# PROCESS SCALE-UP CRITERIA IN PRODUCTION OF RECOMBINANT PROTEINS IN *E. COLI*: A SYSTEMATIC REVIEW

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***ABSTRACT:*** This systematic review provides an overview of the scale-up of recombinant protein production. A systematic search was conducted using pre-established terms in four electronic databases (PubMed, Europe PMC, Science Direct, and Scopus), and included journal articles published between 1999 to 14 November 2019. Studies that met the inclusion and exclusion criteria specified were included. Initially, 665 abstracts were screened, of which 595 were excluded. A further 58 articles were excluded after full-text review and 14 articles underwent quality assessment. Finally, only eight (8) eligible articles were identified and included in this review. Oxygen transfer, shear, and production costs were identified as the main factor that affected the choice and success of a scale-up strategy. The comparison of different fermentation systems revealed that maintaining the $k_La$ constant was found to be effective in all included studies which employed it, and superior in the two studies that compared it to other criteria. Risk of bias assessment revealed that publication bias and reporting bias are significant issues to be considered in scale-up. Overall, these findings provide insight that can assist researchers to improve the scaling up of fermentation processes and achieve a successful scale-up of recombinant protein production using *E. coli* as the host.

***KEYWORDS:*** *Scale-up, Escherichia coli, Fermentation, Recombinant Protein, and Bioreactor*

## 1. INTRODUCTION

### 1.1. Background of study

Genetic engineering has been employed to produce a variety of proteins in amounts required for research, clinical, as well as industrial purposes via microbial cultures [1-2]. *Escherichia coli* (*E. coli*) is the leading toiler and model organism for recombinant protein production, and it has been used to produce numerous recombinant proteins such as enzymes, growth factors, and viral proteins. *E. coli* offers a superior expression system for many fermentations, because of its rapid growth and uncomplicated cultivation [2-3]. Furthermore, there has been a great deal of research effort into the engineering of various strains of *E. coli*, vector development, fermentation media, various modes of bioreactor operation, kinetics modeling, and metabolomics, as well as induction- and expression-related parameters. As such, it is the leading candidate to produce a new recombinant protein [2-3].

A fermentation process is developed in three stages; namely bench or laboratory scale, pilot scale, and full-scale manufacturing. First, fermentation conditions are optimized at the smallest scale to preserve resources and reduce costs associated with the fermentation experiments. Once successful production is established at the laboratory scale, the bioprocess may be upscaled to maintain or improve yield on a larger scale and eventually at an industrial scale to provide large quantities of the protein through a cost-effective, commercially viable manufacturing process. It is only when the desired product yields at both the small-scale and large-scale are similar or are comparable that the scale-up is considered a success. This scale-up, however, is not a straightforward task that can be linearly extrapolated from data at a small-scale. Each fermentation process is a unique and complex system, governed by genetic and environmental factors, that involves a cell that is a complex system that will require an understanding of its metabolic mechanism of response. Factors such as expression strain, fermentation medium, and operating conditions, all play important roles in the process [4]. It is important, therefore, to identify an appropriate parameter or set of parameters that will be critical to the specific fermentation case. Thus, scale-up can be approached in a variety of ways and generally requires a scale-up strategy. Some papers reported successful scale-up from the microplate culture or shake flask culture, however, these studies require separate analysis and cannot be considered together with this study which includes stirred tank bioreactor cultures. This is because the vessel geometry and aeration and agitation methods will not be similar at both scales, thus rendering any comparison difficult [4].

There are many strategies from which researchers can successfully perform a process scale-up, including maintaining a constant power input per unit volume, the volumetric gas flow rate per unit volume of liquid, superficial gas velocity, impeller tip speed, mixing time, and impeller Reynolds number [4]. Although all these criteria are important for a process, it is impossible to control all of them or to maintain them constant simultaneously without the involvement of automated control systems and artificial intelligence. Hence, it is critical to identify which criteria will be more imperative for a process. For an instance, for a fast-chemical reaction, constant mixing time might be more valuable as a scale-up parameter. In the case of aerobic fermentation, more than often one of these scale-up strategies is to maintain constant oxygen transfer or $k_La$. This is because oxygen has limited solubility in water (and fermentation media) and will often be the limiting substrate in the aerobic reaction where a continuous supply is needed. Hence, the oxygen transfer must be determined or predicted for aerobic fermentation. There are many ways that this can be achieved, using models that correlate $k_La$ to another parameter. However, other factors will constrain what will be a realistic process, for example, constraints on power consumption or maximum shear acceptable to the cells. Then, models that consider these constraints on power consumption and impeller tip speed can be used to obtain realistic upscaled processes [1, 4]. Therefore, this paper aims to explore recent studies, highlighting the strategies and criteria employed in the scale-up of recombinant protein production in *E.coli* and their implications. In addition, this paper also includes some studies that attempted to provide solutions to resolve problems faced during scale-up.

### 1.2. Research Questions

The research questions this paper seeks to answer are as follows:

1. What process scale-up methods or strategies have been applied to or used with *E. coli* to produce recombinant proteins?

2. What is the effect of various process and operation conditions on protein production in scale-up?
3. What are the problems experienced in the included papers and how were they resolved?

# 2. MATERIALS AND METHODS

## 2.1. PRISMA Guidelines

This systematic review was carried out by following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [5]. The keywords were determined using the PICOC system, as shown in Table 1.

Table 1: PICOC (Population, Interventions, Comparisons, Outcomes, and Context of study) summary used to develop the search terms

| Study characteristic | Keywords |
|---|---|
| **Population** | recombinant *E. coli* |
| **Intervention** | process scale-up |
| **Comparison** | scale-up strategies |
| **Outcome** | recombinant protein and biomass yield |
| **Context** | fermentation |

## 2.2. Eligibility Criteria

English-language studies published between 1999 to 14 November 2019 were the only articles considered in this study. As only journal articles are included in this study, thus, review articles, conference papers and proceedings, and book sections were excluded. This is to exclude the results of "grey" literature, which may not have been obtained through systematic methods.

## 2.3. Information Sources

The systematic searches were performed in the databases as shown in Table 2. Additional papers were obtained through forward and backward 'snowballing' (citation searching) techniques [5]. This refers to manually browsing the reference lists of accepted articles after the screening had been completed, as well as using suggestions from the databases and search engines that show relevant papers citing the accepted articles.

