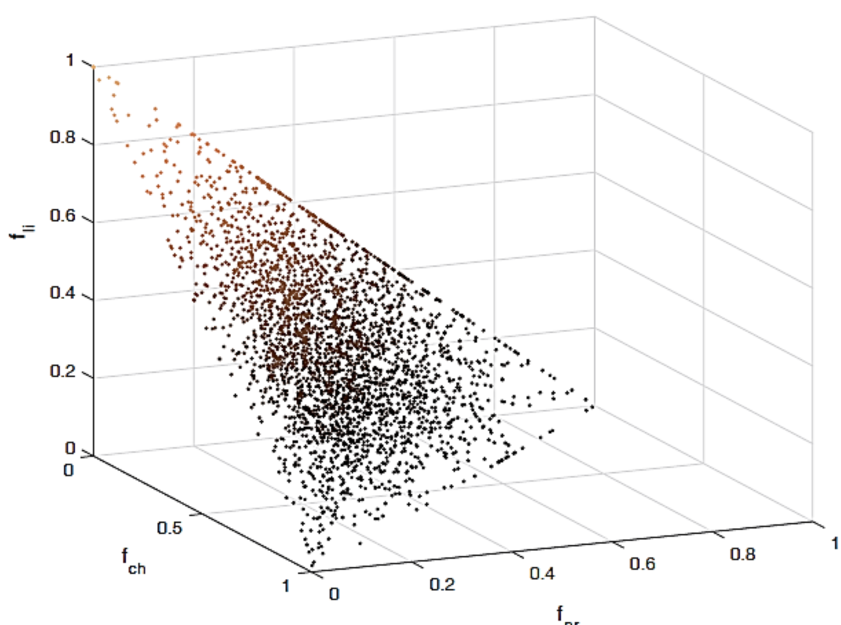

Fig. 4. Combination of f_{ch}, f_{pr}, and f_{li} for 3,000 samples used in Monte Carlo simulations for sensitivity analysis.

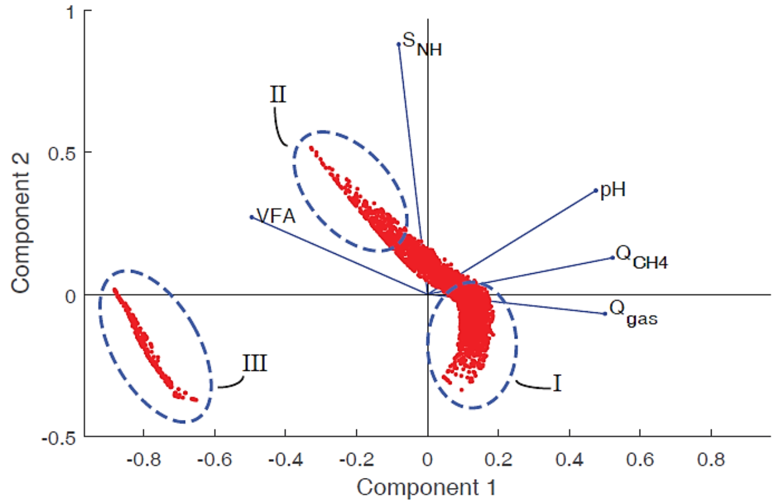

Fig. 5. Principal component analysis for Monte Carlo simulations with varying feed compositions in anaerobic digestion.

characteristics of the anaerobic digestion (AD) effluent. These effects can be favourable, unfavourable, or a mixture of the two, all of which affect the overall outcome of wastewater treatment plants (WWTPs). Thus, simulative approaches with elaborate and mechanistic models are valuable tools to assess the selection of co-substrate, feeding methods and their combined effects on WWTP operation and performance.

