
Application of Hybrid Neural Models to Bioprocesses: A Systematic Literature Review

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Artificial neural network; Bioprocess; Hybrid model; Systematic literature review

Abbreviations

Hybrid artificial neural network (HANN)

Genome-scale Models (GEM)

Monoclonal antibody (mAb)

Design of experiments (DOE)

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)

Radial Basis Function Network (RBFN)

Non-linear programming (NLP)

Chinese hamster ovary (CHO)

Proportional–integral–derivative controller (PID controller)

Process analytical technology (PAT)

Chemical oxygen demand (COD)

Artificial neural network (ANN)

Genetic algorithm (GA)

Feedforward neural networks (FFNN)

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Abstract 53

Due to the complexity of biological processes, developing model-based strategies for monitoring, optimization and control is nontrivial. Hybrid neural models, combining mechanistic modeling with artificial neural networks, have been reported as powerful tools for bioprocess applications. In this paper, a systematic literature review is presented focused on the application of hybrid neural models to bioprocesses by Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) over the last 30 years. This analysis showed that hybrid neural modeling has covered a wide range of microbial processes, animal cells, mixed microbial cultures, and enzyme biocatalysis. Hybrid neural models have been mainly applied for predictive modeling/process analysis, process monitoring/software sensors, open- and closed-loop control, batch-to-batch control, model predictive control, intensified design of experiments, process analytical technology, quality-by-design, and more recently, digital twins. Hybrid modeling experienced a decline in the number of publications after a peak in 2004 and is now surging again. A “model scale” research gap was identified, which will likely narrow by a better integration with deep learning and systems biology in the near future. The biopharma sector is currently a major driver but applications to biologics quality attributes (e.g. glycosylation), new modalities and downstream unit operations are significant research gaps.

1 Introduction

The use of mathematical models for bioprocess monitoring, optimization, and control has a long history in tandem with the progress in computation power and process analytical technology^[1]. Among the many modeling theories applied to bioprocesses, mechanistic modeling has been the preferred approach. As early as mid-70s, Cooney et al. reported an unstructured bioprocess model (elemental material balances combined with macroscopic material balances and off-gas analysis) for computer-based bioprocess monitoring and control^[2]. Bioprocess mechanistic modeling has been however hindered by the lack of fundamental knowledge compared to other engineering fields. With the emergence of systems biology in the early 2000s^[3], several Genome-scale Models (GEM) have been reconstructed for industrially relevant cell factories. GEMs represent significant progress in the mechanistic understanding of cell factories but they are incomplete and difficult to deploy in practice. GEMs are typically large-scale (with 1000s of reactions) with implicit kinetics and regulatory processes that are largely unknown. Due to the complexity of biological systems, hybrid mechanistic/machine learning has emerged as a cost-effective methodology for bioprocess applications^[3-7]. Hybrid models have been classified as semiparametric models as they combine parametric and nonparametric functions in the same mathematical structure^[8]. The parametric functions are derived from knowledge of well-established mechanisms. They have a fixed mathematical structure and a fixed number of parameters with physical interpretation. On the contrary, the nonparametric functions are entirely derived from process data. They have a loose structure without physical interpretation. Examples of the latter are artificial neural networks, which have the job to learn from data the cause-effect relationships lacking mechanistic explanation^[4]. Hybrid modeling may thus be seen as a methodology to augment existing but incomplete mechanistic models with machine learning techniques.

The bioprocess engineering community has embraced this challenge since the early 90s as measured by the growing volume of publications on this topic. Recently, some review papers have examined theoretical and practical challenges directly or indirectly touching the hybrid modeling field. Tsopanoglou et al. recently reviewed mechanistic, statistical, and hybrid modeling strategies for monoclonal antibody (mAb) upstream processing. They compared these three modeling techniques and emphasized the high potential of hybrid models in the era of Biopharma 4.0^[9]. Vinzenz *et al.* (2018) reviewed the state-of-the-art of model-based tools for experimental design and concluded that combining a hybrid model with the design of experiments (DOE) is a powerful tool for process development. The resulting “smart DOE” technique reduced the number of iterations and the overall duration of process validation^[10]. Narayanan *et al.* (2019) reviewed model-based methods for Industry 4.0 emphasizing the potential of hybrid modeling to fulfill Industry 4.0 challenges^[11]. Rajulapati *et al.* reviewed the hybrid modeling field in a systems engineering perspective^[12]. There are different ways to combine mechanistic models with machine learning into hybrid structures with particular identification challenges^[12]. Serial and/or parallel hybrid structures may be static or dynamic, each of them requiring different training methods.

With improved data repositories by new data lakes in the biomanufacturing industry, more opportunities will likely be created for machine learning and hybrid modeling in particular. Managing and taking advantage of hybrid neural models, traditional data, and big data may have a prominent role in the deployment of industry 4.0^[13]. In this study, we present a systematic literature and meta-analyses (PRISMA) explicitly focused on the application of hybrid neural network (HANN) models to bioprocesses. HANNs are a subclass of hybrid models that combine artificial neural networks with mechanistic models. Although other machine learning techniques have been combined with mechanistic modeling, artificial neural networks have been by far the

preferred technique in a hybrid modeling context. The following fundamental research questions guided the present literature review:

Q1: In which bioprocesses have HANNs been applied?

Q2: In which process steps could HANNs be applied and what is the potential benefit?

Q3: What is the state-of-the-art of HANNs applications to bioprocesses?

From the answers to these questions, a research gap analysis is performed and future directions are pointed out.

2 Hybrid artificial neural network (HANN) modeling

HANNs combine parametric functions based on knowledge of well-established mechanisms with artificial neural networks in the same mathematical structure^[8]. Many different hybrid model architectures have been published. The vast majority of HANNs reviewed here are well-represented or are extensions of the general bioreactor hybrid model represented in Figure 1^[14–19]. This model is briefly reviewed for context.

A stirred tank bioreactor is typically modeled by a system of ordinary differential equations (ODEs) derived from macroscopic material balances and/or intracellular material balances, taking the following general form:

$$\frac{dx}{dt} = f(x, \vartheta, u, t) \quad (1a),$$

$$y = h(x, \vartheta) \quad (1b),$$

with t the independent variable time, $x(t)$ the process state vector, $u(t)$ the vector of exogenous inputs, $\vartheta(t)$ a vector of variables with unknown defining functions, and $y(t)$ a vector of observable variables. These equations are of parametric nature with fixed structure stemming from prior knowledge. Some process variables lack mechanistic basis (e.g. biologic kinetics or physiochemical properties of molecular species pertaining to the state vector $x(t)$) are defined as loose nonparametric functions, $\vartheta(\cdot)$. HANN models use artificial neural networks to describe $\vartheta(\cdot)$ as function of the process state, $x(t)$, exogenous inputs, $u(t)$ and process

time, t . As shown in this review, shallow feedforward neural networks (FFNNs) (with 3 layers only) have found wide use in hybrid modeling. Taking the general case of deep FFNNs with arbitrary number of nh hidden layers, the HANN model is completed with the following equations:

$$H^0 = g(x, u, t) \quad (2a)$$

$$H^i = \sigma(w^i \cdot H^{i-1} + b^i), \quad i = 1, \dots, nh \quad (2b)$$

$$\vartheta(\cdot) = w^{nh+1} \cdot H^{nh} + b^{nh+1} \quad (2c)$$

A non-linear pre-processing function, $g(x, u, t)$, may be used to compute the FFNN inputs to improve the training. The $\sigma(\cdot)$ represents the nodes transfer function in the hidden layers. The nodes connection weights $w = \{w^1, w^2, \dots, w^{nh+1}\}$ and $b = \{b^1, b^2, \dots, b^{nh+1}\}$ are calculated during the training of the model. Most HANN studies adopted a training methodology based on the Levenberg-Marquardt optimization, indirect sensitivity equations, cross-validation and \tanh activation function. Recently, hybrid deep approaches have been proposed based on deep multilayered FFNNs, adaptive moment estimation algorithm (ADAM), semidirect sensitivity equations, stochastic regularization and rectified linear unit (ReLU) activation functions in the hidden layers. For further details, the reader is referred to Pinto et al., 2022^[19]. Lastly, the general bioreactor model (Eqs.1-2) may be easily adapted to a wide range of unit operations described by systems of ODEs.

3 Systematic literature review

This review focuses on the application of HANNs to bioprocesses. The preferred reporting items for systematic reviews and meta-analyses (PRISMA) methodology were adopted^[20]. For bibliometric analysis, the Mendeleev application allowed the extraction of metadata and the elimination of duplicates. For network analysis, the VOSviewer software tool (V1.6.18) has been applied to visualize the dataset's extracted information and obtain quantitative and qualitative outcomes. The collection of articles obeyed to the following principles (see appendix for details):

- Select articles from two databases, Scopus and Web of Science, based on the algorithms.