Table 2: Databases used in this review

| Database | Organization/Institution | URL |
|---|---|---|
| Europe PMC | National Center for Biotechnology Information | https://europepmc.org/ |
| PubMed | National Center for Biotechnology Information | https://www.ncbi.nlm.nih.gov/pubmed/advanced |
| ScienceDirect | Elsevier | https://www.sciencedirect.com/ |
| Scopus | Elsevier | http://www.scopus.com |
| Additional sources | - | - |

## 2.4. Search Strategy

The keywords and synonyms presented in Table 3 were considered for formulating the search strings. The search strings in Table 4 were used with their corresponding databases or digital libraries.

Table 3: Keywords and their synonyms used to formulate the database search strings

| Keyword | Synonyms |
|---|---|
| Constant $k_La$ | Constant oxygen mass transfer |
| *E. coli* | *Escherichia coli* |
| Process | Expression, fermentation, production, bioprocess |
| Recombinant Proteins | Heterologous protein |
| Scale-up | Large-scale, pilot-scale, scalable, scaleup, scale-up, upscaled |
| Scale-up strategies | constant $k_La$, constant oxygen mass transfer, constant power, tip speed |
| Yield | - |

Table 4: Databases, keywords, and dates of the latest searches

| Database | Search | Date last searched |
|---|---|---|
| Europe PMC: | KW: "recombinant" AND ("bioreactors" OR "fermentation") AND "coli" AND ("scale-up" OR "scaleup" OR "scale up" OR "upscaled") | 14 November 2019 |
| PubMed: | ((((("scaleup"[Title] OR "scale up"[Title] OR "scale-up"[Title] OR "scalable"[Title] OR "upscale*"[Title] OR "*-scale"[Title] OR "pilot"[Title] OR "industrial"[Title] OR "bench"[Title])) AND (bioreactors [MeSH Term] OR industrial microbiology[MeSH Term] OR fermentation [Title] OR bioprocess[Title] OR production[Title] OR process*[Title] OR kla[Title] OR bioreactor[Title] OR oxygen[Title])) AND (Escherichia coli[MeSH Term] OR coli[Title])) AND (yield [Title/Abstract] OR recombinant [Title/Abstract]) | 14 November 2019 |
| ScienceDirect: | Title-Abstract-Keyword field: "recombinant" AND "coli" AND ("bioreactors" OR "fermentation" OR "bioprocess" OR "production" OR "process" OR "kla" OR "oxygen") Title field: ("scale-up" OR "scaleup" OR "scale up" OR "upscale" OR "scalable" OR "-scale" OR "pilot" OR "industrial") | 14 November 2019 |
| Scopus: | (TITLE ("scaleup" OR "scale up" OR "scale-up" OR "scalable" OR "upscale*" OR "*-scale" OR "pilot" OR "industrial" OR "bench") AND TITLE-ABS-KEY (bioreactors OR industrial AND microbiology OR fermentation OR bioprocess OR production OR process* OR kla OR bioreactor OR oxygen) AND TITLE-ABS-KEY (escherichia AND coli OR coli) AND TITLE-ABS-KEY (yield OR recombinant)) | 14 November 2019 |

## 2.5. Study Selection and Data Extraction

Parsifal, an online tool for systematic literature reviews, was used to manage the methodology planning, conduct screening, and qualitative analysis (https://parsif.al/Xochitl89/process-scaleup-criteria-in-production-of-recombinant-proteins-in-e-coli-a-systematic-review/settings/). Zotero software (Version 5.0.88) was used to manage the bibliography. Duplicates were removed. All titles and abstracts retrieved from the comprehensive search were independently screened and evaluated based on the inclusion and exclusion criteria as shown in Table 5.

Table 5: Inclusion and exclusion criteria

| Type | Criteria |
|---|---|
| **Inclusion** | 1. Must have protein expression |
| | 2. Recombinant *E. coli* fermentation |
| | 3. Studies must report the specific recombinant protein quantities |
| | 4. Studies must involve a fermentation process scale-up or comparison of at least 2 scales |
| **Exclusion** | 1. Reviews, communications, conference papers, theses, and dissertations |
| | 2. Not stirred tank reactors |
| | 3. Not in the English language |
| | 4. Older than 20 years |

The bibliographies of included studies were checked for additional publications. Full texts of selected articles were then obtained. The full texts of accepted articles then underwent full-text eligibility assessment to ensure they comply with the inclusion and exclusion criteria, as some titles, abstracts, and even keywords were unclear or ambiguous. Accepted articles that passed the full-text screening were then subjected to quality assessment.

Stirred tank bioreactors are the most used bioreactor type for a large variety of bioprocesses, both at the laboratory and industrial scale, as they provide high mass and heat transfer rates and excellent mixing. Furthermore, it is more convenient to carry out the scale-up procedure in stirred tank bioreactors as they come in numerous sizes, up to 20, 000 L, with similar geometries. Consequently, this review focused on scale-up studies in stirred tank reactors only [4].

## 2.6. Quality Assessment Checklist

To ensure that the accepted papers are of sufficient standard to be analyzed, the questions in Table 6 were used as guidelines. The possible answers and their respective weights are also shown. The maximum score is 3.0. The cutoff score is 2.5 Therefore, any paper that had a score that was lower or equal to 2.5 was deemed unfit to be included in the discussion of this study.

Table 6: Quality assessment questions and their weighted answers

| Questions | Possible answers | | |
|---|---|---|---|
| | **Yes** | **Partially** | **No** |
| Is the scale-up strategy clearly specified? | 1.0 | 0.5 | 0.0 |
| Are the fermentation parameters reported for each scale? | 1.0 | 0.5 | 0.0 |
| Are the data collection methods adequately detailed? | 1.0 | 0.5 | 0.0 |

## 2.7. Risk of Bias Assessment

Bias, in academic research, may be defined as the introduction of a systematic error into the research methodology, whether in the study design, data collection or analysis, or publication stages, by selecting or encouraging one outcome or answer over others, thus preventing an objective consideration of a research question [6]. The interpretation of bias requires more than a simple yes or no answer, and instead it is important to describe the degree of bias. To assess the risk of bias, accepted papers underwent the risk of bias assessment for individual studies. In addition to the above quality assessment questions, the questions in Table 7 were posed.

