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Wastewater treatment bioprocesses are conventionally modeled using mechanistic, data-driven, or hybrid models. Herein, we identify the knowledge gaps in those models. We also propose potential modeling strategies to incorporate genomic data for handling a large amount of the physical, biochemical, and microbiological data collected from biological wastewater treatment systems, with the overarching goal to achieve real-time monitoring and optimize system performance.

Emerging investigator series: Modeling of Wastewater Treatment Bioprocesses: Current Development and Future Opportunities

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Abstract

For more than a half-century, modelers have developed various modeling strategies to facilitate the transition of wastewater treatment bioprocesses from lab-scale demonstration to full-scale applications. This review presents the mathematical fundamentals of mechanistic models, machine learning algorithms of data-driven models, and hybrid modeling strategies for different biological wastewater treatment systems including activated sludge processes, anaerobic digesters, anammox processes, and bioelectrochemical systems. The discussion is focused on the biological principles in those modeling strategies. The conventional Monod expressions are a prevailing tool to describe the mathematical connection between microbial kinetics and state variables in mechanistic models. Stoichiometric equations and steady-state conditions are also required for the mechanistic modeling approach to predict system performance such as the removal of carbon, nitrogen, and phosphorus. On the other hand, data-driven models statistically link the inputs and outputs for the prediction and optimization of system performance with a minimum requirement of a priori knowledge. Although this strategy shows outstanding learning ability of data interpolation, the predictions are often uninterpretable due to the black-box nature. Hybrid modeling strategies have the potential to dress the inherent limitations of standalone models. Currently, the mechanistic and data-driven components in hybrid models are still structured based on microbial kinetics and trained with physical and biochemical data, respectively. This problem can be potentially solved by incorporating genomics data into model construction to link microbial kinetic to microbial population and functional dynamics. We discuss the perspectives of incorporating genomic data into model construction and propose genomics-enabled hybrid modeling strategies for future research.

Keywords: hybrid model, wastewater bioprocess, data-driven model, mechanistic model, genomics-enabled model

1. Introduction

A variety of wastewater treatment systems are developed to harness microorganisms to remove organic contaminants and nutrients.[1] These include activated sludge processes, anaerobic digesters, and membrane bioreactors that have been extensively applied in full-scale treatment plants, as well as emerging technologies such as anaerobic ammonium oxidation (anammox) and bioelectrochemical systems. By taking advantage of the metabolic versatility of microorganisms, some of those systems can be engineered to achieve sustainable treatment of complex waste streams. For example, anammox as an anaerobic and autotrophic process converts ammonia to nitrogen gas with little energy input,[2] and bioelectrochemical systems can recover energy and resource from waste streams.[3]

Biological wastewater treatment systems are commonly monitored using real-time biochemical data such as biological oxygen demand (BOD), chemical oxygen demand (COD), and mixed liquor volatile suspended solids (MLVSS) as an indication of biomass concentration.[4–6] This is an operationally simple method that leads to a quick assessment of the microbiological activity and system performance. However, those macroscopic state variables sometimes do not help explain the inconsistency in treatment performance caused by environmental and operational perturbations, primarily because they are unable to reflect the complex microbial physiology, community structure, metabolism, and interspecies interactions in the systems.[4] As conventional bioprocesses face challenges from emerging pollutants and more stringent discharge limits over the past decades,[7] real-time monitoring and experimental trials are laborious and frequently fall short to provide insights into system optimization.

The high complexity of bioprocesses and the pressing need to develop more sustainable systems drive environmental researchers and engineers to build computational models to gain mechanistic and predictive understandings of the dynamic behaviors in the systems.[8] A prevailing strategy is to build mechanistic models, also known as white-box or first-principle models, based on the mathematical expressions of the physical/chemical/biological principles of the processes involved.[8] This modeling strategy starts with defining the model purpose followed by model structure selection, input identification, data collection/reconciliation, and model calibration/correction.[9] To improve the robustness of mechanistic models, it is important to understand the fundamental microbial kinetics. Monod expressions are the most well-established tool for modeling microbial growth and substrate utilization kinetics and have been extensively applied to simulate many bioprocesses.[10–15] The kinetic parameters in Monod equations are commonly derived from biochemical measurements.[16] For ill-defined systems that are driven by uncharacterized functional populations, it becomes challenging to determine which biochemical indicators to measure to reliably reflect their growth and substrate utilization kinetics.

Data-driven models seek to establish statistical connections between the inputs and outputs and require little knowledge about the fundamental principles of the processes involved. This is of great interest to the modeling of bioprocesses, in which the lifestyles of many functional populations remain unknown.[17] Data-driven modeling strategies started to attract environmental/biological engineers' attention in the early 90s with the practical implementation of artificial neural networks (ANNs) and major advances in machine learning.[18–20] Using appropriate training datasets and network architecture, neural networks were trained to directly predict wastewater treatment performance,[20,21] as well as the effects of sludge volume index and

total nitrogen concentration on the effluent quality.[22] Recent studies demonstrated the applicability of several machine learning algorithms, including support vector machine, random forest, extreme gradient boosting, and k-nearest neighbors, to full-scale digesters.[23] Those algorithms have also been used to train models with biochemical data collected from literature, presenting a powerful tool for data mining.[24] Despite the outstanding learning performance, data-driven models are black boxes that often generate uninterpretable predictions.[25] This is particularly problematic for bioprocesses when the models are trained solely with physical and biochemical data.[21–23,26]

Hybrid models are built through the integration of mechanistic and data-driven sub-models to improve the shortcomings of those individual modeling strategies.[27] Hybrid models are ideal for ill-defined systems in which only part of the process can be mechanistically described (e.g., the mass balance of measurable biochemical variables), while another part of the process is too complicated to be derived from first principles (e.g. microbial interactions).[28] Depending on the significance of the known and unknown processes in a system, the mechanistic and data-driven sub-models can be integrated through parallel, in series, or a mix of both structures.[29] Such integration allows the designer to structure the model more flexibly based on the availability of the a priori knowledge.[28] Recent studies demonstrate that hybrid models are more accurate and flexible in predicting the dynamic behaviors of wastewater treatment bioprocesses.[30,31]

This review discusses the applications and drawbacks of major modeling approaches that have been developed for simulating wastewater treatment bioprocesses over the past 60 years in chronological order (Figure 1). We first review the mechanistic modeling of several systems

including activated sludge processes, anaerobic digesters, anammox, and bioelectrochemical systems. The discussion of this modeling strategy is focused on the biological principles, in particular microbial growth and substrate utilization kinetics. In the following sections, we review data-driven models capable of capturing the dynamic behaviors of bioprocesses, as well as hybrid models that take advantage of both modeling strategies (Figure 2). We also discuss the potential of incorporating high-throughput sequencing data into model construction to unfold the underlying interactions among microbial kinetics, community structure, and functional dynamics. Finally, we propose a conceptual framework to build hybrid models with omics-based results for robust and interpretable prediction of microbial interactions and wastewater treatment performance.