In the current study, implementation of AcoD within the benchmark simulation model No. 2 (BSM2) was accomplished under the GISCOD

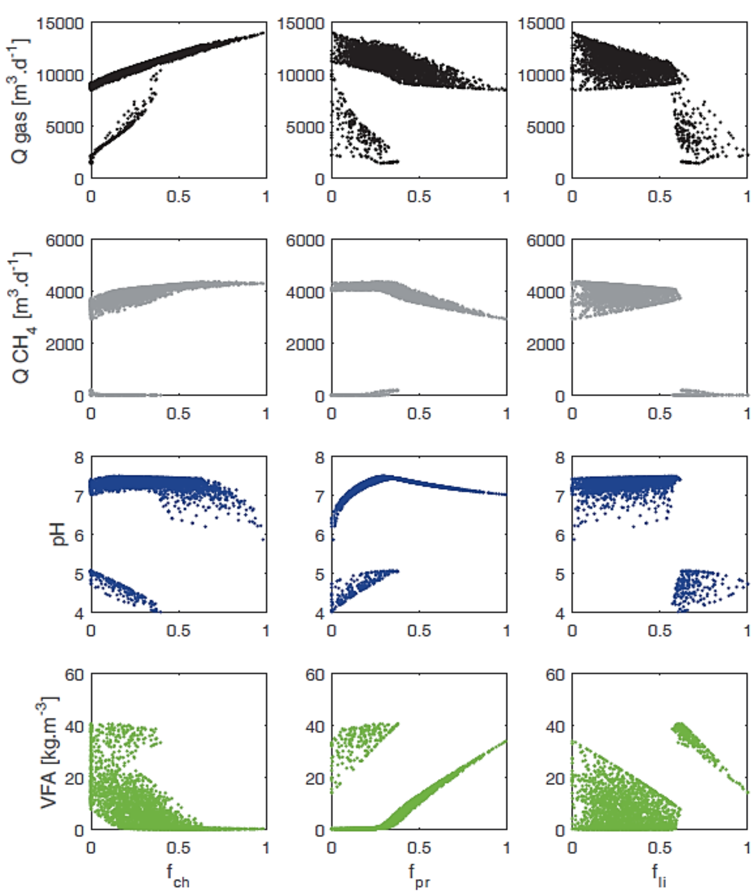

Fig. 6. Output state variables from Monte Carlo simulations. From top to bottom, the rows show Q_{gas}, $Q_{methane}$, pH, and VFA. Each output variable is plotted against the biodegradable COD fractions f_{ch}, f_{pr}, and f_{li} from left to right. Each point represents a simulation where the sum of f_{ch}, f_{pr}, and f_{li} equals 1.

modelling framework. This modelling platform is designed to allow for independent characterization of co-substrates by recognizing substrate-dependent parameters and quantifying chemical oxygen demand (COD) and total nitrogen (TN) fractions for each substrate. Thus, the existing ASM-to-ADM interface for mixed sludge in the BSM2 was maintained as is.

To ease implementation, the hydrolysis reactions were decoupled from the other processes of the ADM1 model. The substrates were dosed to independent reactors with the hydrolysis stage of the ADM1 model only. The products of hydrolysis from the reactors were then mixed and subsequently fed into a mitigation reactor where the remaining processes of the ADM1 model (acidogenesis, acetogenesis, and methanogenesis) took place (see Fig. 8). A small fraction of residual particulate substrates (X_{ch}, X_{pr}, and X_{li}) was passed from the hydrolysis to the ADM1 stage of the model to allow for incomplete hydrolysis scenarios. Only one digester exists physically; however, virtually separating the hydrolysis stage allows for multiple substrates to be fed and is more representative of real systems than only having one substrate type.