Table 7: Risk of bias assessment questions

| Risk of bias assessment question | Related bias |
|---|---|
| Are reports of the study free of selective outcome reporting? | Reporting bias, publication bias |
| Was the statistical analysis for all results reported? | Reporting bias, publication bias |
| Is incomplete outcome data addressed? | Attrition bias |

The level of bias was assessed as a judgment (high, low, or unclear) for individual elements from five areas (selection, performance, attrition, reporting, and other biases). Selection bias is a type of bias that occurs mostly in clinical studies where there is no blinding in choosing the population of study due to convenience or other factors thereby introducing bias. Performance bias occurs due to the knowledge of the researchers or participants involved in the intervention, thus skewing the findings. Attrition bias occurs when some participants in the study do not complete the study. Again, this is often observed in clinical trials. Reporting bias occurs when there is selective reporting of only positive results that would fit the hypothesis. Publication bias is a type of bias that occurs when the results of an experiment or research study influence the decision of whether to publish or otherwise distribute it. This failure in reporting results then affects the ability of accurate synthesis and description of evidence in that area of research. It then leads to wrong conclusions as well as a waste of research opportunities, efforts, and money [7].

### 2.8. Data Extraction and Data Collection Process

The results of each stage of this systematic review were recorded and are presented in the subsequent section. All titles, abstracts, and full-text articles, and performed the quality assessment were screened. The authors concurred, through consensus, on relevance, protocol, findings, quality and risk assessment, and conclusions. Funding information was also obtained for papers included in the systematic review. Table 8 presents the information extracted from the included papers. Recombinant protein yield, biomass yield, and recombinant protein activity (where available instead of protein yield or productivity) at both small and large scales were the main evaluated outcomes. These parameters were used to generate a form using the Parsifal tool to collect and store the data for each included paper.

Table 8: Data extracted from the small-scale and large-scale fermentations in each study

| Type of Variable | Parameter |
|---|---|
| Controlled | *E. coli* strain |
| | Fermentation mode |
| | Fermentation medium |
| | Vessel volume |
| | Working volume |
| | Scale-up strategy (including dimensions, and agitation, and oxygen-related parameters) |
| Measured | Biomass production |
| | Protein production |

## 3. RESULTS

### 3.1. Study Selection and PRISMA Flow Diagram

The systematic search returned a total of 872 citations results, including duplicates. These papers were then imported into the online Parsifal tool, using TexMed and Zotero

tools where a direct import was not possible. Imported studies were made up of 285 records identified through Europe PMC, 2133 records from PubMed, 274 through Scopus, 99 from Science Direct, and one additional paper from references. Of these, 665 abstracts were screened, after the removal of duplicate articles, and 595 were excluded by title and/or abstract screening. The 72 records went through full-text screening and finally, 14 papers underwent quality assessment. A total of eight (8) eligible articles were identified and included in the final review [8-15]. Table 9 shows all the 58 excluded full-text articles and reasons for exclusion and six (6) included articles but whose quality was rejected [16-77]. Fig. 1 summarizes this process and results.

Table 9: Excluded full-text articles and reasons for exclusion (n=58) and those included but whose quality was rejected (n=8)

| No. | Reference | Reason for rejection |
|---|---|---|
| 1 | [16] | Not comparing 2 STR scales |
| 2 | [17] | Not comparing 2 STR scales |
| 3 | [18] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 4 | [19] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 5 | [20] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 6 | [21] | Not comparing 2 STR scales |
| 7 | [22] | Not comparing 2 STR scales |
| 8 | [23] | Not comparing 2 STR scales |
| 9 | [24] | Not comparing 2 STR scales |
| 10 | [25] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 11 | [26] | Not comparing 2 STR scales |
| 12 | [27] | Not comparing 2 STR scales |
| 13 | [28] | Not comparing 2 STR scales |
| 14 | [29] | Not comparing 2 STR scales |
| 15 | [30] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 16 | [31] | Quality rejected |
| 17 | [32] | Not comparing 2 STR scales |
| 18 | [33] | Not comparing 2 STR scales |
| 19 | [34] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 20 | [35] | Quality rejected |
| 21 | [36] | Not comparing 2 STR scales |
| 22 | [37] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 23 | [1] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 24 | [38] | Not comparing 2 STR scales |
| 25 | [39] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 26 | [40] | Not comparing 2 STR scales |
| 27 | [41] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 28 | [42] | Not comparing 2 STR scales |
| 29 | [43] | Not comparing 2 STR scales |
| 30 | [44] | Not comparing 2 STR scales |
| 32 | [3] | Not comparing 2 STR scales |
| 31 | [45] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 33 | [46] | Not comparing 2 STR scales |
| 34 | [47] | Not comparing 2 STR scales |
| 35 | [48] | Quality rejected |
| 36 | [49] | Not STR |
| 37 | [50] | Not comparing 2 STR scales |
| 38 | [51] | Not comparing 2 STR scales |
| 39 | [52] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 40 | [53] | Quality rejected |
| 41 | [54] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 42 | [55] | Quality rejected |
| 43 | [56] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 44 | [57] | Not comparing 2 STR scales |
| 45 | [58] | Not comparing 2 STR scales |
| 46 | [59] | Not comparing 2 STR scales |
| 47 | [60] | Not comparing 2 STR scales |
| 48 | [61] | Not comparing 2 STR scales |

| | | |
|---|---|---|
| 49 | [62] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 50 | [63] | Quality rejected |
| 51 | [64] | Not comparing 2 STR scales |
| 52 | [65] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 53 | [66] | Not comparing 2 STR scales |
| 54 | [67] | Not comparing 2 STR scales |
| 55 | [68] | Not comparing 2 STR scales |
| 56 | [69] | Not comparing 2 STR scales |
| 57 | [70] | Not comparing 2 STR scales |
| 58 | [71] | Not comparing 2 STR scales |
| 59 | [72] | Does not involve a fermentation scale-up study or comparison of at least 2 scales |
| 60 | [73] | Not comparing 2 STR scales |
| 61 | [74] | Not comparing 2 STR scales |
| 62 | [75] | Not comparing 2 STR scales |
| 63 | [76] | Not comparing 2 STR scales |
| 64 | [77] | Not comparing 2 STR scales |