2. Mechanistic Modeling of Wastewater Treatment Bioprocesses

2.1 Early development of microbial kinetic model

Microbial growth is impacted by several factors such as cell metabolic activity, substrate availability, oxygen concentration, temperature, etc. Among them, the substrate is arguably the most important one as it is directly involved in cell metabolism.[32] To model the kinetic behaviors of microbial growth, a greater understanding of the effects of substrate concentration on metabolic quotients, specific growth rate, and yield is required. Between the 1920s and 1960s, many hypotheses and models were proposed to explain the biological mechanisms of substrate consumption for cellular metabolism and storage.[33]

The Monod equation is a microbial kinetic model that describes the hyperbolic growth behavior of microbes in batch systems at exponential and steady-state phases.[16] In the Monod equation,

the specific growth rate of a culture is a function of the concentration of a given substrate,[34] with the substrate saturation constant (i.e., the substrate concentration when the specific growth rate is half of the maximum specific growth rate) indicating the affinity of the substrate to cell growth.[16] The Monod equation and its modified expressions were demonstrated to be robust for modeling different pure cultures under varied conditions.[35] Since then, a variety of mechanistic models have been built on Monod expressions.[10,13,36–38]

Several studies pointed out the inadequacy of the original Monod expression in dealing with substrate inhibition, cell decay, diffusional limitation, etc.[36,39] Substrate inhibition as a common issue arises when the complex composition in wastewater shows different affinity to microbial cells, leading to competition among functional populations and/or inhibition to cell growth. The Haldane-Andrews equation included an inhibition constant in the Monod expression to reflect substrate inhibition.[36] However, because the constant is inferred through the generalized substrate inhibition model, a normal distribution method with an error range, such a modification does not reveal the actual impacts of substrate inhibition on microbial growth.[40]

2.2 Modeling of activated sludge processes

The activated sludge models have been developed for more than 70 years since the early 60s.[11] Early mechanistic models were derived based on steady-state applications in which the cell growth rate remained constant.[34] The biochemical parameters used to quantify cell growth were total organic carbon (TOC), total oxygen demand (TOD), and COD along with 5-day BOD.[41] They are sufficient for modeling steady-state conditions but have severe limitations in real-time situations where cell growth behaves dynamically under the influence of substrate variation.

Oxygen uptake rate and mixed liquor volatile suspended solid were later included to address the limitation.[36,41] Mathematical techniques such as the feedforward-feedback strategy were also applied to control the flowrate disturbance. These modifications allowed mechanistic models to respond to dynamic situations with real-time biochemical data.[41]

In 1982, the International Association on Water Pollution Research and Control published a preliminary model on activated sludge systems. Later, Dold and Marais incorporated the preliminary model to a final version called the Activated Sludge Model No. 1 (ASM1).[12,42] ASM1 is considered a reference model and is generally accepted as a fundamental component for wastewater treatment modeling.[43] In particular, the Monod equation in ASM1 was proven reasonably appropriate to describe the microbial growth and substrate utilization behaviors in wastewater.[44]

The microbiological principles of ASM1 include the growth of aerobic and anoxic heterotrophic organisms, the growth of aerobic autotrophic organisms, the decay of heterotrophs and autotrophs, hydrolysis of slowly biodegradable substrate, ammonification, and hydrolysis of organic nitrogen.[10] In addition, the model also describes the following dynamic mass balances that have impacts on biomass concentration: 1) readily biodegradable substrates, 2) slowly biodegradable substrates, 3) inert particulate substances, 4) particulate organic nitrogen, 5) soluble organic nitrogen, 6) ammonia, 7) nitrate, and 8) oxygen.[10] These state variables serve as explicit indicators of the nutrient removal processes. Among all parameters used in ASM1, the growth and decay rates are of key importance as they control the biomass concentration as a function of the influent substrate concentration. The full description of ASM1 and the

178 comprehensive review of model development can be found elsewhere.[8,10] The robustness of
179 ASM1 has been demonstrated by numerous studies. Forty years after its first implementation,
180 ASM1 is still playing a central role in the mechanistic modeling of bioprocesses and has been
181 incorporated in commercial software as a core structure for the simulation of full-scale
182 wastewater treatment plants.[45]
183
184 Advancing from ASM1, ASM2 incorporates polyphosphate-accumulating organisms (PAO), a
185 functional population enriched during enhanced biological phosphorus removal in activated
186 sludge systems. In addition to common microbial kinetics, the model structure for PAO also
187 includes the storage of glycogen, polyhydroxyalkanoate, and polyphosphate, which are
188 expressed as a function of oxygen availability according to their physiological traits.[46] In the
189 1990s, ASM2d was developed based on ASM2 by including the ability of PAOs to use internal
190 cellular materials for denitrification,[10] thus linking the metabolism of nitrate and phosphorous
191 under anoxic conditions. In the absence of oxygen, PAOs can use nitrate as a terminal electron
192 acceptor for phosphorous uptake.[10,47] In ASM2d, a fraction of the maximum growth rate of PAO
193 is assigned to complete denitrification. The fraction varies depending on PAOs' activity
194 including growth, denitrification, and anoxic phosphorus uptake.[48] ASM2 and ASM2d are
195 comprehensively reviewed by Henze et al.[10]
196
197 ASM3 was modified to improve the prediction of oxygen consumption, sludge production,
198 nitrification, and denitrification. Key modifications include cellular storage of organic substrates
199 and the consumption of dead cells through endogenous respiration (instead of the decay and
200 recycling processes described by ASM1).[37] With these modifications, ASM3 is more accurate in

describing the substrate uptake and storage behaviors, but the enhanced prediction may not be relevant for most treatment plants where ASM1 is sufficient for simulation of general performance.[46] Therefore, ASM3 is needed only when specific metabolic activities are modeled.

There are several commercial software packages available to simulate the activated sludge processes following the development of ASM1/2/3. Olsson and Newell provided a detailed overview of the simulator environments for the bioprocesses in wastewater treatment plants.[49] Some of the simulators, such as MATLAB-based Simulink, serve a general purpose with high flexibility to complete simulation. Other simulators contain a library of predetermined models for specific bioprocesses, and the process configuration is a unit-based simulation environment. Examples of this type of simulator are AQUASIM, BioWin, EFOR, GPS-X, SIMBA, STOAT, and WEST. The computation package can solve mechanistic models with multiple mathematical equations simultaneously.[50] BioWin, for example, is a flexible software tool that includes multiple microbial processes presented in ASMs and anaerobic digestor models (ADMs).[51–55] It has been used to model the biological systems in full-scale wastewater treatment plants,[52–55] including activated sludge,[56] anaerobic digestion,[57,58] and anammox processes.[51] In some of the studies, BioWin provided a good match between the measured and predicted data (difference <10%) for both small-scale batch reactors and full-scale systems,[56–58] and it can perform optimization of sludge retention time and nitrogen removal under different DO and return activated sludge flows.[53] In most of the studies, however, model calibration is required to improve the prediction performance. Improper calibration, for example, with nitrogen, phosphorus, and other microbial inhibitory substances has been reported to cause inaccurate prediction of methane production in anaerobic digestion.[58] Because commercial software still

224 requires both in-depth knowledge of the bioprocess and expertise in modeling, it is not very user-
225 friendly to treatment plant staff.
226
227 **2.3 Modeling of anaerobic digesters**
228 Anaerobic digesters have a highly complex microbial community composed of fermentative
229 bacteria, syntrophs, acidogens/acetogens, and methanogens.[59] The model structure in early
230 models was improved with a greater understanding of the microbial kinetics of those functional
231 populations and the associated biochemical reactions. For example, Andrews applied the
232 Haldane function on the Monod expression to modify the substrate uptake function with
233 inhibition under rate-limiting conditions,[60] Andrews and Graef later proposed to include the
234 effects of pH change and buffering through liquid-gas phase interaction and carbonate
235 equilibrium, leading to more accurate modeling of microbial kinetics.[61] Based on those studies,
236 Hill and Barth further included a function that described the inhibition effects of volatile fatty
237 acids (VFAs) and ammonia on methanogens, as well as charge balance to correct temperature-
238 dependent pH.[62]
239
240 After decades of study of the microbial ecology in anaerobic environments, a task group from the
241 International Water Association consolidated the up-to-date knowledge and formulated
242 Anaerobic Digester Model No. 1 (ADM1) as a common platform model for anaerobic
243 processes.[13] This model involves 4 typical digestion processes (hydrolysis, acidogenesis,
244 acetogenesis, and methanogenesis) and several physicochemical steps including gas-liquid
245 diffusion, ion association, and dissociation. ADM1 describes in total 29 processes and 32
246 variables at dynamic state, 24 of which are based on the Monod equation and first-order kinetics.