3.9. Simulation study to evaluate anaerobic co-digestion at the wastewater treatment plant level

A simulation experiment was carried out to validate the AcoD model generated at the Isfahan WWTP, demonstrating that the effects of AcoD can be evaluated using BSM2. The co-digestion feed was a blend of sludge, blood, and DAF sludge in the ratios of 5:2:1. The average sludge COD loading to the digester was 2.38 kg.m^{-3}.d^{-1}. After that, a mixture of external feed was added, raising the baseline COD load by 50%, followed by two peak load times to stress the system, days 350–410 using blood as the main co-substrate, and days 500–521

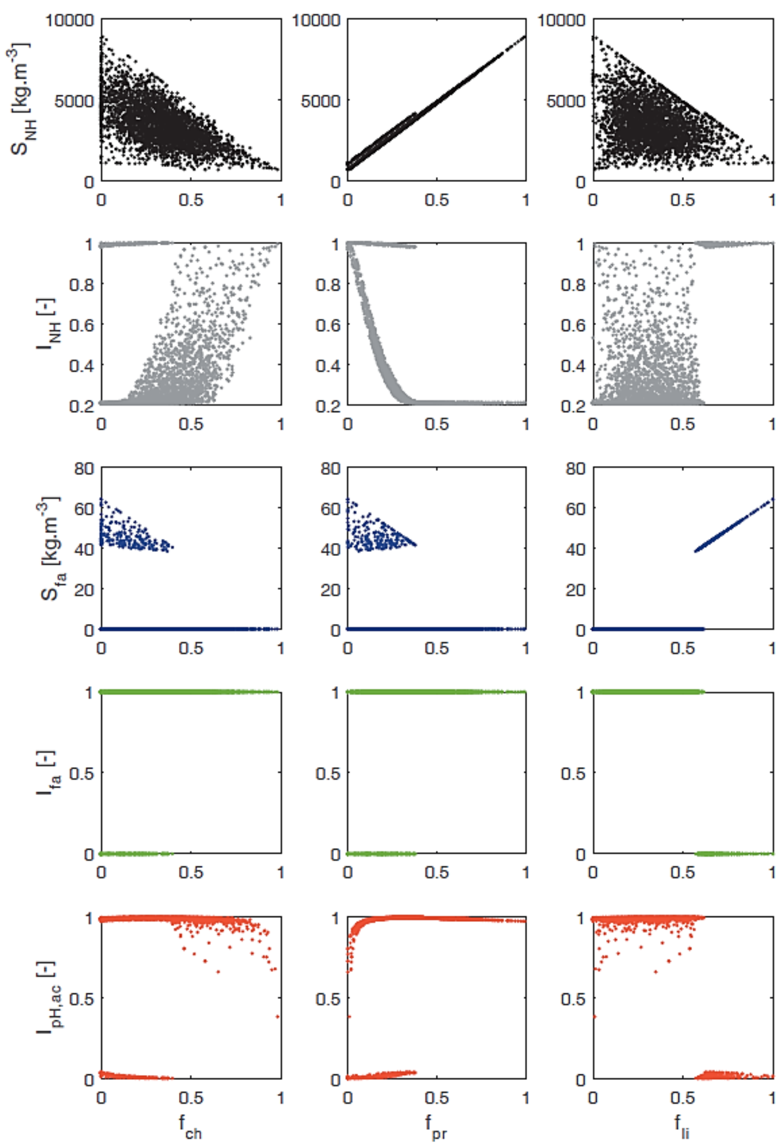

Fig. 7. AD inhibitions and corresponding inhibitor compositions from Monte Carlo simulations. From top to bottom, the rows show S_{NH}, ammonium inhibition (I_{NH}), S_{fa}, I_{fa}, and pH inhibition for acetate uptake ($I_{pH,ac}$). Each state variable is plotted against the biodegradable COD fractions f_{ch}, f_{pr}, and f_{li} from left to right. Each point represents a simulation where the sum of f_{ch}, f_{pr}, and f_{li} equals 1.