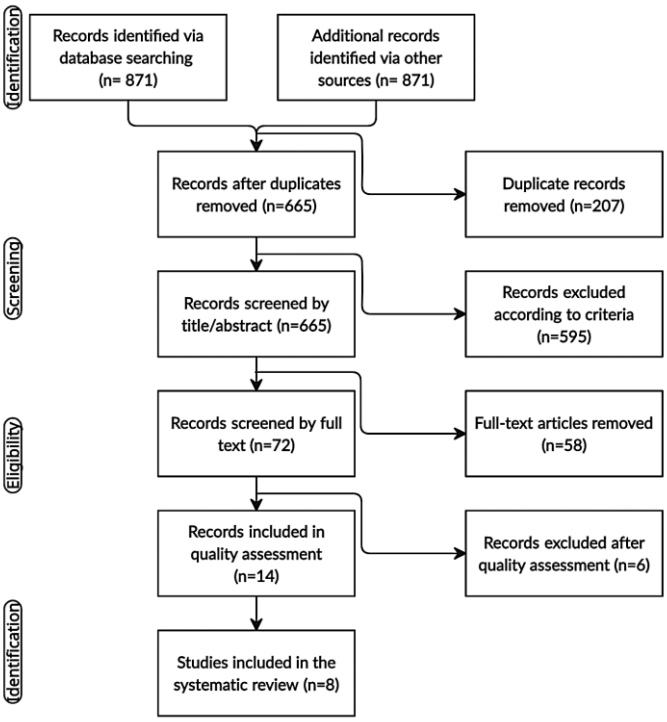


Fig. 1. Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) flow diagram of this study.

### 3.2. Study Characteristics

The findings of each study were analyzed and extracted data were summarized into several tables. Table 10 provides information related to recombinant protein production, scale-up strategies employed, and observations. Table 11 summarizes the fermentation details reported including fermentation mode, media used, carbon and nitrogen sources used, and inoculum sizes. It can be surmised that only batch or fed-batch modes of operation are typically analyzed in scale-up studies. The strains of *E. coli* employed varied, though the BL strain is quite commonly used. The type of media used for the culture also varied across studies. However, glucose was used as the carbon source in all papers indicating it is suitable for growing recombinant *E. coli* of various strains. There is not enough reported data in these papers to analyze inoculum size across studies.

Table 10: Scale-up for recombinant proteins production in *E. coli*

| Study | Protein | Scale-up strategies | Vessel volume and working volume (in liters) | | Measured variables | Observations |
|---|---|---|---|---|---|---|
| | | | **SS** | **LS** | | |
| [8] | Antigen K99 | constant $k_La$<br>constant $Q/V$ | 5 (NA) | 200 (NA) | • K99 productivity (mg/L.h)<br>• cell specific growth rate (/h) | • A slightly reduced yield of biomass and K99 product was noted upon scale-up.<br>• Similar growth rates at both scales. |
| 9] | Antigen K88 | constant $k_La$<br>constant $Q/V$ | 5 (NA) | 50 (NA) | • K88 production (mg/L), OD530 | • Similar at both scales, thus validating the choice of scale-up strategy. |
| [10] | IL-1 receptor antagonist | constant $DO$ | 2 (1.6) | 100 (NA) | • OD600, Productivity | • Reduced cell growth and productivity were observed, due to differences in oxygenation and stress. |
| [11] | Outer membrane protein Opc | constant $P/V$<br>constant $k_La$ | NA (1.5) | NA (50) | • Cell dry weight (g/L)<br>• Opc production (mg/L) | • Using constant P/V, the protein productivity dropped to 26 mg/L/h from 49 mg/L/h. Thus, the aeration rate was recalculated using $k_La$. |
| [12] | P46k protein | constant $nD$i<br>constant $k_La$ /k<br>constant $P/V$<br>constant $Re$ | 2.5 (1.5) | 70 (50) | • Cell dry weight (g/L)<br>• P64k<br>• Protein Production (mg/L) | • Cell dry weight and protein production dropped when a constant Reynolds number was used, and the degree of agitation dropped. In contrast, they improved with other criteria, when compared to small scale and were highest for constant $k_La$. All |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | criteria except constant *Re* resulted in similar agitation speed values, though constant $k_La$ did so without an increase in shear as experienced with constant *P/V*. |
| [13] | Phosphotriesterase-like lactonase | constant *DO* | 2.5 | 150 | • Enzyme production (mg/L)<br>• Final biomass (g $CWW^1$/L) | • Similar, slightly higher yields of the enzyme (37.6 to 39.7) and biomass (108 to 130) were obtained.<br>• Acetate formation, cell density decreased, and product inhibition. Thus, galactose was used as an inducer instead of IPTG. |
| [14] | Granulocyte colony-stimulating factor (GCSF) | geometric similarity<br>constant *DO* | 2 (1) | 13(8) | • Final cell density ($DCW^2$/L))<br>• GCSF concentration (g/L) | • Final cell density and amount of recombinant protein decreased significantly, due to acetate accumulation and heterogeneous conditions.<br>• Plasmid stability decreased from 95% to 90% upon scaleup. This was attributed to a decrease in growth rate due to |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | heterogeneous conditions inside the bioreactor, suggesting that there was a low oxygen concentration in some areas. |
| [15] | Human-like collagen (HLC) | constant p*$k_La$ | 12.8 (6) | 30 (15.4) | • growth (g DCW/L) and HLC concentration (g/L) | • The importance of the initial culture volume was highlighted.<br>• Acetate overproduction was observed. | |

CWW= cell wet weight
[2]DCW= dry cell weight
*SS refers to Small Scale fermentation and LS refers to Large Scale fermentation

Table 11: Fermentation medium and carbon source used for each paper

| Reference | Medium | Carbon source | Strain | Inoculum size | Operation Mode | Biomass production (SS) | Biomass production (LS) | Protein production (SS) | Protein production (LS) |
|---|---|---|---|---|---|---|---|---|---|
| [8] | Defined | glucose | MC1061 | 1% (v/v) | Batch | NA | NA | 29.7 mg/L | 30.1 mg/L |
| [9] | Defined | glucose | BB4 | 2% (v/v) | Batch | NA | NA | 151.4 mg/L | NA |
| [10] | Terrific Broth | glucose | BLR (DE3) | NA | Fed-Batch | 150 | 105 | 8.0 g/L | 6.7 g/L |
| [11] | Modified Media | glucose | K12 GC366 | NA | Batch | 3 g/L | 4 g/L | 418 mg/L | 520 mg/L |
| [12] | Modified Media | glucose | K12 GC366 | NA | Batch | 2.6 g/L | 3.9 g/L | 284 mg/L | 546 mg/L |
| [13] | Semi-defined | glucose | BL21 (DE3) | NA | Fed-batch | 108.1 g CWW/L | 130.2 g CWW/L | 37.6 U/g CWW | 39.7 U/g CWW |
| [14] | Modified M9 | glucose | BL21 DE3 | NA | Batch | 124.0 g DCW/L | 114.0 g DCW/L | 22.0 g/L | 19.0 g/L |
| [15] | Luria Broth | glucose | BL21 pNWCP31 | 10% (v/v) | Fed-Batch | 80.3 g/L | 59.7 g/L | 13.1 g/L | 6.25 g/L |

*SS refers to Small Scale fermentation, and LS refers to Large Scale fermentation.