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- Collect the documents of some well-known authors on this topic and refine them by keyword screening 158
 - Select relevant articles cited by other articles (backward citation). 159

3.1 PRISMA Flow Diagram 160

The articles were selected based on a selection algorithm (Appendix 1) and the PRISMA flow diagram instructions [21], which are divided into four categories: 161

- Scopus: The algorithm initially retrieved 368 publications from the Scopus database and after screening 82 relevant cases were obtained. 163 164
- Web of Science (WoS): The algorithm initially retrieved 201 publications from the WoS database, and after screening 79 relevant cases were obtained. 165 166
- From the well-known authors' search, 685 publications were extracted, and at the final screening step 53 relevant cases were obtained. 167 168
- From the backward citation, 69 relevant cases were obtained. 169

After merging the articles and deletion duplicates, 162 publications were selected for analysis (Figure 2). 170

3.2 Statistical analysis of the PRISMA output 171

In this literature review, we analyzed 162 journal and conference papers published before 18th February 2022. 172

The first report was in 1992, with a distinctive peak in 2004 (15 papers) followed by a decline. The number of papers is surging again (Appendix 2-Figure 4). 173 174

The first ten document sources that have published the highest number of articles, their ranking, publisher, and H-Index are summarized in Table 1(Appendix 2). Among the 162 papers, the ten most cited papers per year are listed in Table 2 (Appendix 2). 175 176 177

It is also apparent in the subject area analytical report of Scopus that over the three decades, two subjects, "chemical engineering" and "Biochemistry, Genetics, and Molecular Biology" stand out with the highest number of publications. "Energy" and "Environmental Science" are two subjects that have attracted more attention since 2000. As expected, in the second decade (2001-2010), research on computer science subjects has grown significantly to develop methods in this field (Appendix 2-Figure 5). 178 179 180 181 182

3.3 Keywords Analysis

The author's keywords (included in the keyword section of the article) were analyzed at first. Additionally, indexed keywords (Indexed keywords are chosen by the database and are standardized to vocabularies derived from thesauri) were also analyzed because some articles did not specify the author's keywords. Keyword analysis and visualization were performed with the help of VOSviewer. Firstly, the cooccurrence of authors' keywords was analyzed with the full counting method and two times occurrences (in two different papers) as the minimum. Then, similar keywords were harmonized, and finally uninformative keywords were omitted, such as hybrid model, artificial neural network, and modeling. As a result, 29 author's keywords were obtained (Appendix 2-Figure 6) (Appendix 2-Table 3).

The visualization showed that hybrid models were first applied to the production of antibiotics. Then, it continued with wastewater treatment, ethanol production, design of experiments (DoE), process analytical technology (PAT), and quality by design (QbD). Recently some subjects, such as biopharmaceuticals and big data, have appeared. The "E. coli", "Bordetella pertussis", and "Bacillus Thuringiensis" are microorganisms that appeared in this analysis.

3.3.1 Keywords Occurrence Over Publication Year

The co-occurrence of all 359 keywords (author's keywords and indexed keywords) was analyzed over time. Figure 3 (Appendix 2) summarizes all keywords' occurrence over publication year. Due to the large number of keywords, three time periods were considered.

3.3.1.1 Keywords occurrence from 1992 until 2000

In this period, 157 keywords were identified, which were reduced to 22 by the previously described keyword analysis algorithm. Based on the information output of keywords occurrence by year overlay visualization (Appendix 2-Figure 7), the HANN modeling was mainly applied to fermentation and enzymatic reaction in this period. Antibiotics and ethanol were the most frequent keywords referring to products. *Saccharomyces cerevisiae* and *Zymomonas Mobilis* were two microorganisms that appeared in this period. Computer

simulation, Kalman filtering, functional link networks, and heuristic methods were combined/compared with the HANNs to control the process or to optimize and/or to estimate process parameters.

3.3.1.2 Keywords occurrence from 2001 until 2010

In this period, 728 keywords were identified, and then reduced to 63 by keyword analysis. Some new keywords appeared such as recombinant proteins, immunoglobulin g1, interleukin2, hydroxybutyrate, mammalian cell, CHO, cell culture, and system biology. Wastewater, immobilized enzyme, and fungal culture are other keywords that appeared during this period (Appendix 2-Figure 8). The “E. coli”, “Saccharomyces cerevisiae”, and “yeast” are subject microorganisms that appeared in this period.

3.3.1.3 Keywords occurrence from 2011 until February 2022

In this period, 935 keywords were identified, and then reduced to 53 by keyword analysis. New keywords appeared such as the design of experiments, critical process parameters, process analytical technology, digital twins, and big data. These are now hot topics for the application of hybrid models to bioprocesses. Moreover, fuzzy neural network, genetic algorithm, deep neural network, and intensified design of experiment are keywords that appeared in this period. This result suggests a growing interlink between the areas of machine learning and hybrid modeling (Appendix 2-Figure 9). The “E. coli”, “Bordetella pertussis”, “yeast”, “microalgae”, and “Saccharomyces cerevisiae” are subject microorganisms that appeared in this period.

4 Discussion

4.1 The onset of hybrid modeling

The first HANN application to bioprocesses was reported by Psychogios and Ungar (1992), where the authors compared hybrid modeling, Kalman filtering, non-linear programming (NLP), and standalone neural networks for predictive modeling and state estimation in a fed-batch bioreactor^[4]. The HANN model

consisted of a feedforward neural network (FFNN) (with a single hidden layer) connected with a system of ordinary differential equations derived from macroscopic material balances (well represented by the general bioreactor hybrid model of Fig. 1). The indirect training method using the sensitivity equations was introduced in this study, which allowed to train the FFNN by error backpropagation in a similar fashion to a standalone FFNN. The authors concluded that extended Kalman filtering and NLP estimation performed better than the hybrid approach when a detailed mechanistic model is available. If however, the mechanistic model is incomplete or unreliable, then the hybrid model outperformed the Kalman filtering and NLP estimation. The key messages of this pioneering study were: 1) effective indirect training of the FFNN using the sensitivity method, 2) hybrid models are more flexible (better interpolation) than standalone mechanistic models, and 2) hybrid models have better generalization properties and are easier to interpret than standalone neural network models.

Shortly after, Thompson and Kramer (1994) conducted a study in which they applied a hybrid model to a fed-batch penicillin fermentation. The hybrid model consisted of a Radial Basis Function Network (RBFN) connected in parallel with a mechanistic kinetic model to calculate specific kinetic rates. The RBFN worked as residual model to correct the output of the fundamental kinetic model. The kinetic rates were then fed to the bioreactor material balance equations (connected in parallel with the kinetic models). This pioneering study has framed hybrid models as parallel and/or serial semiparametric mathematical structures that may grow in complexity depending on prior knowledge. They also concluded that less data is required for parameter estimation, and more accurate and consistent prediction of the hybrid model are obtained in comparison to the standalone mechanistic or neural network models [6].

Schubert et al. (1994) studied the application of hybrid models for state and parameter estimation, feed rate optimization (open-loop control problem), and closed-loop control of a fed-batch baker's yeast process. While Psychogios and Ungar^[4] and Thompson and Kramer^[6] used synthetic data, Schubert et al. (1994) addressed a real-life problem. The hybrid model consisted of a simultaneously serial and parallel structure composed of a FFNN (with 12 inputs, single hidden layer with 10 nodes and 3 outputs), dynamical ordinary differential

equations and a fuzzy expert system to decide on which process conditions the FFNN predictions are reliable. This pioneering study pointed out for the first time to the need of reliability monitoring of the neural network outputs outside the training domain and to adjust the model accordingly. A simple rule-based expert system was adopted for this purpose. It was concluded that process optimization and control based on hybrid models have a higher benefit/cost ratio than other methodologies [22,23].

4.2 Microbial culture

In the early years, hybrid models were mainly applied to traditional microbial processes such as Baker's yeast [22,23], antibiotics production [24] and beer/bioethanol production [25–29]. Preusting et al. (1996) addressed the historical penicillin process at the production scale [24]. The hybrid model structure was similar to Schubert et al. (1994) [22], using an expert system to weight the outputs of the neural network and fundamental kinetic model depending on the reliability of the FFNN. This model was used to optimize (open-loop control problem) the large-scale production of penicillin using the HYBNET software.

Simutis *et al.* (1998) developed a simple hybrid model of *S. cerevisiae* showing successful predictive modeling of Diacetyl formation during beer production in a production plant [30]. In the study by da Silva Henriques et al (1999) a hybrid model for the alcoholic *Zymomonas mobilis* fermentation was developed [28]. The model consisted of a 3-layers FFNN (describing the kinetic rates) connected in series with macroscopic mass-balance equations. Meleiro et al. (2000) applied a hybrid model for dynamic modeling and control of an industrial-scale *S. cerevisiae* fermentation process for bioethanol production. They combined a 3-layers FFNN with macroscopic material balances and simple Monod-type kinetics. The hybrid model provided online estimations of key process state variables and kinetic parameters based on reliable and easily accessible measurements. It enabled the implementation of efficient automatic control strategies [25]. Recently, hybrid modeling of bioethanol production was revisited by Da Silva Pereira et al. (2021) [31]. Bioethanol was produced by a flocculating yeast grown on cashew apple juice. A hybrid model was built consisting of three shallow FFNN combined with mass balance equations (biomass, substrate, and product). Particle swarm optimization (PSO)

was adopted to optimize bioethanol production (open-loop/dynamic optimization problem) at aid of the hybrid model, thereby achieving high yield and productivity.

Hybrid modeling of the *Pichia pastoris* yeast for recombinant protein production was first addressed by Ferreira et al. (2014) Ferreira^[17]. The authors applied a serial hybrid model (3-layers FFNN combined with material balance equations in series) for dynamic modeling of *P. pastoris* GS115 expressing scFv in a pilot 50L bioreactor. The hybrid model was subsequently employed for iterative batch-to-batch control showing a four-fold titer improvement after 4 optimization cycles. Batch-to-batch control using hybrid models and evolutionary programming was addressed in a simulation study by Teixeira et al. (2006)^[32]. Constraining the optimization design space depending on the reliability of the FFNN was shown to be essential to ensure stable convergence to the global optimum. Another important point is that the hybrid model should not include “wrong” mechanisms or otherwise an off-set to the global optimum is observed. Recently, hybrid modeling of *P. pastoris* was revisited by Pinto et al. (2022) using state-of-the-art deep learning methods^[19]. FFNN networks with varying depths and rectified linear unit (ReLU) nodes were combined with material balance equations in the form of deep hybrid models. Deep learning techniques, namely the adaptive moment estimation method (ADAM), stochastic regularization and depth-dependent weights initialization were evaluated in a hybrid modeling context. The semi-direct training method was proposed to reduce the CPU time of the sensitivity equations, which then become independent of the size and depth of the neural network. The CPU time for training deep HANN models was significantly reduced.