247 The model also allows modifications for specific applications such as sulfate reduction,
248 phosphorous conversion, and mineral precipitation.[13] ADM1 has been successfully implemented
249 to simulate effluent characteristics with various types of substrates and operating conditions. The
250 most common function of this comprehensive model is to predict and optimize biogas production.
251 Satpathy et al. applied ADM1 to simulate biogas production from rare substrates such as chicken
252 manure.[63] Other applications of ADM1 include the prediction of effluent quality from systems
253 fed with winery wastewater[64] or treating phenol from olive mill waste.[65] For example, Ozkan-
254 Yucel and Gokcay applied ADM1 to a full-scale anaerobic digester under varying organic
255 loading rates to predict total VFAs and COD in the effluent.[66] Modified ADM1 can also help
256 troubleshoot operational problems caused by inhibition effects,[48] in particular, the accumulation
257 of VFAs.[68]
258
259 Despite successful implementation for specific applications, the extensions of sulfate reduction
260 and mineral precipitation can cause a significant computational burden. This is because the
261 precipitation of multiple components ($CaHPO_4$, struvite, and other unknown compounds) and
262 their release mechanisms involve a large number of processes, in which the fundamental
263 knowledge is not available for the model to perform *ab initio* prediction.[69] Another critical issue
264 is that ADM1 is still not able to fully recapitulate the actual functional populations due to the
265 lack of an in-depth understanding of the microbial community. Specifically, ADM1 is structured
266 without considering the production of different short-chain VFAs, alcohols, and hydrogen. The
267 model is thus not able to meet the growing interest in those value-added products.[64] Shi et al.
268 attempted to solve this problem by redefining the pseudo-stoichiometric dynamic parameters of
269 VFAs and alcohols corresponding to the hydrogen partial pressure.[70] The modified model

successfully predicted the concentrations of acetate, propionate, butyrate, ethanol, and hydrogen with standard errors < 0.04. However, there remains some discrepancy between the predicted and observed hydrogen levels and effluent COD when the system was fed with high-strength streams.[70] In terms of methane production, the model did not include the methanogenic population via direct interspecies electron transfer,[71] which was recently found to be a ubiquitous electron transfer mechanism in many engineered and natural environments.[72] A reaction-diffusion-electrochemistry model composed of activation and ohmic losses predicted that methanogenesis could be an order of magnitude faster via direct interspecies electron transfer than via the classic route of interspecies hydrogen transfer.[73] Modified ADM1 further predicted over one-third of the CH_4 produced via this novel electron transfer mechanism,[74] underpinning its critical role in methane production.

2.4 Modeling of emerging bioprocesses

Discovered in the 90s, anammox has been applied as an alternative technology for biological nitrogen removal.[75] Anammox can be engineered as a single-step process, which is efficient in terms of energy, space, and cost compared with conventional two-step nitrification/denitrification.[76] In anammox systems, part of the ammonium is oxidized by ammonium oxidizing bacteria through partial nitrification to nitrite, which serves as the electron acceptor for anammox bacteria to oxidize the remaining ammonium to nitrogen gas.[77] Due to the unique bioprocesses, a previous study built an anammox model from fundamental processes including diffusion, hydrolysis, and microbial kinetics of anammox bacteria for simulation of long-term nitrogen and COD removal by a granular up-flow anaerobic sludge blanket reactor.[15] Because the model only assumed cell growth under optimum conditions, the predicted and

observed nitrogen removal did not correlate well. The anammox process was later modeled using modified ASM1. Through experimental calibration, the model yielded satisfactory prediction of nitrogen removal efficiency of a laboratory-scale bioreactor.[78] Interestingly, anammox is better simulated when coupled to other bioprocesses, e.g., sulfur-driven denitrification,[14] as the kinetic parameters in the model can be more accurately estimated by varying both sulfur (sulfite, sulfur, and sulfate) and nitrogen (ammonium, nitrogen, nitrite, and nitrate).[14] Although the models have demonstrated the potential of anammox systems, the modeling of anammox is still challenging because the system performance is highly dependent on the dynamic interactions between ammonium/nitrite oxidizing bacteria and anammox bacteria.[14] A reliable method is to calibrate the maximum growth rates of ammonium oxidizing bacteria, nitrite oxidizing bacteria, and anammox bacteria using experimental data from the full-scale bioreactors and validate the calibration based on the sensitivity analysis.[79] In addition, the substrate affinity coefficients should be adjusted based on reported literature to fit the microbial substrate utilization under the possible effects of mass transfer in flocs.[79]

In addition to predicting and optimizing the performance of well-developed bioprocesses such as anammox, mechanistic models can also be implemented for emerging biotechnology such as bioelectrochemical systems to gain an in-depth understanding of their potential and facilitate practical applications. A typical bioelectrochemical system is composed of a cathode and an anode in which the electrochemical reactions are catalyzed by microorganisms. It is challenging to model bioelectrochemical systems primarily because of the close yet uncharacterized connections among microbial kinetics, extracellular electron transfer mechanisms, and electrochemical factors (e.g., internal/external resistance).[80] A few mathematical models for this

316 novel system have been developed and reviewed elsewhere.[17,81–83] Noticeably, the growth and
317 substrate utilization rates of the functional groups are still based on the Monod expression and
318 corrected with electron mediator concentration, which in turn is expressed as a function of the
319 substrate utilization rate of electroactive bacterial.[18,31,84–86] Meanwhile, the Nernst-Michaelis-
320 Menten equation was used to calculate the electron transfer rate in the system[87] and the Nernst-
321 Planck equation was introduced to represent ion diffusion through the membrane between anode
322 and cathode.[88,89]
323
324 **2.5 Inherent drawbacks of mechanistic modeling**
325 The model structure in response to intracellular biochemical reactions is oftentimes inadequate,
326 which represents a major limitation of mechanistic models. Structural inadequacy stems from the
327 generalization of microbial growth and substrate utilization kinetics with Monod expressions.[8]
328 Ideally, the Monod expression for the growth rate of a functional population should consider all
329 degradable and inhibitory compounds and the corresponding factors. The substrates in practical
330 applications (e.g., wastewater) contain various degradable and inhibitory compounds that result
331 in highly complex microbial communities, in which many of the functional populations are
332 uncharacterized.[90–92] Therefore, it is not clear which substrates should be experimentally
333 measured to reflect their growth and substrate utilization kinetics. The absence of expressions for
334 novel methanogenesis mechanism via direct interspecies electron transfer is an example of
335 inadequate model structure.[57] Such an issue is more problematic for emerging bioprocesses. The
336 models for bioelectrochemical systems were typically structured with fermentative, electroactive,
337 and methanogenic populations. Although the microbial community in bioelectrochemical
338 systems is much more diverse than those in anaerobic digestors,[93,94] uncharacterized populations

cannot be incorporated into the model structure because their physiological traits related to growth and substrate utilization are poorly understood.[95–97]