Table 12 reports the findings of the included studies, in terms of biomass yield and recombinant protein yield at both the small and large scales. It also includes the strategies employed and whether they were reported as successful or not. Most of the strategies were reported as successful except for two strategies [11-12]. The former study [11] reported 257 mg/L Opc protein yield using constant *P/V* as a scaleup strategy, whereas the starting yield in the small-scale was 418 mg/L as the aeration rate was too low. The latter, [12], keeping the Reynolds number constant in scaleup resulted in a P64k protein yield of only 168 mg/L whereas the small-scale production had yielded 284 mg/L P64k protein. This is due to severely reduced agitation speed, and $k_La$. By comparison, all other strategies,

yielded values equal to 383 mg/L or higher. All studies were able to achieve successful scale-up using at least one criterion, even though in some cases some strategies attained lower protein yields on the large scale [10, 14-15] compared to the small scale. In the subsequent section, the specific results for each of the studies reviewed are elaborated.

Table 12: Scale-up parameters applied and resulting biomass and protein production (assessed by protein yield or activity)

| Reference | Constant Parameter | Biomass production in Small Scale | Biomass production in Large Scale | Protein production in Small Scale | Protein production in Large Scale | Reported as successful |
|---|---|---|---|---|---|---|
| [8] | $k_La$ and Q/V | NA | NA | 29.7 mg/L | 30.1 mg/L | Yes |
| [9] | $k_La$ and *Q/V* | NA | NA | 151.4 | NA | Yes |
| [10] | *DO* | $OD_{600}$ = 150<br>29 g DCW/L | $OD_{600}$ = 105<br>NA | 8.0 g/L | 6.7 g/L | Yes |
| [11] | $k_La$ | 3 g/L | 4 g/L | 418 mg/L | 520 mg/L | Yes |
| | *P/V* | 3 g/L | 2 g/L | 418 mg/L | 257 mg/L | No |
| [12] | $k_La$ /k | 2.6 g/L | 3.9 g/L | 284 mg/L | 546 mg/L | Yes |
| | *Re* | 2.6 g/L | 1.4 g/L | 284 mg/L | 168 mg/L | No |
| | *ND*i | 2.6 g/L | 2.7 g/L | 284 mg/L | 338 mg/L | Yes |
| | *P/V* | 2.6 g/L | 3.0 g/L | 284 mg/L | 383 mg/L | Yes |
| [13] | *DO* | 108.1 g CWW/L | 130.2 g CWW/L | 37.6 U/g CWW | 39.7 U/g CWW | Yes |
| [14] | *DO* | 124.0 g DCW/L | 114.0 g DCW/L | 22.0 g/L | 19.0 g/L | Yes |
| [15] | $p^*$ $k_La$ | 80.3 g/L | 59.7 g/L | 13.1 g/L | 6.25 g/L | Yes |

Table 13 highlights all the problems encountered during the fermentation or scale-up process for each included study. For those that were resolved, the solution is also reported in Table 13. In some papers, the issues were mentioned but not resolved [9-10].

Table 13: Problems encountered, and solutions applied

| Reference | Issue during/before scale-up | Resolution method |
|---|---|---|
| [8] | Initial carbon source (glucose, yeast extract) concentrations had a negative influence on product yield. | Optimization of carbon source concentrations was carried out prior to scale-up. |
| [9] | Plasmid stability | Not Resolved<br>The difference between the two scales was not significant. |
| [10] | Hypoxia<br>Lower growth and productivity | Not Resolved |
| [11] | Very low productivity (53% of the small-scale productivity) was obtained using calculated parameters for constant aeration number. | The aeration rate was recalculated using constant $k_La$ criteria. |
| [12] | None mentioned. | Not mentioned. |
| [13] | Growth inhibition | Inducer changed to galactose |
| [14] | A decrease in biomass yield | Substrate feeding strategy |
| [15] | Acetate overproduction<br>Low yield in larger scale | Not Resolved |

## 4. DISCUSSION

This review aimed to provide an overview of the process scale-up of recombinant protein production and to address the research questions that have become the key subheadings in this section, as shown below.

### 4.1. Summary of Evidence

To answer the first research question, the process scale-up methods or strategies that have been used with *E. coli* to produce recombinant proteins are summarized in Table 14. The constant $k_La$ strategy was used in a total of five papers. Constant volumetric power consumption (*P/V*) was used in three included papers. Constant aeration as a scaleup strategy was employed in two papers as constant *Q/V* and three of the included papers as constant *DO*. Constant Reynolds number and constant tip speed strategies were each used in one paper. Constant geometry as a strategy was mentioned only in two of the included papers.

Table 14: Scale-up strategies employed in each study

| Reference | Scale-up criteria | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Constant *DO*** | **Constant $k_La$** | **Constant *P/V*** | **Constant *Q/V*** | **Constant *Re*** | **Geometrical similarity** | **Constant tip speed ($nD_i$)** |
| [8] | | ✔ | | ✔ | | | |
| [9] | | ✔ | | ✔ | | ✔ (implied) | |
| [10] | ✔ | | | | | | |
| [11] | | ✔ | ✔ | | | | |
| [12] | | ✔ | ✔ | | ✔ | | ✔ |
| [13] | ✔ | | ✔ | | | | |
| [14] | ✔ | | | | | ✔ | |
| [15] | | ✔ | | | | | |
| **Frequency** | ***3*** | ***5*** | ***3*** | ***2*** | ***1*** | ***2*** | ***1*** |

From the definition of the process scale-up, *i.e.*, maintaining the production of the desired product at the large scale, it is possible to evaluate the success of the scale-ups conducted in each included study. This can be assessed by calculating the ratio of the biomass yields at the large scale to that of the small scale. Similarly, this ratio can be calculated for the recombinant protein product. A value greater than or equal to one indicates that the scale-up was successful. The calculation of scale-up efficiency, calculated using the ratio of recombinant protein yield in the larger scale to that of the small scale, as well as that of the biomass yields, is presented in Table 15.