Recombinant *E. coli* was addressed by von Stoch *et al.* (2016,2017), who introduced the methodology of intensified design of experiments (iDoE) coupled with dynamic hybrid modeling^[33,34]. This approach was applied to an industrial *E. coli* process expressing a therapeutic protein. iDoE is a dynamic design of experiments based on intraexperiment step changes of design factors (such as pH, temperature and feed rates) and dynamic modeling. Contrary to standard DoE, different combinations of process conditions are explored step-wise in the same experiment. A serial hybrid dynamic model (FFNN + Material balance equations) was adopted to captured the dynamic relationship between step-wise variations of design factors and process

response variables. The authors concluded that intraexperimental variations of process conditions could reduce the number of experiments by a factor, which in limit would be equivalent to the number of intraexperimental variations per experiment. Bayer et al. (2020) further explored the iDoE methodology in a 20L fed-batch *E. coli* process expressing hSOD. The hybrid model could accurately predict the endpoint biomass concentration and product titer as well as the respective time-resolved trajectories^[35]. These studies emphasized the potential of hybrid modeling to address the challenges of Process Analytical Technology (PAT) and Quality by Design (QbD) in the biopharma sector.

Hybrid modeling of *biopolymers* production by bacteria has been addressed in several studies^[36-39]. One of the first studies addressing Polyhydroxyalkanoates (PHA) production by bacteria was reported by Peres et al (2004)^[39]. A competitive hybrid structure was applied where a gating system was trained with the expectation maximization (EM) algorithm to learn in which regions of the input space the FFNN performs better than the competing mechanistic model. This hybrid approach was further detailed in a follow-on paper applied to baker's yeast^[40]. Production of Poly- β -hydroxybutyrate (PHB) by *Ralstonia Eutropha* has been addressed by Patnaik et al. (2008)^[41]. The authors demonstrated the superiority of the hybrid model (H-model) to the neural-cum-dispersion model (D model) and to the neural network model (N-model). Recently, Luna et al. developed a HANN model with four layers of five nodes to describe the continuous production of PHA by *Pseudomonas Putida* GPo1^[42]. The hybrid model was shown to describe the process in a wide range of operating conditions, including single and dual nutrient-limited growth conditions.

4.3 Animal cell culture

Animal cells are ubiquitous in the biopharmaceutical industry and are getting momentum in the hybrid modeling community^[7,35,43-47]. One of the first hybrid modeling studies of mammalian cell culture was reported by Dors et al. (1995)^[48]. Fu and Barford (1996) reported one the first consistent HANN model applications to animal cells' monoclonal antibody production. The HANN model predicted substrate consumption, toxic by-product accumulation, cell growth, cell composition, and metabolic product formation^[49]. They reached a

better result compared to the standalone mechanistic model or artificial neural network model. Teixeira et al. (2005) developed a hybrid model for BHK-21 cultures expressing the fusion glycoprotein IgG1-IL2^[50]. The model consisted of a simultaneously serial and parallel structure for dynamic predictive modeling. A shallow FFNN was connected in parallel with a fundamental kinetic model. The experience measure technique was adopted to automatically switch between the fundamental model and FFNN predictions depending on the reliability of the latter. The hybrid model was used to optimize the feeding strategy (dynamic optimization / open-loop control problem) of glucose and glutamine. Later on, Teixeira et al (2007) extended the hybrid model to incorporate knowledge of the metabolic network using the concept of elementary flux modes^[15]. The resulting hybrid model predicted simultaneously extracellular concentrations and intracellular fluxes. The model was adopted for on-line optimizing control, which delivered a 10% titer increase in relation to the control experiment. This was one of the first attempts to include metabolic detail in hybrid models. Recently, Maton et al. (2022) applied a similar elementary flux modes hybrid model to a hybridoma HB-58 cell line^[51]. Aehle et al. (2010) developed a serial hybrid model for on-line estimation of viable cell concentration in fed-batch CHO cultures^[52]. The authors concluded that the hybrid model outperformed other data-based and model-based techniques. Narayanan et al. (2019) developed a serial hybrid model (FFNN with one hidden layer connected to material balances) for a CHO fed-batch process (81 batches in a 3.5-liter bioreactor) ^[53]. They used the mass balance equation as the mechanistic part of the hybrid model to predict the process variables (substrate and metabolite concentration, cell density, and product concentration). The hybrid model revealed a superior capability to predict CHO dynamics using only the initial and process conditions as inputs, in comparison to other statistical modeling methods. Kotidis *et al.* (2020) developed a complex serial hybrid model to describe N-glycosylation of recombinant proteins in CHO cultures ^[54]. The hybrid model utilized the extracellular concentration of metabolites and certain amino acids as inputs. A metabolic module calculated the specific growth rate and the specific antibody production rate. These rates were then fed to a nucleotide sugar donors module that calculated the respective intracellular concentration. The nucleotide sugar donors concentrations were then inputted to a 4-layers FFNN, which calculated glycans distribution. The overall hybrid model successfully simulated the glycoforms distribution of four different proteins (two IgGs and two

fusion proteins, EPO-Fc and Fc-DAO) expressed in three CHO cell lines (GS-CHO, CHO-K1, CHO-S). This study was one of the first addressing hybrid modeling of glycosylation. As follow up of the previous E. coli study, Bayer et al (2021) applied the same iDoE/hybrid modeling approach to reduce the validation burden of CHO cultures in a PAT and QbD context^[55]. They also investigated the transferability of hybrid modeling along process scales (300 mL shaker-scale and 15 L bioreactor). The authors concluded that the hybrid model trained on 300 ml bolus feeding shake flask DoE could be used to correctly estimate the cell behavior and product formation in a 15 L stirred-tank bioreactor.

4.4 Mixed Microbial Cultures

Mixed microbial cultures (MMC) are of widespread use in waste treatment plants commonly termed as activated sludge. Reducing wastewater treatment costs has long been of interest, and modeling has proven an essential tool to optimize wastewater treatment plants (WWTP). Due to the intrinsic complexity of WWTP, some researchers combined a hybrid artificial neural network with other methods or models to estimate and/or control process parameters. Côté et al (1995) reported one of the first studies where a hybrid model was applied to a WWTP^[56]. A mechanistic model was combined with a three-layer FFNN in parallel. The job of the FFNN was to extract cause-effect patterns from the mechanistic model residuals, thereby correcting its outputs (residual modeling strategy). The parallel coupling of the mechanistic model with the FFNN provided more accurate simulations of five key variables of the activate sludge process. Zhao et al. (1997) developed a hybrid dynamic model of a Sequencing Batch Reactor (SBR) consisting of a simplified mechanistic model and a FFNN connected in parallel also following the residual modeling approach^[57]. Anderson et al. (2000) applied different hybrid mechanistic/FFNN models for dynamic modeling of WWTPs and process control^[58]. They concluded that hybrid models do not necessarily produce superior control results. Sung Lee et al. (2002) applied a parallel hybrid model to a full-scale WWTP^[59]. The authors reported more accurate predictions with good extrapolation properties of the hybrid model compared with other modeling approaches. Fang and Dai (2003) developed a simple hybrid model for chemical oxygen demand (COD) prediction^[60]. Azwar et al. (2006) proposed a hybrid FFNN/Proportional Integral controller of dissolved oxygen concentration in a SBR^[61]. The hybrid control scheme consisted of a basic FFNN controller in parallel with a proportional integral

(PI) controller. This approach was shown to outperform other nonhybrid control schemes. Peres et al. (2006) reported a hybrid modular model applied to a phosphorous removal WWTP^[62]. The hybrid model consisted of a mixture of experts (ME) network and a gating system connected in series with material balance equations. This serial/parallel hybrid structure with competing expert networks was trained with the EM algorithm. The final ME network was shown to better represent the cellular kinetics structure, which resulted in higher accuracy and generalization capacity of the hybrid model. Xiao et al. (2020) developed a hybrid model of dark fermentation for biohydrogen production. A NARX-BP hybrid neural network consisting of a two-stage model was developed, which combined NARX (nonlinear autoregressive exogenous) and BP-NN (Back propagation neural networks). The model could predict biogas production with high accuracy^[63]. Cheng et al. (2021) proposed a complex hybrid model that combined the Activated Sludge model (ASM) (knowledge-based model) and deep neural networks (data-based model)^[64]. For the latter, a convolutional neural network (CNN) was combined with a long short-term memory network (LSTM). The CNN was used to extract data spatial features whereas the LSTM was used to extract temporal features. The integration of knowledge- and data-based models in parallel was achieved with a FFNN model connected in series. This complex hybrid model was applied to a sewage treatment plant. It showed an improvement in the prediction accuracy in comparison with the typical existing models. The authors could also prove the hybrid model's stability by applying it to different datasets. This paper is one of the first hybrid modeling studies incorporating state-of-the-art deep neural networks and the ADAM training method.

4.5 Enzymatic bio-catalysis

Enzyme reaction mechanism can be quite complex and difficult to model mechanistically. A few studies applied HANNs to enzymatic conversion processes. van Can et al. (1998, 1999) addressed the problem of enzymatic conversion of penicillin G to 6-ami-nopenicillanic acid (6APA) and phenyl acetic acid (PhAH) by the enzyme penicillin acylase^[65,66]. The extrapolation properties of hybrid models combining FFNNs, white-box kinetics and macroscopic material balance equations were investigated. It was concluded that when the

macroscopic material balances are correctly formulated, the identification data only have to cover the amplitude domain of the rate terms without taking into account the future frequency domain of the complete model. Silva et al. (2008) developed a hybrid model of penicillin G acylase immobilized in chitosan for the production of amoxicillin^[67]. Three kinetic models were compared, namely a mechanistic, a semi-empiric, and a hybrid-neural model. It was shown that the hybrid model could accurately predict the reaction rates for conditions where the semi-empiric model failed (e.g. at low substrate concentrations occurring at the end of the fed-batch industrial process). This study did not explicitly consider the reaction-diffusion problem typically occurring in immobilized catalysis.