Another challenge lies in the unmeasurable kinetic parameters in Monod expressions. Most of the kinetic parameters such as inhibition constant, maximum growth rates, half-saturation constants, and substrate utilization rates can only be derived from biochemical measurements (e.g., substrate concentration).[98] From an experimental perspective, when microbial kinetics responds to changing influent characteristics in real-time, it is unrealistic to derive the kinetic parameters throughout the variation. As the operating condition changes, biochemical measurements can vary significantly, and the estimation of kinetic parameters becomes conditional.[27] The identifiability of unmeasurable kinetic parameters, i.e., the possibility to derive a unique set of values for the parameters from experimental data, is then of great concern. Firstly, uncertainty issues arise because the derivation of the kinetic parameters is obtained through rate-controlling experiments with specific temperatures, pH, BOD, and COD, etc.,[34] while in wastewater treatment processes, microbial cells are exposed to varied substrates. Those parameters need constant calibration. An uncalibrated model is not likely to yield accurate predictions. As a result, it is not certain to what extent the predictions can be used to explain the observed physical, chemical, and biological mechanisms..[99] Secondly, to identify the kinetics parameters, the identification of the biochemical and physical parameters responsible for biomass concentrations must be achieved first. Flotats et al. applied the Taylor Series Expansion to four state variables (acetate, propionate, valerate, and methane) of ADM1 to identify the parameters related to valerate consumption and biomass concentration.[100] Such a method has only been reported to solve simple models with a few unidentifiable parameters.[101,102] For

362 complex models such as second-order models, parameter identification was handled with the
363 asymptotic behavior of the maximum likelihood estimator and multiple shooting approach
364 described in Muller et al.[103] Those previous studies collectively show that the identification and
365 computation processes of kinetic parameters are cumbersome and uncertain in reality.
366
367 **3. Data-Driven Modeling of Wastewater Treatment Bioprocesses**
368 **3.1 Neural networks**
369 Neural networks, first reported in 1943, are arguably the most prevailing data-driven models
370 across various research fields.[104,105] A neural network is composed of multiple layers of
371 interconnected nodes (neurons), through which the inputs are propagated to the final output
372 layer.[106] Each input to a neuron has a weight factor that determines the interconnection strength
373 to the next neuron. By adjusting the weight factors, a neural network can be properly trained to
374 perform problem-solving. The training algorithm can be divided into three types: supervised,
375 unsupervised, and hybrid training. In supervised training, neural networks are trained with a
376 labeled dataset that provides feedback about the prediction accuracy.[106] Unsupervised training
377 allows networks to be trained with unlabeled data, and the algorithm extracts features and
378 patterns on its own.[106] The hybrid training strategy uses unsupervised training for the hidden
379 neurons and supervised training for the output neurons.[107]
380
381 Neural networks were first implemented for continuously stirred bioreactors to predict the
382 fermentation products and pH in the effluent.[108] Boger applied the modeling strategy to full-scale
383 wastewater treatment plants and showed that neural networks could be a solution for simulating
384 expert rules, a set of boundary values that confined the neural network prediction, from historical

385 operating data.[19] Traditionally, neural networks were trained in a feed-forward fashion, meaning
386 that the feed was directed forward-only through layers of training. Yang and Linkenst found that
387 the back-propagation method, which fed outputs from a random layer back to a previous layer,
388 could help the network lower the error rate of prediction.[109] Backpropagation thus became a
389 prevailing algorithm of neural network modeling. Several studies used this method to predict
390 effluent COD, biogas production, and NH_4^+-N removal in different biological wastewater
391 treatment systems including activated sludge processes,[21] up-flow anaerobic sludge blanket
392 reactors,[26] sequencing batch reactors,[110] and anaerobic digesters.[111]
393
394 Previous studies have combined neural networks with other types of data-driven models to
395 improve the simulation of ill-defined systems. One of the strategies is to use the genetic
396 algorithm to select the initial dataset for downstream neural network training, thereby identifying
397 the optimal training parameters and reducing the computational burden.[112] Bagheri et al. have
398 successfully applied the genetic algorithm to optimize the weights and thresholds of neural
399 networks to accurately predict sludge volume index.[22] Neural networks can also be coupled to
400 particle swarm optimization, a population-based optimization technique that searches for optimal
401 weights and biases through multiple iterations of particle positions in a given search space. This
402 coupling approach was intended to lower the training time and computational cost for finding an
403 optimal neural network structure.[113] Compared to the genetic algorithm, neural networks coupled
404 to particle swarm optimization is more memory-efficient in searching optimal weight parameters
405 but is less practical because it has no crossover and mutation in its operator.[114]
406

Neural networks have been proven feasible for interpolation within the training data range.[91] Insufficient training data can result in low prediction accuracy, particularly when prediction is performed with a dataset of fewer than 10 samples.[93] Another issue is that the model structure is often determined based on a trial-and-error approach, leading to significant time consumption and computational cost.[92] The fact that neural networks are black box built based on data fitting rather than the mechanistic understanding of the processes suggests that their outputs cannot be used to explain the mechanisms where the inputs are sourced.[25]

3.2 Random forest

Random forest is initially developed as a stochastic discrimination approach for classification purposes in the 90s.[115] Later, the approach was extended to combine bagging and random selection features to construct a collection of decision-making trees with control variance.[116] To use the random forest algorithm, the input data are classified through layers of tree branches consisting of a variety of features and classes, and multiple trees composed of the same number of features and classes are collectively used for prediction.[116]

In the wastewater treatment field, the random forest algorithm has been implemented for activated sludge processes, anaerobic digesters, membrane bioreactors, and anammox processes.[117–120] The main use of random forest-based models includes the prediction of system performance, fault finding, big data handling, model comparisons, and exploration of datasets with applicable reservations and constraints.[121] Although random forest-based models, similar to other data-driven models, are not able to integrate biological principles, these models allow for the identification of key features and conditions that are most influential on the process. The

inference can shed light on the underlying biochemical mechanisms. Song et al. implemented this modeling approach with wastewater treatment inputs as multivariate datasets to predict N_2O emission from the aerated zones of activated sludge processes.[118] Based on the model inference, they identified inorganic carbon concentration and specific ammonia oxidation activity as two of the dominant factors that determined treatment performance.[118] The model was further used to identify the different mechanisms of N_2O generation in oxic and anoxic environments and demonstrated the key role of N_2O in those zones in promoting niche-specific biochemical reactions.[122]

Random forest can be combined with other algorithms such as principal component analysis (PCA) and neural networks to improve the prediction of effluent quality. Preprocessing of data using PCA could enhance the robustness of random forest-based models, leading to a more accurate prediction of membrane flux in membrane bioreactors as compared with neural networks.[117] When coupled to neural networks, random forest-based models could be trained to predict the settleability in the biological reactor chamber,[119] as well as to evaluate the effects of key operating factors on treatment performance.[123] The results suggested that such a combined strategy could help achieve real-time monitoring and optimize operating conditions.[124]

3.3 Fuzzy logic

Fuzzy logic is an if-then algorithm that can be used to develop a set of flexible rules for diagnosis and control. A fuzzy logic-based system has four robust components: a fuzzifier, a fuzzy rule-base, an inference engine, and a defuzzifier.[125] The fuzzifier is responsible for converting crisp inputs into fuzzy sets, which are mapped by the inference engine to produce