Table 15: Evaluation of the success of scale-up

| Reference | Constant Parameter | Scale Ratio ($V_{T,LS}/V_{T,SS}$) | Biomass Yield Efficiency[1] of scale-up | Product Yield Efficiency[2] of scale-up |
|---|---|---|---|---|
| [8] | $k_La$ and Q/V | 40 | NA | 1.0135 |
| [9] | $k_La$ and *Q/V* | 10 | NA | NA |
| [10] | *DO* | 50 | 0.7 | 0.84 |
| [11] | $k_La$ | 28 | 1.33 | 1.24 |
| | *P/V* | 28 | 0.67 | 0.61 |
| [12] | $k_La$ /k | 28 | 1.5 | 1.92 |
| | *Re* | 28 | 0.54 | 0.59 |
| | *NDi* | 28 | 1.04 | 1.19 |
| | *P/V* | 28 | 1.15 | 1.35 |
| [13] | *DO* | 60 | 1.20 | 1.06 |
| [14] | *DO* | 6.5 | 0.92 | 0.86 |
| [15] | p* $k_La$ | 2.3 | 0.74 | 0.48 |

[1]Biomass yield efficiency here refers to the ratio of the biomass yield value for the large-scale fermentation to the biomass yield value for the small-scale fermentation. It is unitless. A value greater than or equal to one indicates a successful scale-up.

[2]Product yield efficiency here refers to the ratio of the product yield value for the large-scale fermentation to the biomass yield value for the small-scale fermentation. It is unitless. A value greater than or equal to one indicates a successful scale-up.

## 4.2. Scale-up Strategies Employed in the Included Studies

The seven parameters which were kept constant in the included studies were geometrical similarity, constant volumetric oxygen transfer coefficient ($k_La$), constant volumetric power input (ratio of agitation power input per volume of liquid) ($P/V$), constant impeller tip speed (or maximum shear stress), constant $Q/V$ (volumetric airflow rate per unit volume), constant impeller Reynolds number, and constant dissolved oxygen concentration. The selection of these parameters as scale-up criteria was mentioned in four studies [8, 12, 14-15] while the remaining four studies did not clearly justify the reason for the choice(s). The effects of inner vessel pressure and heat transfer were not mentioned in any of the included studies. Table 16 shows the selected scale-up criteria of some studies [4, 78-81] by highlighting their corresponding applications and limitations of each criterion.

Table 16: Scale-up criteria used and their features

| No. | Scale-up criteria | Related equation | Applications | Limitations |
|---|---|---|---|---|
| 1 | Geometrical similarity | $D_{T2}/D_{T1}=(V_{T2}/V_{T1})^{1/3}$ | • Most fermentation processes for alcohol and organic acid production have followed the concepts of geometric similarity and constant power per unit volume.<br>• This is a prerequisite assumption for other scaleup approaches [4]. | • This assumes constant impeller geometry or diameter and the number of impellers.<br>• It is difficult to maintain geometrical similarity when it is used with other criteria in scale-up [4]. |
| 2 | Volumetric oxygen transfer coefficient ($k_La$) | $(k_La)_1 = (k_La)_2$ | • The majority of aerobic systems have been scaled-up on the $k_La$ basis. | • $k_La$ values vary throughout the fermentation and are difficult to obtain a reliable value for it. Thus, the scale-up is complex and process-specific [79]. |
| 3 | Constant impeller Reynolds number | $(ND_i^2)_1= (ND_i^2)_2$<br>$Re = \rho ND_i^2 /\upsilon$<br>$Re \propto ND_i^2$ | • It is useful when the heat transfer rate from the fermentation broth to the cooling coils inside the bioreactor vessel is vital, as in the case of the use of thermophilic microorganisms. | • Since the effect of aeration is not considered, often this basis does not work well in scale-up [4]. |
| 4 | Constant maximum shear | Impeller tip speed= $\pi ND_i$ | • This scale-up criterion is used for possibly shear-sensitive fermentations where a maximum shear rate is calculated to prevent shear damage that can lyse the cells or influence cell growth and protein production.<br>• On the other hand, it is used in cases where the cells tend to form dense flocks, the minimum shear rate required to break-up these flocks must be known and applied.<br>• Useful for branched yeast and filamentous bacteria [4]. | • This is less useful for single-cell yeast and bacterial cultures.<br>• Often, $P_g/V_L$ is lowered, and this affects aeration efficiency and thus must be coupled with keeping $k_La$ constant [4, 80]. |
| 5 | Constant Gassed or Ungassed Power Per Unit Liquid Volume ($P/V_L$) | $(P/V_L)_1= (P/V_L)_2$<br>$P/V \approx N^3/D^2$ | • It is efficient for turbulent flows in single-cell *E. coli* and yeast cultures [78].<br>• Several aerobic fermenter systems have been scaled-up on the P/V basis including many | • Scale-up based on constant power per unit<br>• liquid volume can have an unnecessarily large motor size, making it impractical, |

| | | | | |
|---|---|---|---|---|
| | | | antibiotic fermentations [81]. | economically or technically, to apply [4]. |
| 6 | Constant *Q/V* (constant *VVM*) | $N_A=Q/ND_i^3$ | • It is useful when a simple approach that considers aerations is required, and sparger characteristics are not known [82]. | • The resulting superficial velocity values must be checked for reasonableness as foaming and flooding could occur at very high airflow rates [4]. |
| 7 | Constant minimum dissolved oxygen (*DO*) | - | • It is easy to achieve this by controlling operating conditions for facilities that have existing installed equipment [4]. | • The scale-up of methods to control the dissolved oxygen concentration, such as aeration and agitation rate, must be considered [4]. |

### *4.2.1. Constant Power per Unit Volume*

Power consumption per unit volume can be used to represent the turbulence and degree of mixing in a bioreactor. As the culture scale increases, power requirements decrease as a result of an increase in pressure inside the vessel. By keeping the power per unit volume constant, many fermentations have been successfully scaled up. However, other consequences such as high costs and/or high shear effects must also be considered [82]. Generally, this criterion worked very well in the included studies. However, in one case the protein productivity decreased markedly when using this scale-up strategy [11]. Hence, power consumption per unit volume can be applied to recombinant protein production, along with another scale-up strategy for comparison of attainable protein yield.