4.6 Downstream applications

The keyword analysis in this study clearly shows that only a few hybrid modeling publications have addressed downstream unit operations. One of the early studies was that by Piron et al. (1997), who applied a parallel hybrid model to cross-flow microfiltration in a baker's yeast process^[68]. The hybrid model consisted of a static FFNN connected in parallel with a dynamic material balance equation. They concluded that a recurrent neural network provided better approximation of process dynamics than the hybrid model and questioned the validity of the material balance equation. Rajabzadeh et al. (2012) estimated the filtration time and total solid concentration in the biomass leachate in permeate flux during reverse osmosis^[69]. They applied a FFNN with four neurons in a single hidden layer. The standard Levenberg–Marquardt algorithm was chosen to train the FFNN. The model predicted more than 80% rejection efficiency of calcium, magnesium, phosphorus, and silica (for three types of biomass leachate samples) in the reverse osmosis permeate. *Nagrath et al.* (2004) applied hybrid models to represent complex preparative chromatographic systems, thereby significantly reducing the computational time required for simulation and optimization^[70]. Other recent applications of hybrid models in chromatography are optimization, cleaning, and resin aging^[43,47,71]. Narayanan et al. (2021) compared a HANN model with a mechanistic lumped kinetic model. They applied these models to in-silico data, and the results showed a higher prediction accuracy of the HANN model^[72].

4.7 Research Gap

Based on information extracted from Scopus analysis (Figure 4), the publications number shows a peak in 2004 followed by a decline and recovery. HANN modeling is surging again with expected high growth in the near future. Neural networks applications to bioprocesses have shown similar publication dynamics, with an explosion in the 80s/early 90s followed by a prolonged decline. The resurgence of neural networks was triggered by advances in deep learning techniques, particularly the ADAM method with stochastic regularization that enabled efficient training of deep neural networks with innovative configurations such as the CNN and the LSTM. Most of the hybrid modeling studies so far applied simple shallow neural networks. With a significant delay, hybrid modeling is now incorporating some of the advances in deep learning^[19,64]. Pinto et al (2022) recently compared traditional shallow hybrid modeling (using the Levenberg-Marquardt training coupled with the indirect sensitivities, cross-validation and tanh activation function) with a novel hybrid deep modeling approach (using ADAM training, semidirect sensitivities, stochastic regularization, multiple hidden layers and ReLU activation functions)^[19]. A clear advantage of adopting hybrid deep models both in terms of predictive power and in terms of computational cost in relation to the shallow hybrid case is shown in this study. A significant result was that for the same problem the hybrid deep approach systematically generalized better than the shallow hybrid model. An “explosion” of hybrid deep modeling will likely be observed in the near future incorporating state-of-the-art neural network architectures and deep learning algorithms. As for the neural network research gap, published hybrid modeling studies also seem to be limited to relatively simple mechanistic models. There seems to be an overall “model scale” research gap. Large-scale hybrid models embodying complex and highly dimensional mechanistic models are almost absent in the literature. The penetration of systems biology techniques in routine bioprocess operation will likely challenge novel hybrid modeling methods and applications in the future. Genome-scale models (GEMs) of industrially relevant cell factories are continuously improving. Hybrid genome-scale models will likely fill this gap in the near future. An important tool will be the encoding of hybrid deep models in Systems Biology Markup Language (SBML).

Hybrid modeling applications in the biopharma sector are currently boosting particularly for Process analytical 458
technology (PAT)^[13,73-75] and quality by design (QbD)^[7,34,35,45]. Industry 4.0^[13,76], big data^[13,77], and digital 459
twin^[47,78] are recently added subjects that introduce new concepts that challenge the application of hybrid 460
modeling to the digitalization of biopharmaceutical processes. A report from an expert panel discussion of 461
European academics and industrialists has addressed the drivers, challenges, and enablers of hybrid modeling 462
applications in the biopharmaceutical industry^[45]. Several recommendations were drawn to enhance the ap- 463
plication of hybrid models for PAT and QbD. One of them was the need to increase the number of industrial 464
case studies describing the optimization of business relevant process variables. Significant achievements have 465
been reported on process validation using hybrid models and iDOE for *E. coli* and CHO culture^[33,46]. There is 466
however a clear gap in the application to new modalities such as cell-based therapies and nucleotide-based 467
therapies. Most of published studies are still focused on yield and productivity of upstream processes. Very 468
few studies incorporate critical quality attributes (CQAs) of biotherapeutics related to molecular properties 469
such as glycosylation patterns^[54], charge variants and aggregates. A major future challenge is the implemen- 470
tation of “platform hybrid models”. Platform hybrid models should bear the capacity to learn with experience 471
across different molecules and/or therapies. For platform models, a multi-scale vision is need that links the 472
molecular properties of the target biologic, the cellular biology of the host cell line and the macroscopic scale 473
of the production equipment. Hybrid modeling is in principle a strong candidate to address such complex 474
modeling problems filling this gap. 475

Lastly, the present literature review shows that most applications of hybrid models are found in upstream 476
operation steps. Few exceptions are found for membrane unit operations and chromatography. There is thus a 477
noticeable potential to apply hybrid models in downstream processing. Narayanan et al. (2020) noted that this 478
could be explained by the availability of well-established mechanistic models of many downstream 479

processes^[11]. Therefore, the benefit/cost ratio of hybrid modeling of downstream processes is potentially lower than for upstream processes. On the other hand, informative data of some downstream steps (such as lyophilization) is not easily available to academic researchers. Smyth *et al.* emphasized the difficulties to collect informative data sets in production facilities due to regulatory constraints^[79]. Lab or pilot scale data is more easily accessible but not necessarily representative of production-scale. Bourlès et al. (2019) did research on lyophilization scale-up challenges^[80]. The authors emphasized that some parameters, such as the vial heat transfer coefficient and equipment's sublimation capability are important parameters for model-based scale-up.

4.8 Study Limitation

This systematic literature review is focused on two databases (Scopus and Web of Science). To choose the synonyms of “hybrid model” as a search keyword, a large number of common synonyms were chosen that however do not prevent missing records. For example, synonyms such as "composite model," were not considered. Although we could only find one paper^[81] that contains a "composite model," bioprocess, and neural network, there might be some limitations in the search algorithm. To mitigate the possibility of missing relevant publications the study was complemented with well-known authors' search by their names. The well-known authors' publication records were then added to the repository of relevant cases. Two additional problems were identified; 1) Some of the articles did not have the author's keywords (even recent articles). 2) Although some articles had keywords, the databases' search engines could not find them. It seems that doing a systematic literature review and automatically choosing keywords may have some bugs in categorization.

5. Conclusions

In this paper, a systematic literature review on the application of hybrid neural models to biological processes is presented using the PRISMA method, which shows a structured vision of the research developed on the subject. Statistical analysis regarding the number of articles, subject area of interest, and keywords occurrence in the last 30 years was performed. Hybrid neural network modeling has covered a wide range of microbial,

animal cells, mixed microbial, and enzyme biocatalysis in different industries such as wastewater treatment, clean energy, biopolymers, and biopharmaceutical manufacturing. Hybrid models were mainly applied for process analysis, process monitoring, open- and closed-loop control, batch-to-batch control, model predictive control, intensified design of experiments, process analytical technology, and quality-by-design. Some recent “hot” topics such as big data, deep learning, industry 4.0, and digital twins are major drivers of hybrid modeling applications mainly in the biopharma sector. These topics will likely drive hybrid models to incorporate deep neural networks, deep learning methods, and systems biology tools in the near future. A significant research gap is identified in the application of hybrid models to downstream operations. Some very recent publications have addressed membrane processes and chromatographic processes. There is significant potential for research in applying hybrid models to filtration, adsorption, chromatography, membrane separation, lyophilization, and many more. Closing this gap will likely enable hybrid models to evolve towards plant-wide digitalization platforms integrating multiple up- and down-stream operations as the next big step in the future.

Author Contributions

Conceptualization, methodology, software, data curation, investigation, writing original draft preparation, visualization, and resources, Roshanak Agharafeie; writing review and editing, João R. C. Ramos; writing review and editing and supervision, Jorge M. Mendes, and Rui Oliveira; All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are openly available in “Mendeley Data” at <http://doi.org/10.17632/gng5bdjbmr.1>^[82].