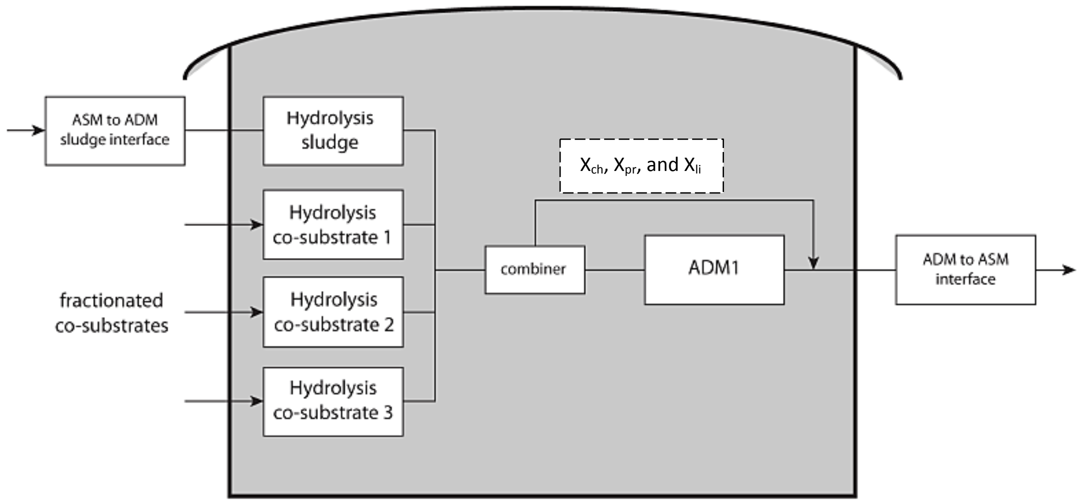

Fig. 8. Schematic of the anaerobic digester block in benchmark simulation model No. 2 (BSM2) with the integrated GISCOD model for anaerobic digestion.

using DAF sludge as the co-substrate. The total OLR time series is presented in Fig. 8.

The results of the main simulation, compared with the default BSM2 parameters, are shown in Table 2. The dynamic profiles can be seen in Figs. 9 & 10, which show the methane production, OLR, internal nitrogen load, and nitrate nitrogen (NO_3-N) in the effluent.

The co-substrates were able to improve methane production, and the total Operational Cost Index (OCI) was lower at 10,490 compared to the default at 11,630. However, because the supernatant was recirculated back into the anaerobic digestion (AD) chamber, the Effluent Quality Index (EQI) increased from 5,330 to 5,970.

This was a decrease in OCI that represented approximately a 10% decrease in the total operational costs of the WWTP. This represents a significant cost savings due to a dramatic 92% increase in revenue of electricity generated from the increase in methane production, while the aeration energy consumption and sludge production only increased by 6% and 39%, respectively.