453 another fuzzy set as the output. The fuzzy rule base is a collection of rules that guide the fuzzy
454 engine to produce the outputs. Finally, the defuzzifier transfers the output fuzzy sets back to
455 crisp values.[125]
456
457 Fuzzy logic can be applied to numerous scenarios in wastewater treatment bioprocesses such as
458 diagnosis and control of sequencing batch reactor processes,[126] simulations and prediction of
459 phosphorus removal,[127] as well as design, evaluation, and decision optimization of activated
460 sludge processes.[128] Robles et al. developed a fuzzy logic-based controller to optimize biogas
461 production and VFA concentration at varied influent flow rates.[129] The designed controller was
462 able to help prevent acidification in a closed-loop setting.[129]
463
464 A combination of fuzzy logic and neural networks could bring together the learning powers of
465 both algorithms, enabling fault tolerance during the modeling of complex systems.[107,108] For
466 example, the adaptive neuro-fuzzy inference system that pairs neural networks with fuzzy logic
467 allows modelers to insert *a priori* knowledge into the neural network structure as rules for
468 training. The combined modeling strategy was used to predict suspended solids, COD, pH and
469 DO levels in activated sludge systems.[108–110] Using the adaptive neuro-fuzzy inference system,
470 Essienubong et al. obtained a strong correlation between the experimental and predicted biogas
471 production with temperature, pH, substrate/water ratio, and hydraulic retention time as the
472 inputs.[134] The work by Hosseinzadeh et al. also demonstrated a higher sensitivity of the adaptive
473 neuro-fuzzy inference system when predicting water flux in an osmotic membrane bioreactor.[112]
474 In addition to neural networks, fuzzy logic was coupled to genetic algorithms and particle swarm

optimization, which outperformed the adaptive neuro-fuzzy inference system during the prediction of BOD, ammonium, and suspended solids in specific bioprocesses.[136]

Although fuzzy logic models are powerful tools for predicting system outputs using observable environmental data and human-like logic, these data-driven models cannot capture the behaviors of the complex kinetic reactions in engineered biological bioprocesses, which presents the major criticism among other concerns of implementation.[137] From an engineering point of view, detailed descriptions of the chemical, physical, and microbiological principles in bioprocesses and computing-based predictive methodology are equally important.[138] Unfortunately, fuzzy logic systems, like most of the data-driven models, are incapable of providing mechanistic insight into troubleshooting and system optimization due to their black-box nature.

4. Hybrid Models That Address the Limitations of Conventional Modeling Strategies

The concept of combining mechanistic and data-driven sub-models for hybrid modeling was first proposed in the early 90s and immediately implemented for a fermentation bioprocess to reduce the dependence on microbial kinetics.[29] Hybrid modeling strategies were further examined with activated sludge processes and anaerobic digesters through parallel or serial combinations of the mechanistic and data-driven components.[139,140]

In a parallel structure, the outputs from the mechanistic and data-driven components are combined primarily through pure superposition (i.e., summation of the outputs).[106,140–142] Weighing functions can be introduced to adjust the weight of the outputs, thereby improving the overall prediction accuracy.[143] It should be noted that the prediction performance of a parallel-

498 structured hybrid model is highly dependent on the robustness of the individual sub-models.[141] In
499 cases when a biological system is too dynamics/nonlinear and some of the biochemical data are
500 too expensive to collect in real-time (e.g., H_2 in anaerobic digesters), neither the mechanistic nor
501 the data-driven components could predict accurately, leading to poor prediction performance of
502 the hybrid model. It is therefore argued that the sub-models in a parallel structure are not well
503 integrated due to the lack of interactions (e.g., cross-feeding of the outputs as done in a serial
504 structure).
505
506 In a serial structure, the data-driven component acts as a parameter simulator and estimates
507 kinetic parameters for the mechanistic component to complete simulation.[29,140] Serial coupling of
508 the sub-models leverages the prediction power of the data-driven component and is sometimes
509 capable of extrapolating system outputs outside of the observation range. A mixture of both
510 structures has been implemented for chemical systems but not bioprocesses.[106] As the selection
511 of the structures depends on the availability of the mechanistic information,[142] we argue that with
512 more functional populations being characterized in wastewater biological systems,[144] a serial or
513 mixed structure may better reflect the underlying biological mechanisms whilst accurately
514 predicting the system performance.
515
516 One of the advantages of hybrid modeling is that unmeasurable mechanistic parameters, in
517 particular microbial growth and substrate utilization rates, can be determined by the data-driven
518 component (Figure 2).[29,145] This was done in the first study of hybrid modeling of engineered
519 bioprocesses, in which a neural network was trained to estimate the specific growth rate of the
520 overall microbial community, and the outputs were used to establish a biomass balance, resulting

521 in more accurate predictions than those from a standalone neural network.[29] Compared to
522 conventional mechanistic models that perform one-time estimation of the kinetic parameters, the
523 data-driven component in hybrid models allows the microbial kinetics to be updated in a timely
524 manner based on the collected data, thereby making the hybrid model more robust under varied
525 conditions.[29] Another benefit is that the data-driven component can capture more dynamic data
526 to compensate for the prediction of the mechanistic component. For instance, a neural network
527 was used to estimate the operational data at time t+1 based on the influent and operational data at
528 time t, and the outputs were subsequently used to correct the mechanistic predictions at time
529 t+1.[140] In this way, the hybrid model could capture the disturbance caused by shock loadings of
530 toxic compounds and deliver more accurate prediction of the effluent composition.[140]
531
532 Similar to standalone mechanistic models, hybrid models still contain a Monod expression-based
533 model structure. As previously discussed, Monod expressions approximate the physiological
534 traits of functional populations (i.e., microbial growth and substrate utilization) by assuming a
535 homogeneous culture.[16,35] Real wastewater and sewage sludge are highly heterogeneous with
536 various degradable and inhibitory compounds that result in diverse microbial populations.[43,55]
537 For well-characterized populations with known functions, unmeasurable kinetic parameters are
538 predominantly derived from biochemical measurements.[14,16,34,35,40] Although the data-driven
539 component can help correct the estimates and improve the prediction performance, the estimated
540 kinetic parameters do not necessarily reflect the actual activity of those populations. This is even
541 more problematic for uncharacterized functional populations, whose microbial activity
542 information can be inferred with the data-driven component but does not help interpret the final
543 prediction because those populations are frequently overlooked in the mechanistic component.

Therefore, conventional hybrid models constructed with biochemical and physical data still suffer from interpretation issues. A potential solution is to incorporate microbial population and functional dynamics directly into the mechanistic and data-driven components.

5. Genomics-Enabled Modeling Strategies for Accurate and Interpretable Prediction

5.1 Genomics-enabled data-driven modeling

The rapid development of high throughput sequencing techniques and bioinformatics has led to a greater understanding of the microbiomes in wastewater treatment bioprocesses.[146–149] Recovering the 16s rRNA sequences allows us to unfold the taxonomy and phylogeny of the core populations in activated sludge, anaerobic digester, and many other systems.[146] Meanwhile, metagenomic and meta-transcriptomic data have greatly advanced our knowledge about the genetic potential and functional dynamics of uncharacterized populations.[147,148] The findings gained with those powerful tools have validated the mechanistic structure of existing models formulated with known functional populations.[149] The million-dollar question now is how to incorporate those genomic data into modeling in a more direct manner (Figure 3).

Two pioneering studies integrated 16S rRNA amplicon sequencing data with machine learning algorithms (neural networks and Bayesian networks) to reconstruct the microbial communities in natural ecosystems.[150,151] Following similar strategies, several machine learning-based models were trained with microbial taxon abundance and generated semi-interpretable predictions of system performance and stability.[152,153] The inferences suggested that for specific systems, classifying taxonomic data at the family level could enhance the prediction accuracy, whilst abundances of specific genera could act as better predictors, highlighting the potential to improve

the prediction interpretability with proper data preparation. Before training Bayesian networks, Yuan et al. prepared the genomic data collected from a bioelectrochemical system by selecting dominant taxa at the phylum, genus (Figure 4A), and operational taxonomic unit levels.[154] The genomics-enabled data-driven modeling approach was rigorously cross-validated using three validation strategies.[154] Firstly, the difference between the predicted and observed relative abundances of the selected populations remained within an acceptable range as indicated by a relative root-mean-square error (RMSE) of ~20%. Secondly, the microbial communities reconstructed with the predicted abundances of the selected populations shared high Bray-Curtis similarity with the observed communities at all taxonomic levels. Finally, the predicted system outputs agreed well with the experimental data. For example, current production as the most important system performance for bioelectrochemical systems was predicted with high accuracy at the order level (R^2 = 0.97 for prediction vs. observation, Figure 4B). After validation, the model was used to predict current production as a function of operating conditions (e.g., substrate salinity, Figure 4C) and provided practical insights into system optimization.