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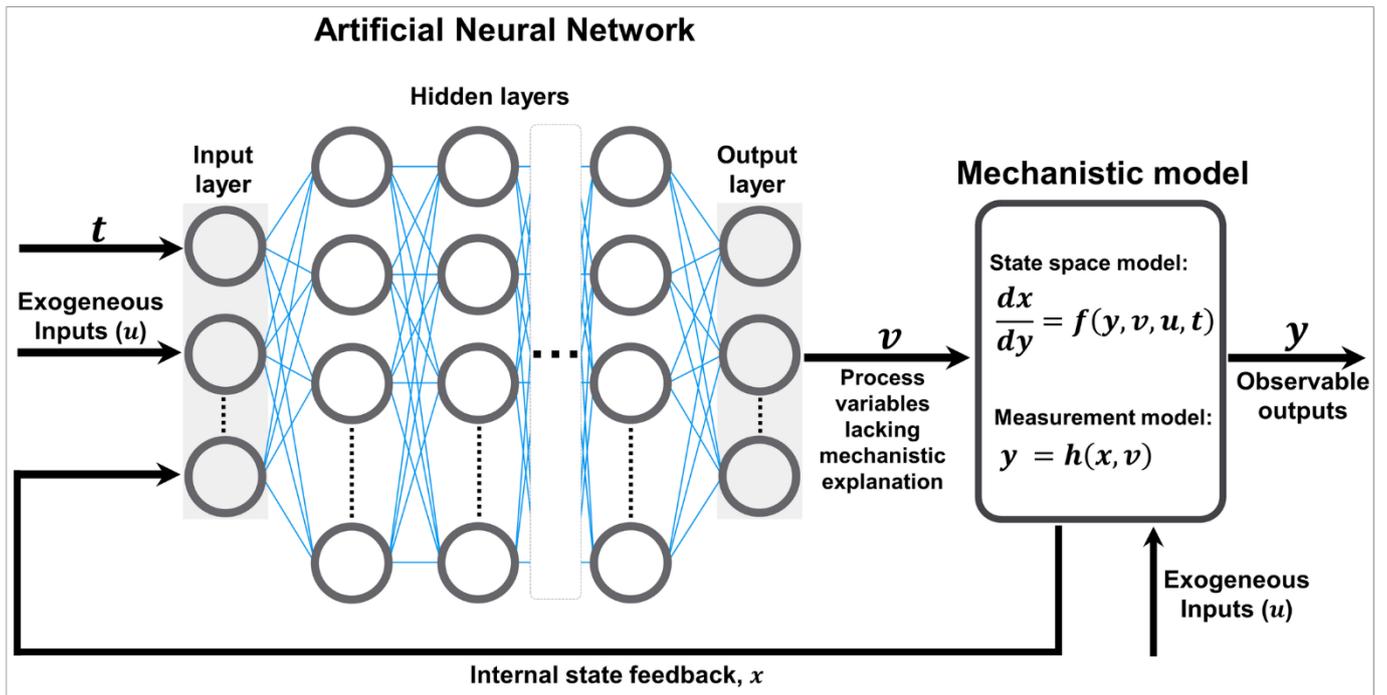
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Figures

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Figure 1- General deep hybrid model for bioreactor systems. The model has parametric functions (functions $f(\cdot)$ and $h(\cdot)$) with fixed mathematical structure (typically material/energy balance equations). Some process properties lacking mechanistic explanation are modelled by a feedforward neural network (FFNN) as function of the process state, x , exogenous inputs, u , and time, t . FFNN is a nonparametric function with loose structure that must be identified from process data given the absence of explanatory mechanisms for that particular part of the model. The model is dynamic in nature with state vector, x , and observable outputs, y .

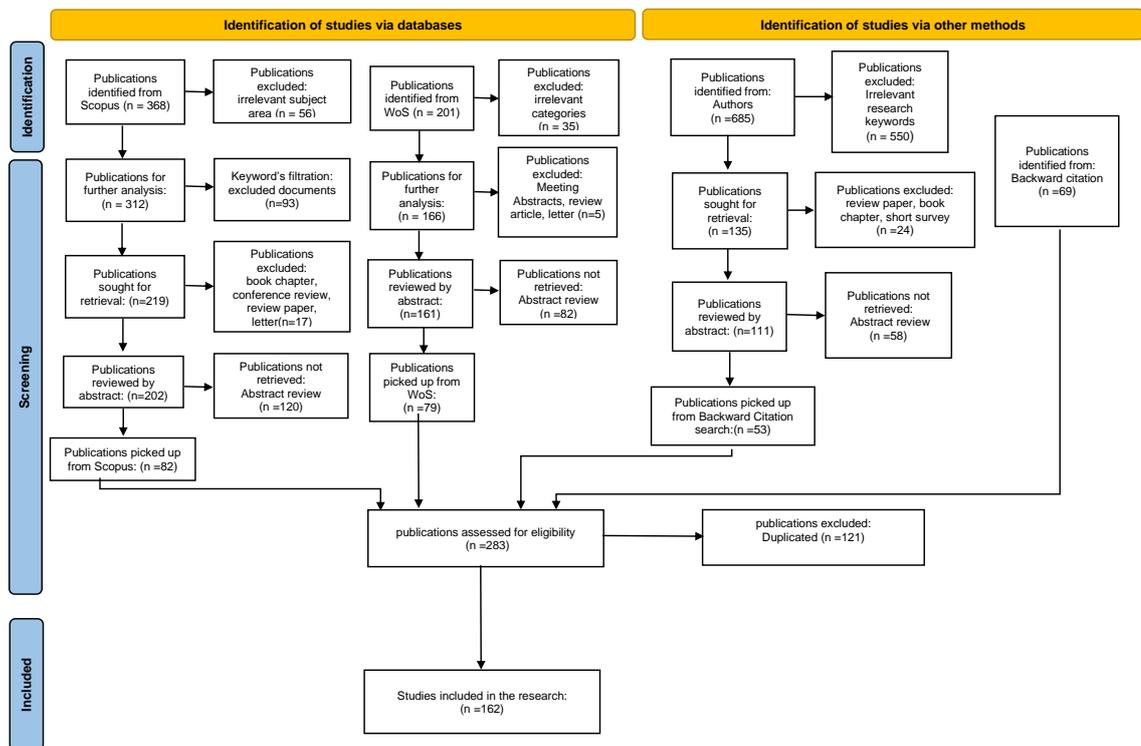
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Figure 2-PRISMA flow diagram summarizes the selection of the articles based on the algorithm (Appendix 1).

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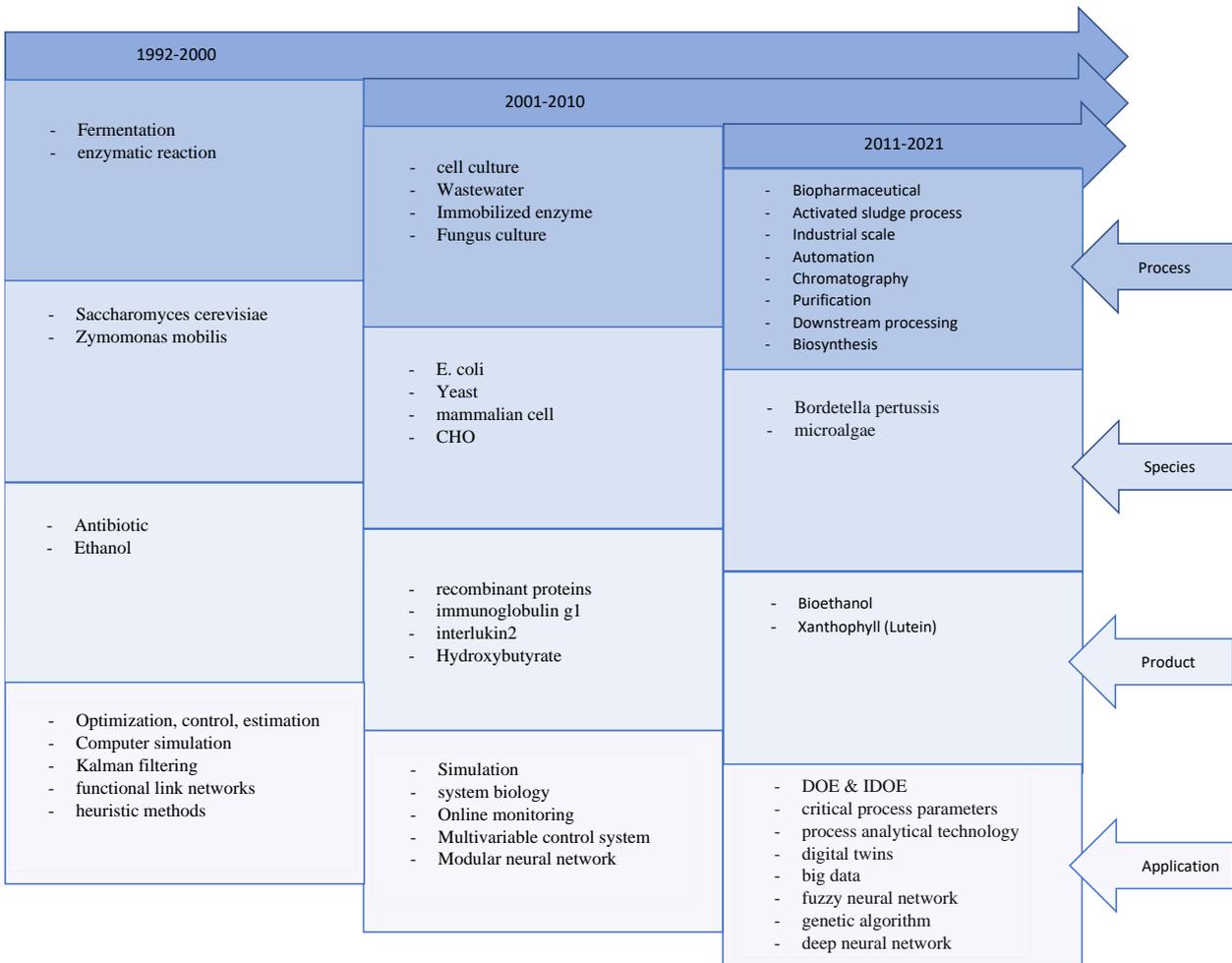


Figure 3-All Keywords (author's keywords and indexed keywords) Occurrence Over the Years

Appendixes

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Appendix 1- Algorithm for article selection

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For this systematic literature review, the relevant articles were selected based on a computerized search, backward citation, and some well-known authors' works.