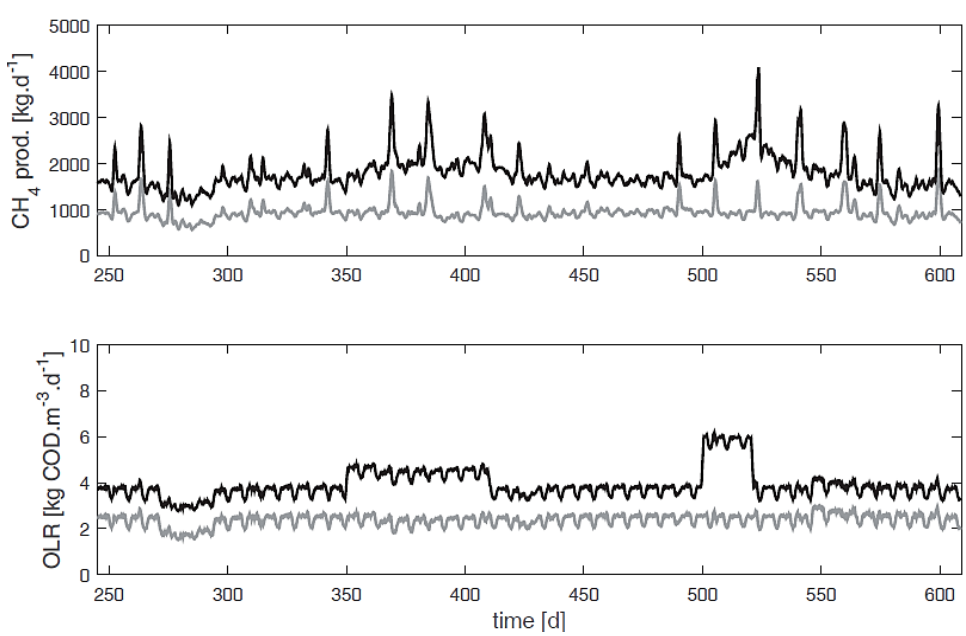

Fig. 9. Simulated effects of anaerobic co-digestion on the anaerobic digester in benchmark simulation model No. 2 (BSM2). Methane production is shown in the upper figure, and the organic loading rate (OLR) is shown in the lower figure. Gray lines represent the standard BSM2 results without co-digestion, and black lines represent the simulated scenario with co-digestion (filtered values). Time indicates simulation days, where day 245 corresponds to July 11.

Table 2. Simulation results for BSM2 with co-digestion compared to default values.

Parameter	BSM2 default	BSM2 with AcoD
Operational cost index	11,600	10,050
Effluent quality index, kg pollutant/d	5,330	5,970
Average aeration energy, kWh/d	4,130	4,380
Methane production, kg/d	935	1,800
Sludge production, kg TSS/d	3,480	4,730
Effluent NH, g/m^3	0.49	0.44
Effluent NO (S_{NO}), g/m^3	9.81	12.9
Effluent TN, g/m^3	12.3	15.4
Time in violation TN, %	0.17	15.0

Increased total nitrogen (TN) concentrations in the AD effluent (15.4 g.m^{-3}) due to additional nitrogen loads from the protein-rich co-substrate during the AcoD test were primarily responsible for the overall increase in EQI. This resulted in an increase in ammonium nitrogen concentrations (S_{NH}) in the returned AD supernatant from 290 g.m^{-3} on average to 1,610 g.m^{-3} (Fig. 10), leading to 15% violation of the effluent TN discharge limit, as compared to only a 0.17% violation without AcoD.

These effluent limit violations could be considered a potentially significant barrier to AcoD implementation and illustrate the importance of thoroughly evaluating substrate selection and dosing strategies.

Increased TN concentrations in the effluent can be primarily attributed to increased nitrate nitrogen (SNO) concentrations resulting from sufficient carbon availability that promoted nitrification rather than poor nitrification performance (see Table 2 and Fig. 10). This can be partly attributed to the default closed-loop dissolved oxygen (DO) control strategy being employed in the BSM2 activated sludge unit, which, in response to increased ammonia loads, maintains a fixed dosing rate of methanol.

Effluent limit violations could potentially be reduced through simulated adaptive or increased dosing of methanol substrate strategies. These mitigation strategies could be easily explored and optimized via future simulations.

This case study illustrates that incorporating AcoD simulation in WWTP models supports the design of optimal substrate combinations and feeding strategies while enabling assessment of their operational and environmental consequences. In this respect, Nordell and Wiberg [32] showed in pilot- and full-scale AcoD studies at Linköping WWTP, Sweden, that utilizing low-nitrogen co-substrates may have a beneficial effect on treatment plant performance and achieved improved nitrogen removal due to biosolid uptake.

4. Conclusions

The main findings of this study are summarized as follows:

- Developing precise kinetic models and parameterization is vital to improving energy recovery through anaerobic co-digestion in wastewater treatment plants. The first-order kinetic model had superior predictive-reliability and operational simplicity than Monod-type models for cumulative methane predictions, permitting better systems to be designed and operated.