### *4.2.2. Constant Oxygen Transfer*

For aerobic fermentation, the dissolved oxygen concentration depends on the oxygen transfer rate (OTR) from the gas phase to the liquid, the oxygen transport rate from the liquid into the cell, and the oxygen uptake rate (OUR) by the microorganism for cellular functions such as cell growth, maintenance, and recombinant protein production. In turn, these factors are governed by the cell culture conditions within the bioreactor and the physical aspects of the bioreactors [4]. As the culture scale increases, it becomes more difficult to ensure available dissolved oxygen availability throughout the culture and oxygen often becomes the limiting nutrient for the fermentation process. Thus, parameters related to oxygen transfer become of high interest in scale-up to ensure that the oxygen is always above a critical level. This is often achieved using constant $k_La$, but sometimes a minimum dissolved oxygen setpoint is controlled instead.

In the studies reviewed, sufficient oxygen transfer is the main problem in scale-up and takes priority. Three common methods can be used to correlate $k_La$, namely:

1. Energy input criterion relating $k_La$ to the power input ($P_g$) and the superficial gas velocity ($v_s$), or $Q/V$, volumetric flow rate over volume,
2. The use of dimensionless numbers such as the Froude number ($Fr$), the gas flow number ($FlG$) and the ratio of the impeller and tank diameters ($D_i/D_T$), and
3. The gas hold-up in using the dispersion parameter, $N/N_{cd}$, where $N_{cd}$ represents the minimum impeller speed required for all the medium to be in contact with the sparged gas.

The correlations that have been used to determine $k_La$ in the included studies are presented in Table 17. The actual value of $k_La$ was not provided in the included papers, except in [12].

Table 17: Correlations for $k_La$

| Reference | Correlation for $k_La$ | Value |
|---|---|---|
| [8] | $k_La = k(P_g/V_L)^{\alpha}(v_s)^{\beta}$<br>$k_La = 0.00048N^{2.2}D_i^{2.4}Q^{0.52}(1/V)^{0.7}B^{0.8}J^{0.3}$ | NA |
| [9] | $k_La = k(P_g/V_L)^{\alpha}(v_s)^{\beta}$<br>$k_La = 0.00048\ N^{2.2}\ D_i^{2.4}\ Q^{0.52}(1/V)^{0.7}\ B^{0.8}\ J^{0.3}$ | NA |
| [10] | NA | NA |
| [11] | $k_La = 2\ x\ 10\text{-}3\ (P/V)^{0.7}(4Q/\pi D_T^2)^{0.2}$ | NA |
| [12] | $k_La = k(P_g/V_L)^{\alpha}(v_s)^{\beta}$<br>$k_La/k = (4P_g/\pi D_T^2 H_L)^{0.4}(4Q/\pi D_T^2)^{0.2}$<br>$k_La/k = (4/\pi D^2)^{0.6}(N_p r D_i^5 N f_c f_p/H_L)^{0.4}n^{1.2}Q^{0.2}$ | 8.7 $W^{0.4}\ m^{-1}\ s^{-0.2}$ |
| [13] | NA | NA |
| [14] | NA | NA |
| [15] | $k_La = k(P_g/V_L)^a\ (v_s)^b v^c$<br>$k_La = K\ Q^x\ N_y\ D_i^{2.4}\ V_L^{-y/3.15}$ | NA |

All the empirical correlations in Table 17 use a similar pattern that is the Van't Riet equation, relating power consumption and superficial gas velocity to $k_La$. These empirical correlations were probably chosen because of ease as compared to determining the values through various chemical or physical methods, providing comparable values to experimental values. As can be seen from the reviewed studies, it is best, in scale-up, to obtain $k_La$ values themselves as scale-up criteria instead of factors the $k_La$ value is affected by. However, obtaining accurate $k_La$ values is still an issue, and the empirical models may not always give good estimates for $k_La$ since it is influenced by many factors such as the geometry of the bioreactor, operating conditions, broth rheology, and the measurement method employed [4]. In the included studies that involved a comparison of several strategies, constant $k_La$ remained the best option, giving the highest yield upon scale-up. This is in line with the literature available on the subject [83] and therefore it is best to determine *kLa* value, even when another strategy is applied.

### *4.2.3. Constant Aeration and/or Constant DO levels*

Another way to scale-up by considering oxygen transfer focuses on constant aeration number, flow rate per unit volume or vessel volume, or constant oxygen saturation concentration. In the included studies, two papers considered constant *Q/V* as a scale-up criterion [8-9]. In both cases, the scale-up was reported as successful. Similarly, constant DO was used in three included studies [13, 14]. While this method seems simple, it had involved adjusting the agitation speed by trial and error, and manual adjustment, to control DO levels and ensure they are above a certain value. Even though a cascade can be programmed to control the bioreactor's DO above a certain level, the agitation requirement might be too high and this can cause shear-related problems if not set properly. Hence, this method is not very clear-set and the DO value may vary greatly throughout the fermentation.

#### *4.2.4. Constant Reynold's Number*

Scaling up using Reynold's number as a constant criterion resulted in a sharp decrease in impeller speed, volumetric power input, and $k_La$ values compared to the small-scale values. This effect can affect mixing adversely, as was observed in terms of the large-scale biomass yield and protein yield [12]. Usually, applying constant Reynold's number does not provide a successful scale-up [12]. Hence, only one included study had considered it here. The findings of the study agree with the available literature on the constant Reynolds number strategy [78, 83]. In conclusion, this strategy should not be a used for recombinant protein production using *E. coli* unless the protein product great affects flow properties in the bioreactor.

#### *4.2.5. Constant Shear*

As the volume of the bioreactor increases, it becomes more difficult to maintain homogeneity within the bioreactor, and thus mixing properties are also of interest. While it is of interest to have an impeller speed that will provide ample mixing, shear effects must also be considered as the cells may be disrupted, experience morphological changes, and growth and product formation slowed down, due to the high shear forces. Similarly, power consumption must also be considered when increasing speed. By considering the maximum allowable shear, the agitation speed can be controlled during scale-up to provide adequate mixing without damaging the cells. Constant shear as a criterion gave satisfactory yield values upon scale-up [12] and this scaleup strategy was therefore successful.

### 4.3. Challenges Encountered During Scale-Up

To answer the second research question of this review, regarding the effect(s) of various process and operation conditions on protein production in a scale-up, more papers must be available for synthesis. The number of papers and the omissions they contain, do not provide the researchers with enough data to confidently conclude the effects of the various process and operation conditions. Similarly, the included papers do not report the challenges faced during scale-up in detail and, in many cases, do not show a troubleshooting methodology. However, from the issues faced during scale-up, some considerations that could assist researchers can be learned. Besides considering a change of strain, at the lower scale, a change in carbon source might also be necessary. The first is to give more consideration to the working volume or bioreactor at both scales.