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1.1. Algorithm for selection of articles from Scopus database

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The paper selection algorithm from the Scopus database started with keyword screening in the “title, abstract, and keywords” of documents. Firstly, the advanced search performed by keywords; ("gray-box model*" OR "hybrid neural model*" OR "hybrid semiparametric model*" OR "hybrid semi-parametric" OR "hybrid neural network*" OR "hybrid mechanistic model" OR "hybrid white box model" OR "hybrid black box model" OR "hybrid parametric model" OR "hybrid nonparametric model" OR "Hybrid Artificial Neural Network" OR "Hybrid Process Model") AND (bioproc* OR biopharma* OR biofuel OR bioreact* OR ferment* OR biologic* OR biopolym* OR bioseparation* OR wastewater OR cell OR microorganism OR yeast OR bacteria OR mammal* OR animal OR "systems biology" OR bioinformatics OR biotech* OR biomass OR "Escherichia Coli" OR "Recombinant Protein" OR "Recombinant Protein prod*" OR "e.coli" OR "microbial fuel" OR "biologic* wastewater treatment" OR bioethanol OR biodiesel) and retrieved 368 publications.

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In the next step, some records were excluded based on the irrelevance of the subject areas (“Psychology”, “Economics, “Econometrics and Finance”, “Dentistry”, “Health Professions”, “Business, Management and Accounting”, “Social Sciences”, “Neuroscience”, “Physics and Astronomy”, “Earth and Planetary Sciences”) which resulted in 56 excluded documents and 312 publications.

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Afterward, some records were excluded based on the irrelevance of the keywords (“Pattern Recognition”, “Blood”, “Photovoltaic Cells”, “Diagnosis”, “Sewage Pumping Plants”, “Nerve Cell Network”, “Neurons”, “Fuel Cells”, “Electrodes”, “Sewer”, “Forestry”, “Geometry”, "PID Controllers", "Paget Bone Disease", "Partial Discharges", "Plasmid", "Power Control", "Power Spectral Density", "Pressure Effects", "Pressure Filter", "Pressure Filters", "Program Processors", "Battery State Of Charge", "Behavior-finding",

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"Behavioral Research", "Behaviour", "Blood Glucose", "Blood Pressure", "Blood Pressure (BP)", "Blood Pressure Estimation", "Blood Pressure Measurement", "Blood Pressure Monitoring", "Bone", and the outcome was 93 excluded documents and 219 eligible publications (At this step, if we doubted whether the keyword was related to the topic or not, we would have reviewed the abstract of the articles containing the keyword). Then the resulting documents were refined by the document's type ("Book chapter", "Review paper", "conference review paper", and "Letter") and 17 documents were excluded because of the document type, and 202 papers were remaining articles. Finally, 82 relevant cases were obtained by manually reviewing the abstracts and contents of eligible publications (120 were excluded).

1.2. Algorithm for selection of articles from Web of Science database

The paper selection algorithm from the Web of Science database also started with keyword screening in the Topic (title, abstract, and keywords) of documents and retrieved a total of 201 publications. Regarding the differences between Scopus and Web of Science, we refined the documents by the Web of Science Categories and excluded the irrelevant categories; "Telecommunication", "Computer Science Hardware Architecture", "Radiology Nuclear Medicine Medical Imaging", "Transportation Science Technology", "Oceanography", "Cardiac Cardiovascular Systems", "Engineering Civil", "Information Science Library Science", "Geography Physical", "Physics-Condensed Matter", "Optics", "Imaging Science Photographic Technology", "Forestry", and "Robotics" and resulted in 35 excluded documents and 166 publications for further analysis.

Then the resulting documents were refined by the document's type ("Review Articles", "Meeting Abstracts", and "Letter") and 5 documents were excluded. Finally, 79 relevant cases were obtained by manually reviewing the abstracts and contents of eligible publications (82 publications were excluded).

1.3. Algorithm for selection of articles from Authors' work

Authors whom we reviewed their works were "Carrondo, M.J.T.", "Simutis, R.", "Lübbert, A.", "Oliveira, R.", "Galvanauskas, V.", "von Stosch, M", "Teixeira, A.P.", "Peres, J.", "Gnoth, S.", "Sokolov, M.", "Feyo de Azevedo, S." and 685 publications were extracted from their works. The documents were refined by keywords screening ("hybrid model*", "hybrid neural*", "hybrid artificial neural*", "hybrid gray box*", "hybrid

semi-parametric*", "hybrid mechanistic*", "hybrid black box*", "hybrid white box*", "hybrid parametric*",
"hybrid nonparametric*") in "title, abstract and keywords" and 135 were sought for retrieval. Then 24 docu-
ments were excluded because of the document type ("Book chapter"; 12, "Review paper; 11", and "Short
Survey"; 1) and 111 papers were remaining articles. Finally, 53 relevant cases were obtained by manually
reviewing the abstracts and contents of eligible publications.

1.4. From the backward citation, 69 papers were obtained.

The results of merging the papers from computerized search, well-known authors' work, and the backward
citation were 283 publications. After omitting the duplicated papers, 162 papers were selected for this study
(Figure 1). We put all articles in a list in Scopus to have the opportunity to use the analytical reports of Scopus.

Appendix 2- Tables and Figures

2.1 Tables

Table 1- Specifications of the first ten document sources that have published the highest number of articles

No.	Source Title	Source Type	Documents	Country	Publisher	H-Index	Quartiles
1	Computers And Chemical Engineering	Journal	14	United Kingdom	Elsevier BV	139	Q1
2	Computer Aided Chemical Engineering	Book Series	11	Netherlands	Elsevier	25	Q4
3	Bioprocess And Biosystems Engineering	Journal	10	Germany	Springer Verlag	68	Q2
4	Biotechnology And Bioengineering	Journal	9	Germany	Wiley-VCH Verlag	189	Q1
5	Journal Of Biotechnology	Journal	7	Netherlands	Elsevier	156	Q2
6	Biotechnology Progress	Journal	6	United States	Wiley-Blackwell	129	Q2
7	Brazilian Journal of Chemical Engineering	Journal	6	Brazil	Braz. Soc. Chem. Eng.	52	Q3
8	IFAC-Papers Online	Journal	5	Austria	IFAC Secretariat	72	Q3
9	AIChE Journal	Journal	4	United States	Wiley-Blackwell	173	Q1
10	Applied Biochemistry and Biotechnology Part A Enzyme Engineering And Biotechnology	Journal	4	United States	Humana Press	119	Q2

Table 2- The ten most cited per year articles

No	Authors	Title	Year	Source title	Cited by	Cited /year
1	Wang et al.	A machine learning framework to improve effluent quality control in wastewater treatment plants	2021	Science of the Total Environment	36	36
2	Sansana et al.	Recent trends on hybrid modeling for Industry 4.0	2021	Computers and Chemical Engineering	30	30
3	von Stosch et al.	Hybrid semi-parametric modeling in process systems engineering: Past, present and future	2014	Computers and Chemical Engineering	189	24
4	Narayanan et al.	Hybrid Models for the simulation and prediction of chromatographic processes for protein capture	2021	Journal of Chromatography A	20	20
5	Psichogios et al.	A hybrid neural network-first principles approach to process modeling	1992	AIChE Journal	589	20
6	Gargalo et al.	Towards smart biomanufacturing: a perspective on recent developments in industrial measurement and monitoring technologies for bio-based production processes	2020	Journal of Industrial Microbiology and Biotechnology	37	19
7	Zhang et al.	Hybrid physics-based and data-driven modeling for bioprocess online simulation and optimization	2019	Biotechnology and Bioengineering	45	15
8	Thompson et al.	Modeling chemical processes using prior knowledge and neural networks	1994	AIChE Journal	415	15
9	Narayanan et al.	A new generation of predictive models: The added value of hybrid models for manufacturing processes of therapeutic proteins	2019	Biotechnology and Bioengineering	44	15
10	Narayanan et al.	Hybrid-EKF: Hybrid model coupled with extended Kalman filter for real-time monitoring and control of mammalian cell culture	2020	Biotechnology and Bioengineering	27	14

Table 3- Top 29 keywords with at least two times occurrences

No	keyword	occurrences	total link strength
1	optimization	14	8
2	fed batch	11	6
3	fermentation	10	4
4	control	7	4
5	estimation	6	5
6	dynamic models	5	5
7	ethanol	5	3
8	genetic algorithm	4	2
9	penicillin g	4	4
10	adaptive control	3	3
11	<i>E. coli</i>	3	3
12	QbD	3	5
13	artificial intelligence	2	2
14	<i>Bacillus thuringiensis</i>	2	2
15	big data	2	2
16	biopharmaceuticals	2	2
17	<i>Bordetella pertussis</i>	2	1
18	cephalosporin c production	2	0
19	chromatography	2	1
20	DOE	2	2
21	downstream processing	2	1
22	immobilized enzyme	2	4
23	intensified design of experiments	2	5
24	optimal control	2	2
25	PAT	2	4
26	poly- β -hydroxybutyrate	2	1
27	upstream bioprocess development/optimization	2	5
28	wastewater	2	2
29	β -lactamic antibiotic	2	4