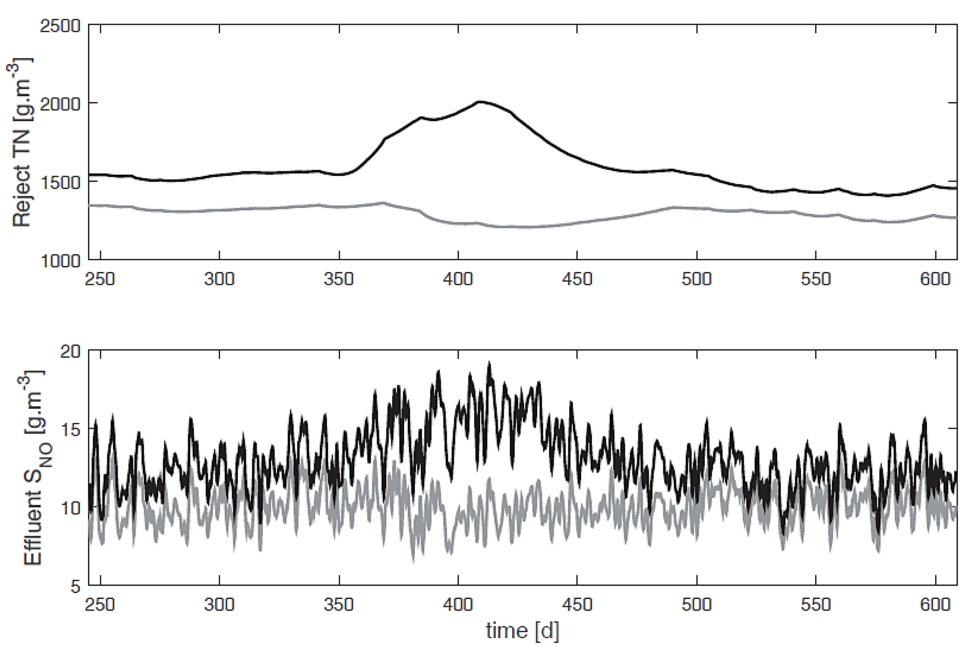

Fig. 10. Simulated effects of anaerobic digestion on the benchmark simulation model No. 2 (BSM2) process. The total nitrogen (TN) concentration in the anaerobic digester supernatant is shown in the upper figure, and the effluent nitrate (S_{NO}) concentration is shown in the lower figure. Gray lines represent the standard BSM2 results without co-digestion, and black lines represent the simulated scenario with co-digestion (filtered values). Time indicates simulation days, where day 245 corresponds to July 11.

- Accurate characterization of the substrate, combined with explicit inhibition functions, improved predictive accuracy. A reliable, well-proven, and cost-effective method of chemical oxygen demand (COD) fractionation enhanced substrate characterization for operational decision making and planning the use of multiple waste mixtures.
- The operating simulations indicated that co-digestion strategies could be designed to provide an opportunity to save on processing costs while providing a net benefit overall sustainable economics; however, careful attention to feedstock types is necessary for the potential risks associated with excessive nitrogen and fat contents, as they can have the potential to destabilize the process and decrease methane yield. We identified the protein and lipid fractions as the factors having the greatest influence on the overall performance of the process.

The study provides a complete framework to enable relevant end-users to quickly execute and continue the refinement of co-digestion methods in operationally based full-scale wastewater treatment plants. The limitation of this study was the relatively small range of feedstock types examined, along with the variability of on-site operational conditions, which may limit the generalization of findings beyond the setting of this study. Future research should explore effective methods of mitigation of ammonia and lipid-derived inhibition, as well as consideration of innovative feedstock combination strategies to maximize performance and sustainability of the systems.

CRediT authorship contribution statement

Hamidreza Shiran: Conceptualization, Data curation, Methodology, Formal analysis, Writing – original draft.
Gholamreza Nabi Bidhendi: Supervision, Project administration, Writing – review & editing.
Nasser Mehrdadi: Supervision, Validation, Writing – review & editing.
Amirhossein Choopani: Writing – review & editing.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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