### 4.4. Risk of Bias Assessment in Individual Studies

The risk of bias assessment for individual studies is presented in Table 18. A study on the large-scale production of a recombinant protein [8] did not report on the yield obtained; however, it was qualitatively stated that the recombinant protein yields were comparable. Another study by Wong [9] was unclear and it was not possible to extract precise data from the graphics as published; however, graphically it was shown that the recombinant protein yields at both scales were comparable.

Table 18: Risk of bias in individual studies

| Reference | Selection Bias | Detection Bias | Performance bias | Reporting Bias | Attrition bias | Statistical analysis | Funding | Evidence |
|---|---|---|---|---|---|---|---|---|
| [8] | Unclear | Unclear | Unclear | High | Unclear | Yes | NA | Biomass yield was measured, but actual values were not reported. |
| [9] | Unclear | Unclear | Unclear | High | Unclear | Yes | NA | Biomass yield was measured, but actual values were not reported. |
| [10] | Unclear | Unclear | Unclear | High | Unclear | NA | Funded | The choice of the scale-up strategy was not justified. |
| [11] | No | Unclear | Unclear | Unclear | Unclear | Yes | NA | Two scale-up strategies were compared. |
| [12] | No | Unclear | Unclear | Unclear | Unclear | Yes | NA | Four scale-up strategies were compared. |
| [13] | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Funded | The choice of the scale-up strategy was not justified. |
| [14] | Unclear | Unclear | Unclear | Unclear | Unclear | NA | Funded | The choice of the scale-up strategy was not justified. |
| [15] | Unclear | Unclear | High | Unclear | Unclear | Yes | Funded | The choice of the scale-up strategy was not justified. |

### 4.5. Risk of Bias in the Review Process

The AMSTAR tool's checklist was used to assess the risk of bias in the writing of these articles. There is a risk of publication bias as well as selective reporting as statistically significant findings are more likely to be published. Thus, publication bias could have distorted the interpretation of the findings of this review. One reviewer reviewed all the publications and made the decision to exclude or include them, and this contributes to a risk of bias as well.

### 4.6. Limitations

Publication bias, as well as reporting bias, have significantly impacted this review. It is understood that many more scale-up studies have been conducted. However, they are usually carried out on behalf of certain companies and therefore they were not published in academic journals. Of the 72 full-text scale-up articles initially screened, numerous studies were relevant but were considered to be of low quality as they did not clearly state their scale-up criteria and parameters, report their scale-up methodology, including fermenter and impeller specifications, and/or challenges faced, even though they had scale-up as one of their keywords. Hence, they could not be included as quality articles. This issue has impacted the findings of this review as well. Publications on process scale-

up should report unbiased strategies, calculations, challenges, errors as well as complete findings, to help other researchers better understand scale-up. Publication bias, as well as reporting bias, have significantly impacted the number of publications included in this review, even though data from two decades were considered.

It is noted that the outcome for the scale-up is measured differently in different papers. For instance, some papers [14] reported dry cell weight (DCW) to $OD_{600}$, while others reported wet cell paste [13]. Some papers report protein activity instead of yield in grams per liter. This makes it difficult to compare different studies and synthesize results. It might be preferable in some cases to report productivity as a better assessment of production, as it includes fermentation time in the consideration.

## 5. CONCLUSION

The conclusions that may be drawn from this systematic review are that (1) choice of scale-up criterion or criteria significantly alters yields of recombinant protein from *E. coli*, and (2) The comparison of different fermentation systems revealed that the criterion of maintaining $k_La$ constant was found to be effective in all five included studies which employed it, and superior in terms of yield of protein and biomass in the two studies that compared it to other criteria. This can be explained by the importance of oxygen transfer in aerobic fermentation, as oxygen becomes the limiting substrate due to its low solubility [4]. Furthermore, with the effect of scale, heterogeneities such as dissolved oxygen concentration become more apparent and significant and $k_La$ accounts for them. This is in line with scale-up recommendations for aerobic fermentation in general. A constant impeller Reynolds number, on the other hand, resulted in an unsuccessful scaleup. This is consistent with previous findings in the literature, as the effect of aeration is not considered in this strategy. The evidence described in this review suggests such recommendations are warranted and it fortifies them.

There is a need to identify and include unpublished data, that may better fit the research questions posed. As such authors should strive to address this issue in scale-up research to allow for more reliable conclusions to be made. Scale-up from shake flasks to pilot-scale bioreactors were not included in this study, but they can be addressed in a separate study. This study can be extended to include other types of bioreactors, as well as single-use bioreactors.

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## NOMENCLATURE

| | |
|---|---|
| $C^*$ | Liquid *DO* concentration at saturation |
| $C_L$ | Measured liquid *DO* concentration |
| $N$ | Impeller tip speed, m/s |
| $D$i | Impeller diameter, m |
| $D_T$ | Vessel diameter, m |
| *DO* | Dissolved oxygen |
| *Fr* | Froude number |

| | |
|---|---|
| $H_L$ | Height of liquid, m |
| $H_T$ | Height of vessel, m |
| $k_La$ | Volumetric oxygen transfer coefficient, mmol/L.h |
| LS | Large-scale |
| $N$ | impeller speed, rpm |
| $N_A$ | Flow aeration number |
| $N_{cd}$ | minimum impeller speed for all medium to be in contact with sparged gas |
| $N_i$ | Number of impellers |
| $N_P$ | Power number |
| $OTR$ | Oxygen transfer rate, mmol/L.h |
| $OUR$ | Oxygen uptake rate, mmol/L.h |
| $P_g$ | Gassed power input |
| $Po$ | Ungassed power input |
| $P_g/V$ | Gassed power input per unit volume |
| $Po/V$ | Ungassed power input per unit volume |
| $Q$ | Volumetric air flow rate, L/min (lpm) |
| $Q/V_L$ | Volumetric air flow rate per unit vessel liquid volume, L/min (vvm) |
| $Re$ | Impeller-based Reynolds number |
| SS | Small-scale |
| $T_{mix}$ | Mixing time, s |
| $v_s$ | Superficial velocity, cm/s |
| $V_L$ | Working volume of the vessel, L or $m^3$ |
| $V_T$ | Total volume of the vessel, L or $m^3$ |
| $X$ | Cell density, g/L |
| $Y_{xO2}$ | Cell yield on oxygen, g/mmol/L |
| $\rho$ | Density, $g/cm^3$ |
| $\upsilon$ | Liquid effective viscosity, g/cm.s |
| $\mu$ | Growth rate, /h |

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