Functional genomic data as the training input can improve prediction interpretability. A previous study trained ANNs with gene expression levels to infer metabolic behaviors, resulting in a plausible explanation of microbes' stress adaptation behaviors under environmental perturbations.[155] Using a similar but more dynamic modeling strategy, Yuan et al. trained Bayesian networks with meta-transcriptomic data to explain the contribution of interspecies hydrogen transfer and direct interspecies electron transfer to methanogenesis.[30] It is highly desired to develop a predictive understanding of the involvement of the two mechanisms due to the lack of measurement techniques.[73,74] To prepare data for model training, the genes for alcohol

metabolism, hydrogen metabolism, extracellular electron transfer, and methanogenesis were extracted from the metagenome-assembled genomes of the dominant microbes. A Bayesian network trained with those genes is composed of two components (Figure 5A): upstream gene-gene interactions that predict the expression level of the relevant genes in methanogens, and a downstream sub-network that links the genes encoding methanogenesis to methane. A complete network could accurately predict methane production (R^2 = 0.96 for prediction vs. observation, Figure 5B). To statistically infer the contribution of the electron transfer mechanisms, relevant genes were manually silenced. When the simulation was performed without the genes for hydrogen metabolism, the prediction accuracy was significantly compromised as evidenced by the noticeable difference between the predicted and observed methane production and high RMSE (ΔIHT in Figure 5B). In contrast, the prediction remained accurate with *in silico* knockout of the genes for direct interspecies electron transfer (ΔDIET in Figure 5B). The results thus implied a more critical role of hydrogen-mediated electron transfer in methane production.

5.2 Genomics-enabled hybrid modeling

Thus far, genomic data have only been used to train data-driven models for semi-interpretable prediction. There is a growing interest to incorporate it into hybrid modeling to predict the underlying mechanisms for system design and optimization. One potential strategy is to infer unidentifiable kinetic parameters from microbial population and gene dynamics. The mechanistic component can be formulated following conventional modeling procedures to estimate kinetic parameters, and the estimates can be combined with microbial taxon abundance and operating conditions to train the data-driven component. The resulting hybrid model is therefore a kinetics

612 simulator that statistically infers kinetic parameters, which can then be fed back to the
613 mechanistic component for prediction of system performance.
614
615 The concept has been proven valid by a recent study,[31] in which a genomics-enabled hybrid
616 model was implemented for bioelectrochemical systems based on 77 samples collected from 13
617 publications. The mechanistic component of the hybrid model was built to estimate the
618 maximum growth and substrate utilization rates of three functional populations: fermentative,
619 electroactive, and methanogenic microbes, which were subsequently combined with the relative
620 abundances of 38 core taxa at the genus level to train a hybrid Bayesian network (Figure 6A).
621 When examined with six new samples that were not included in network training, the hybrid
622 model achieved the most accurate prediction of current production (Hybrid + Mechanistic in
623 Figure 6B) compared with standalone data-driven models. The enhanced prediction performance
624 of the hybrid model likely results from the close connection between population dynamics and
625 microbial kinetics.
626
627 An alternative strategy to incorporate genomic data into hybrid modeling is to replace Monod
628 expression-based model structures in conventional mechanistic models by simulating microbial
629 population dynamics. This can be achieved with an iterative strategy, in which the data-driven
630 component trained with processed genomic data infers instantaneous biochemical and microbial
631 intermediates, and the intermediates are fed into the mechanistic component to predict steady-
632 state biochemical outputs. This novel strategy mimics microbial community assembly driven by
633 environmental perturbations in engineered systems:[156–158] operating conditions and biochemical
634 inputs impose selective pressure and together shape the microbial community structure, and the

enriched functional populations produce biochemical intermediates that are rapidly mixed with the inputs to form a new steady-state, causing the community structure to further shift until the biochemical outputs reach equilibrium. The proposed strategy thus frees models from microbial kinetics-based structures, while the abiotic and microbial processes and their interplay are revealed in each iteration. Successful modeling with this strategy relies on the ability of hybrid models to act as a community simulator to predict microbial taxon abundance and reconstruct microbial communities *in silico*. For example, Bayesian networks could infer the relative abundances of dominant taxa, resulting in Bray-Curtis similarity of over 90% between the simulated and observed microbial communities at the phylum level.[156] However, the similarity dropped to 83% at the order level and 69% at the OTU level, likely because of the presence of functionally redundant taxa in the small data pool. The potential of the proposed strategy warrants investigation with big data collected from global databases.

6. Conclusions

This review focuses on three major types of models: mechanistic, data-driven, and hybrid models. Mechanistic models can provide fundamental insights but need laborious calibration because the Monod-based model structure is inadequate to reflect the biological principles whilst the microbial kinetic parameters are largely unidentifiable. As a result, a mechanistic model built for a specific system frequently falls short when applied to other bioprocesses. Data-driven models can provide predictive insights but yield uninterpretable predictions due to their black-box nature. Hybrid models are believed to overcome the issues of structural inadequacy, parametric unidentifiability, and uninterpretable prediction of the standalone models. Recent biotechnological development such as high throughput sequencing data and omics-based analysis

658 can further enable the incorporation of microbial population and functional dynamics into the
659 model to directly reflect the biological principles. Genomics-enabled hybrid modeling strategies
660 require the mechanistic and data-driven components to be integrated interactively. Two strategies
661 are proposed: kinetics simulator and community simulator, and their applicability warrant further
662 studies. Although hybrid models can potentially overcome the drawbacks of standalone models,
663 the main rationale of modeling selection and design is largely dependent on its intended use.
664 Additionally, the availability of omics-based data and computational cost require more effort in
665 preparation, collection, process, and analysis, which demands technical labor, time, and financial
666 investment. All these factors need to be taken into consideration when modelers design and
667 modify existing models.
668