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2.2 Figures

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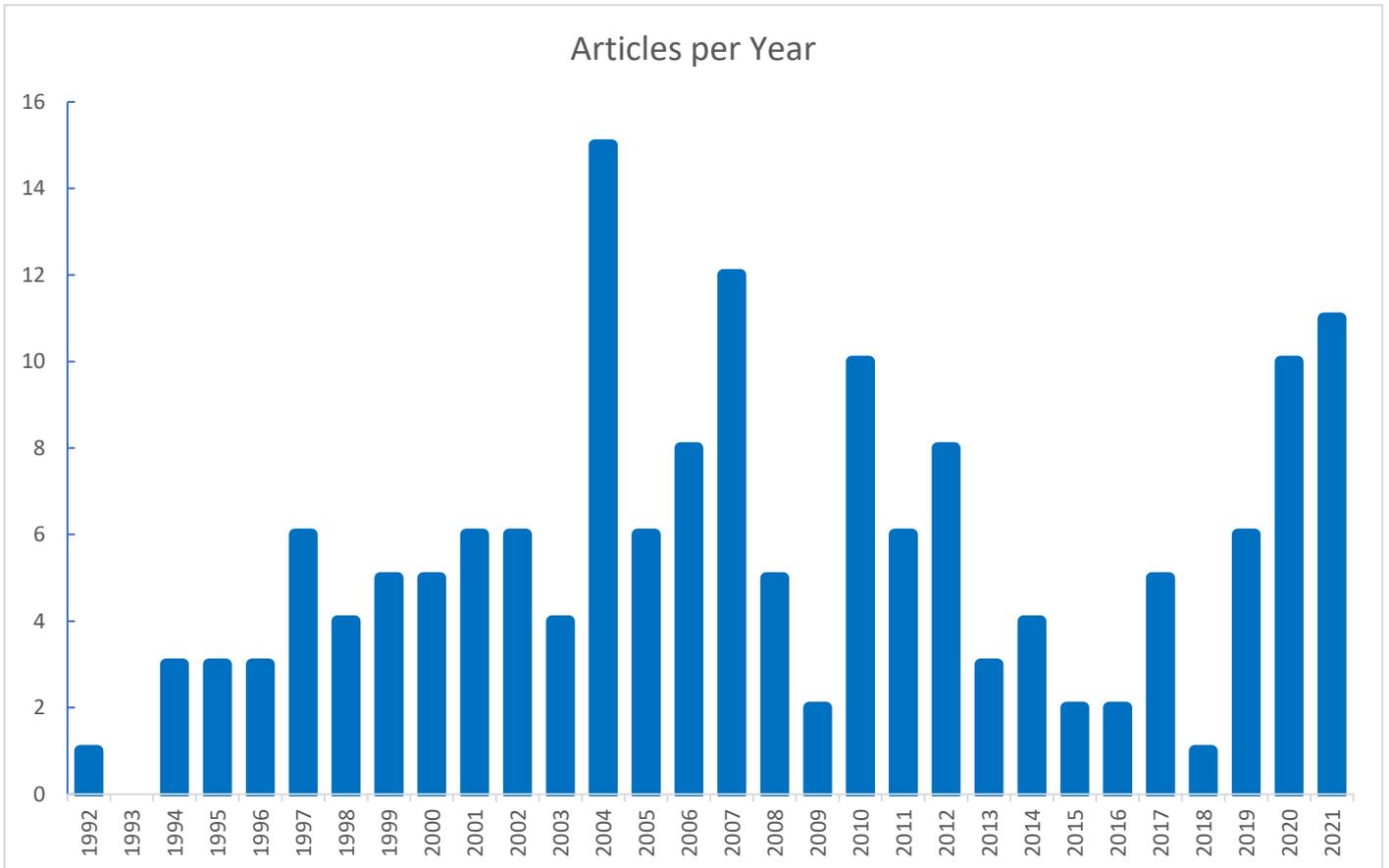


Figure 4-Trend of articles number

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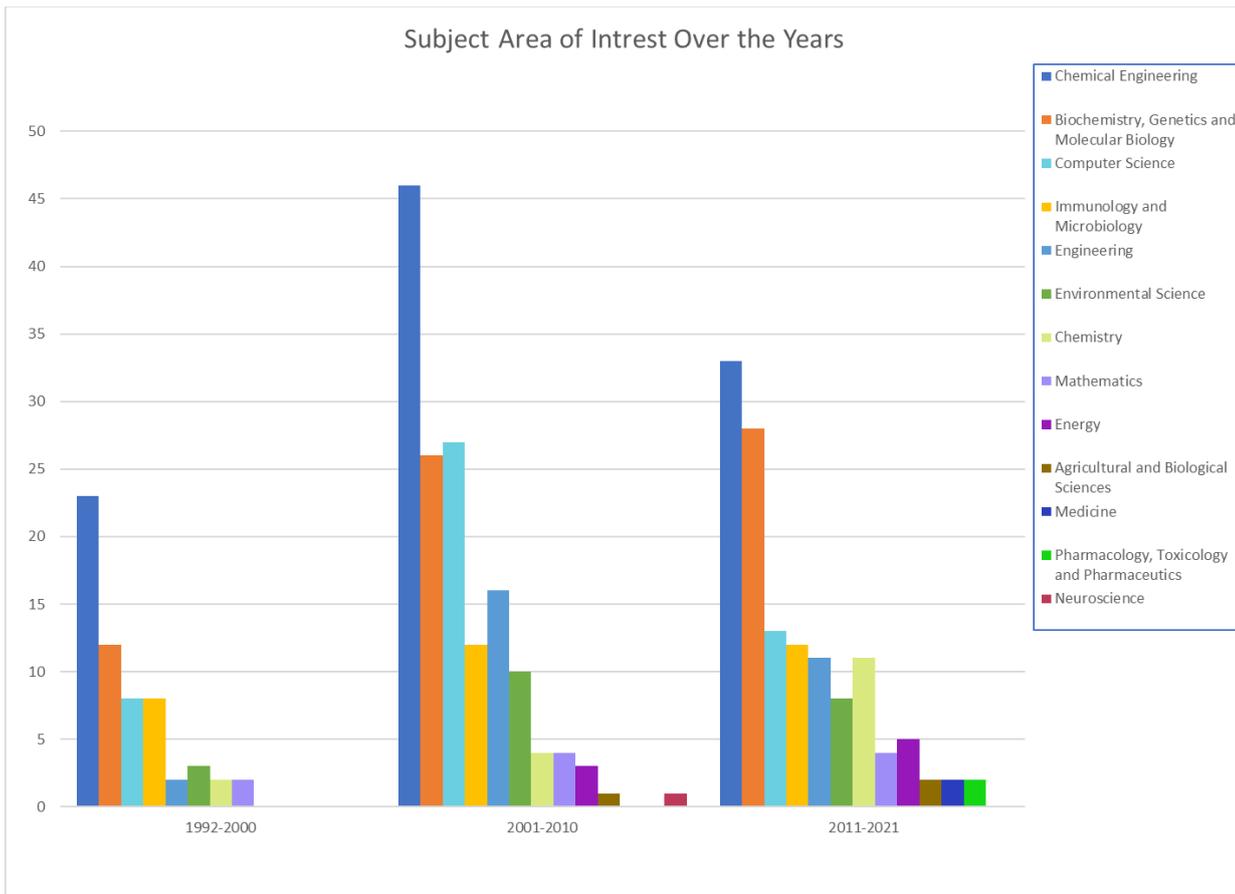


Figure 5-subject areas of interest over the years based on the Scopus analytical reports

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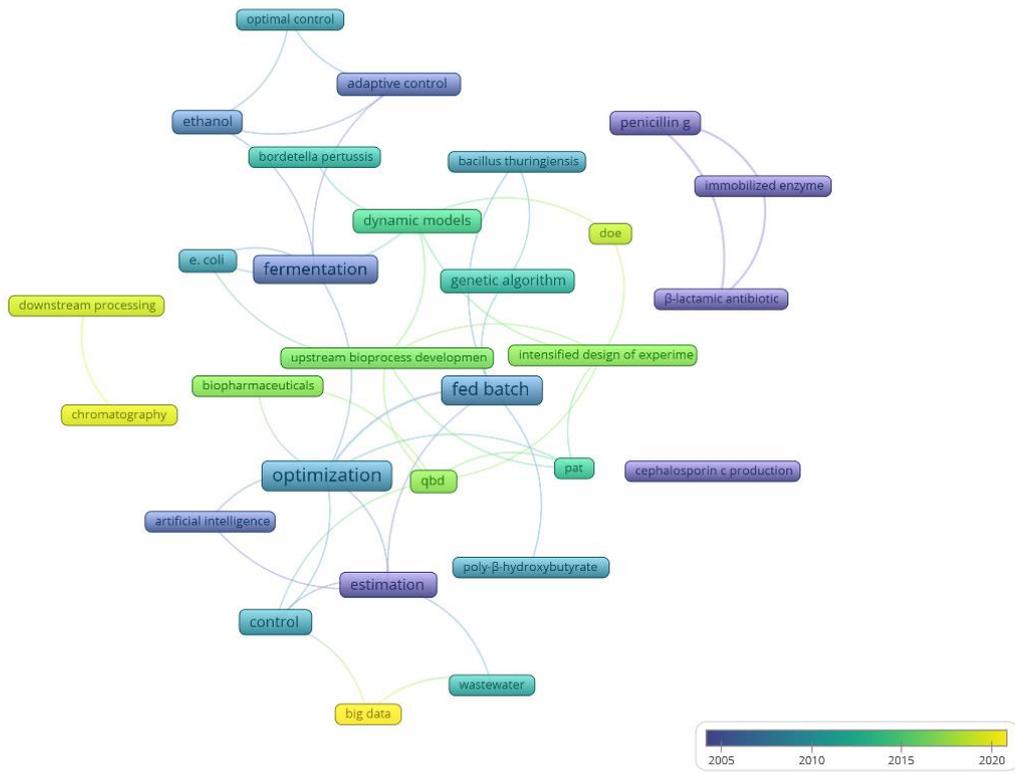