669 **Conflicts of Interest**

670 There are no conflicts of interest to declare.
671

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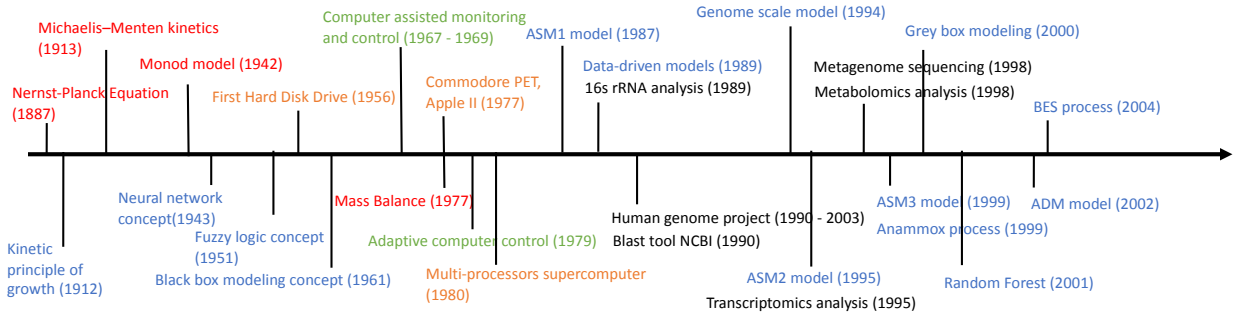
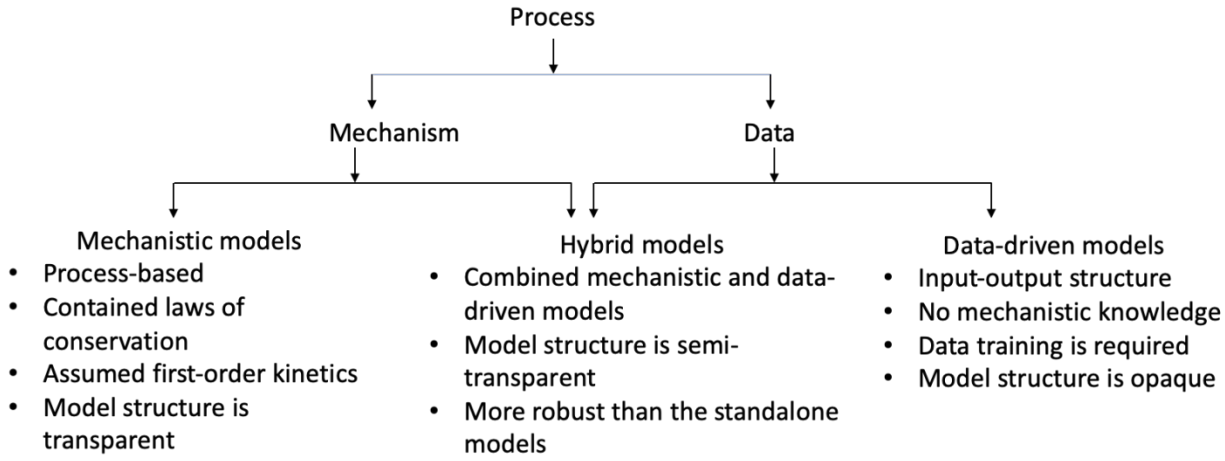


Figure 1. Timeline of model concepts (red), model strategies (blue), control and monitoring development (green), computational infrastructure (orange), and development of molecular biology and bioinformatics (black).



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1097 Figure 2. The characteristics and advantages of mechanistic, data-driven, and hybrid models

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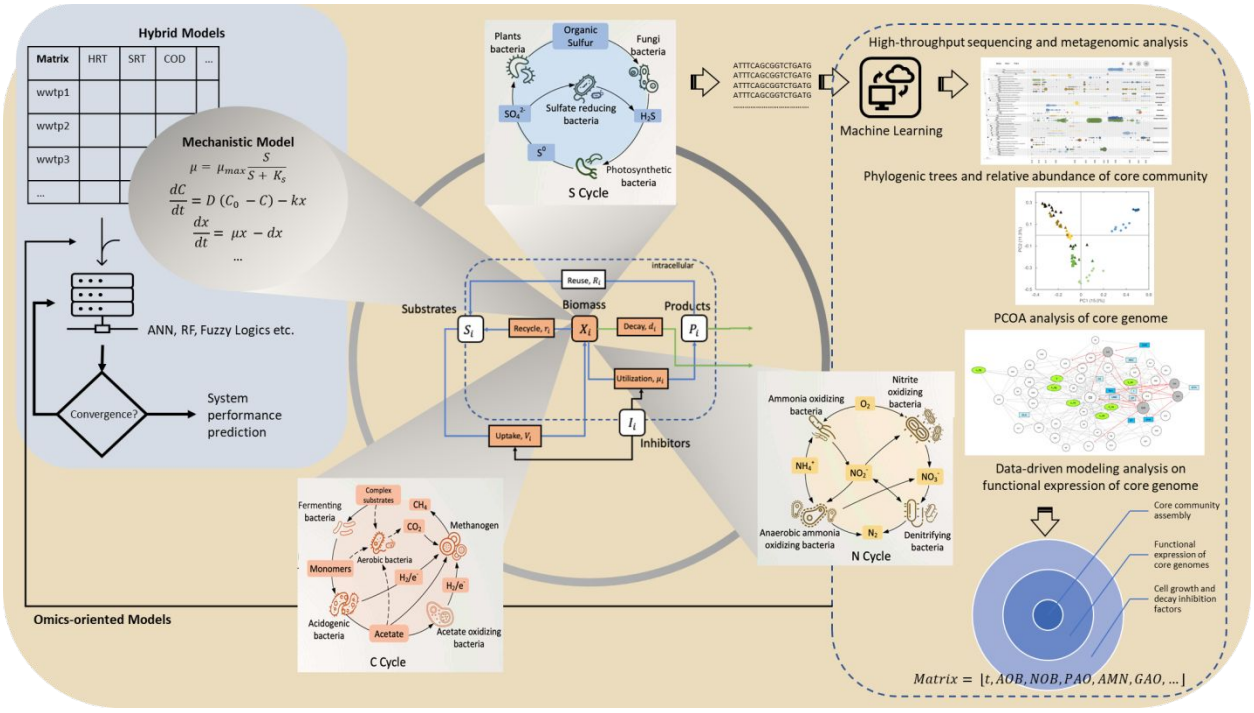
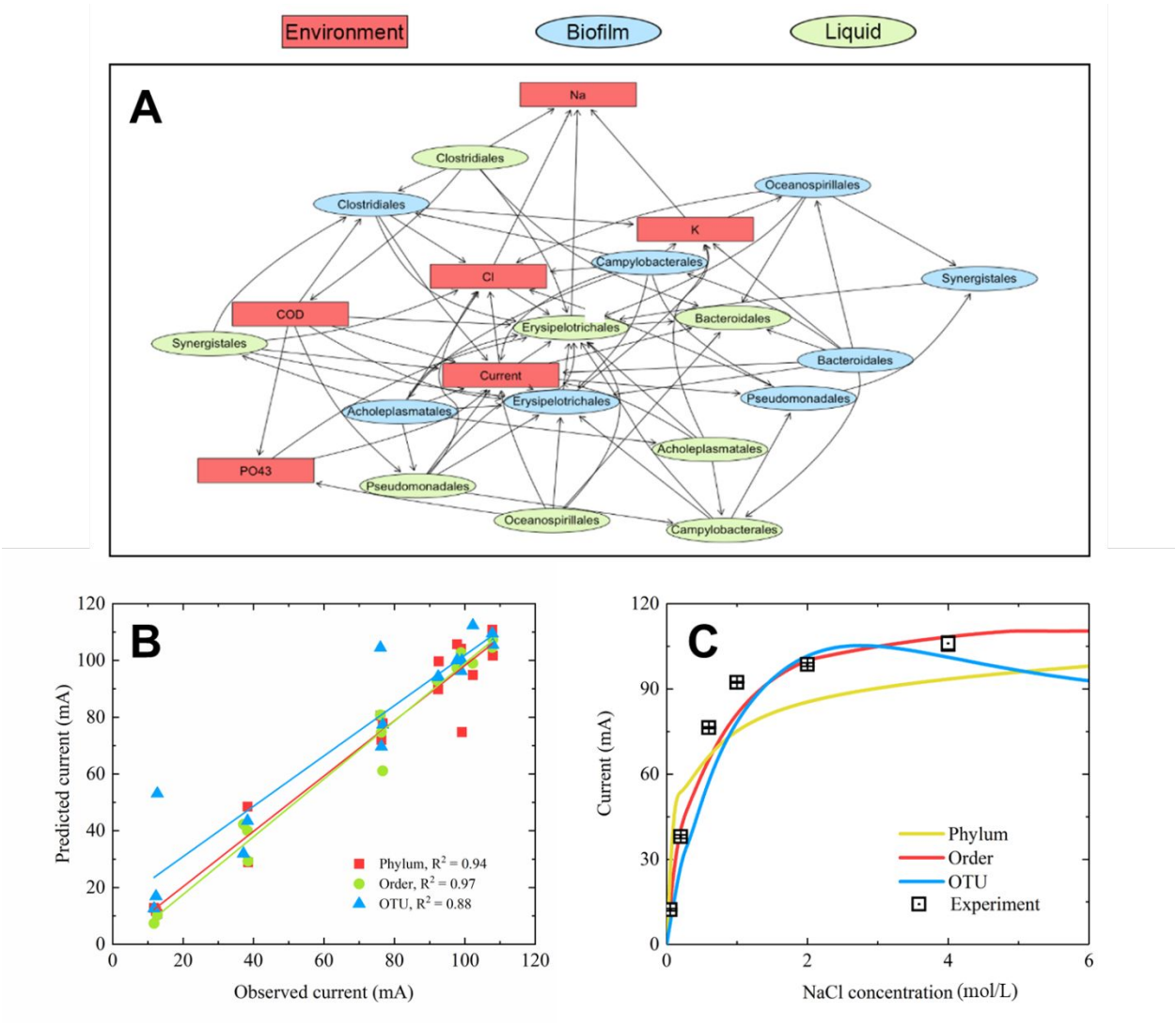


Figure 3. Incorporation of genomic data into model construction. The diagrams of S-, N-, and C-cycle are originated from the study of Wu and Yin[7]. The figures of phylogenic trees, PCoA, and data-driven modeling analysis on functional expression are based on the study of Cheng et al.[31]



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1104 Figure 4. (A) A Bayesian network trained with the microbial population dynamics at the order
1105 level in a bioelectrochemical system. (B) Predicted vs. observed current production. (C)
1106 Prediction of current production as a function of substrate salinity. Figures adapted from Yuan et
1107 al.[154]

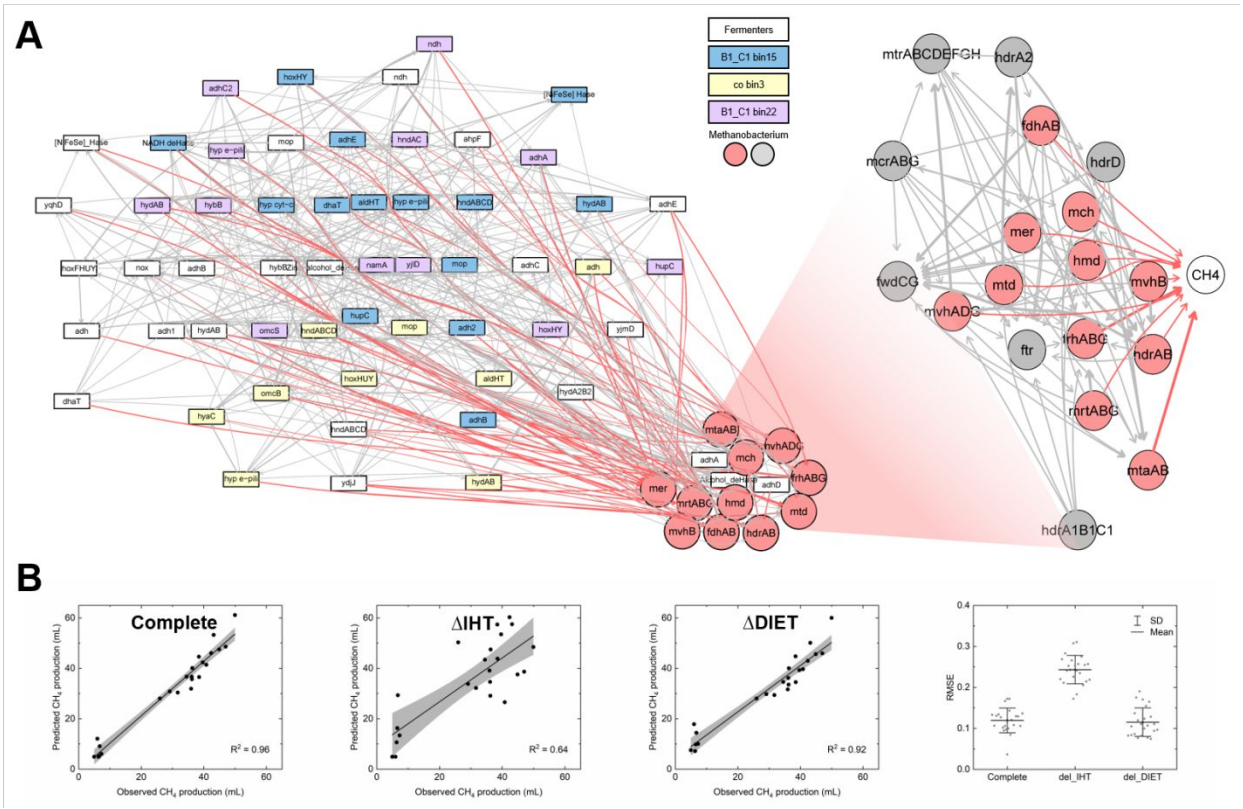

Figure 5. (A) A Bayesian network trained with the genes for alcohol metabolism, hydrogen metabolism, direct interspecies electron transfer, and methanogenesis from dominant microbes. (B) Prediction of methane production with a complete Bayesian network and in-silico knockout of relevant genes. Figures adapted from Yuan et al.[30]

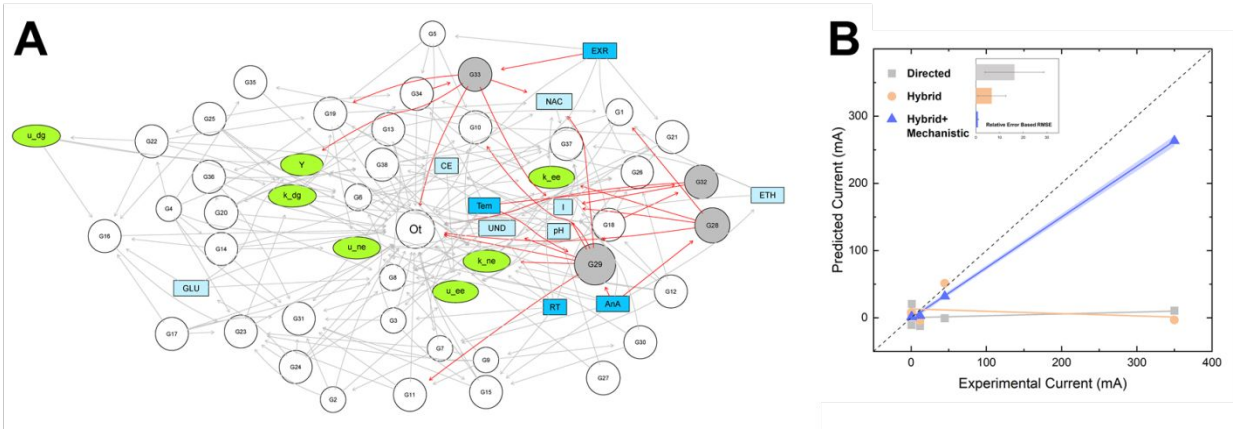

Figure 6. (A) A Bayesian network as the data-driven component of the hybrid model was trained with microbial population dynamics and microbial kinetic parameters estimated from the mechanistic component (green oval nodes). (B) Predicted vs. observed current production. Figures adapted from Cheng et al.[31]