Figure 6- Author's keywords occurrence analysis by year overlay visualization

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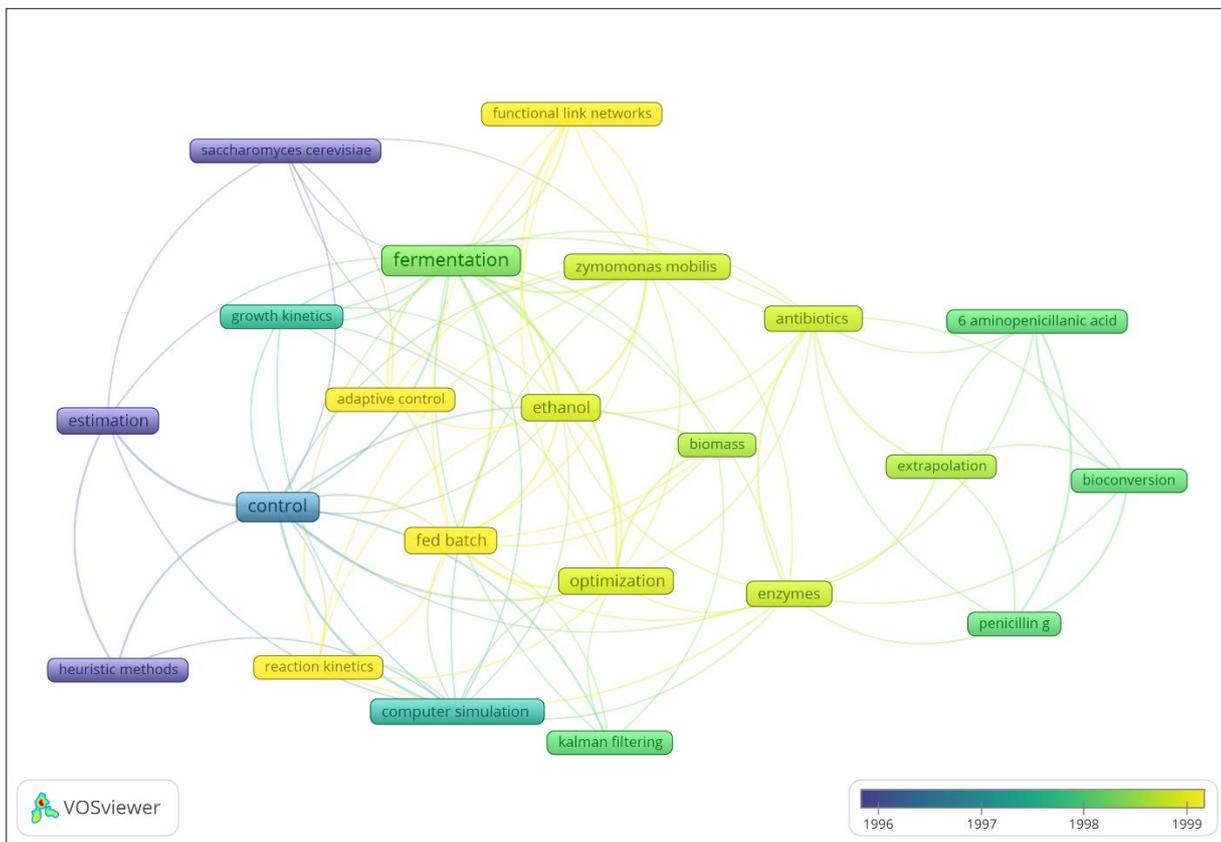


Figure 7- All keywords occurrence from 1992 until 2000 by year overlay visualization

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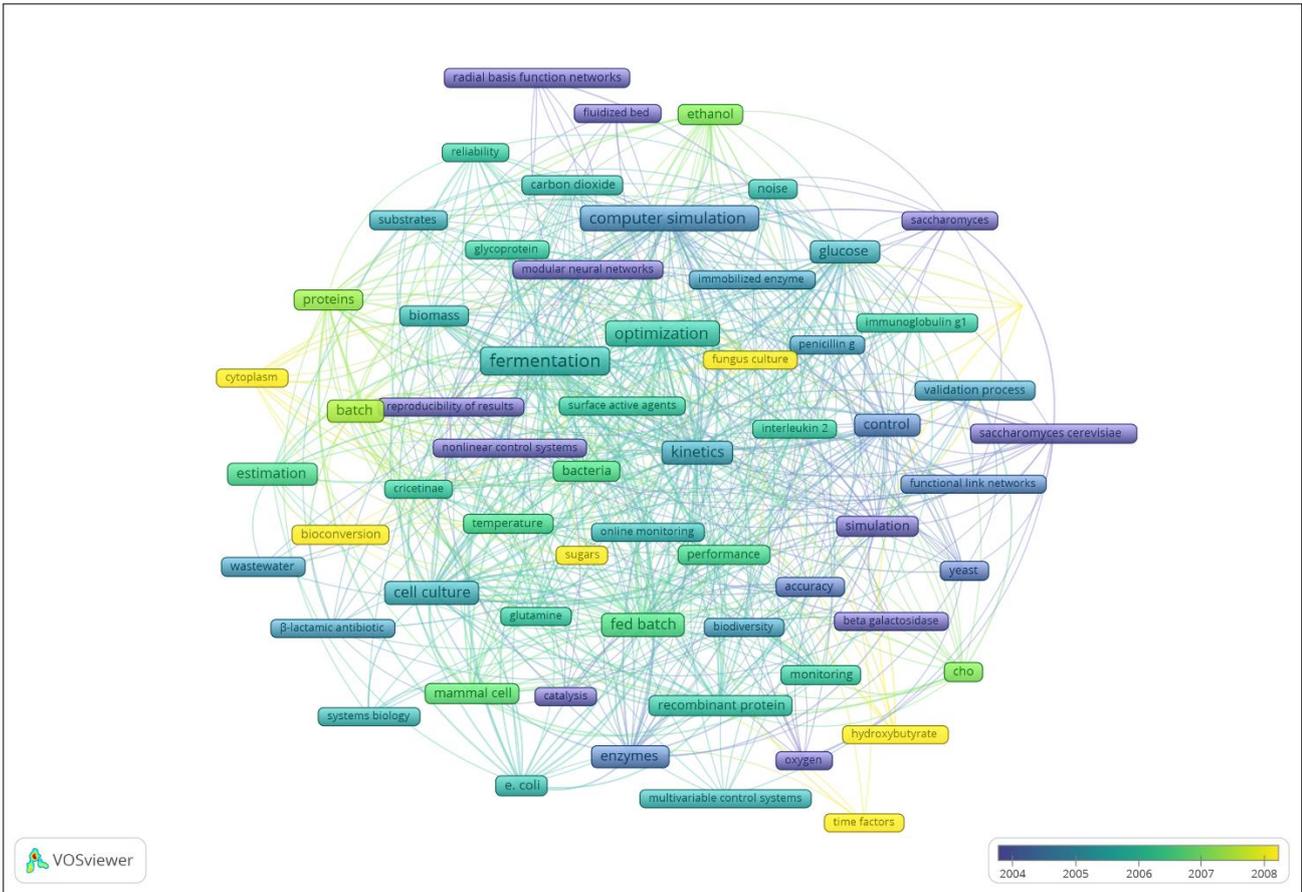


Figure 8- All keywords occurrence from 2001 until 2010 by year overlay visualization

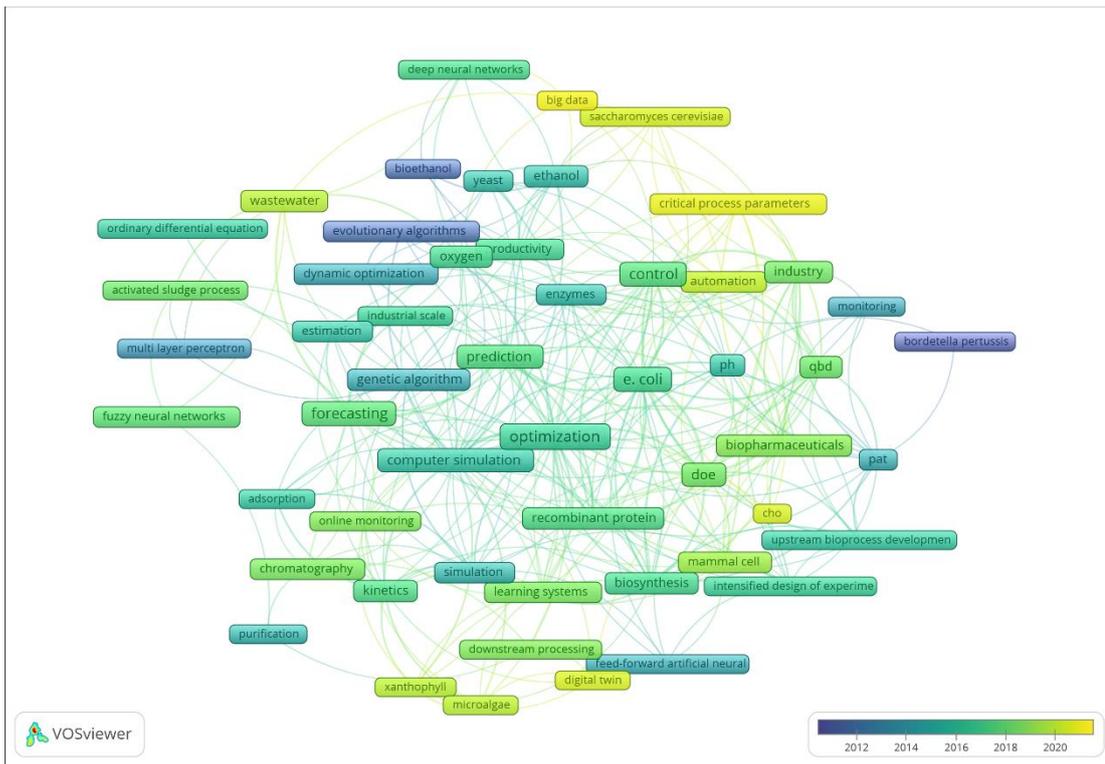


Figure 9- All keywords occurrence from 2011 until 2021 by year overlay visualization

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