Improvement of growth rates through nucleoside media supplementation of CHO clones

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Abstract Chinese Hamster Ovary (CHO) cells are used for the production of therapeutic proteins. This work examines improving passaging growth rate of two CHO clones. Growth rates were significantly improved for both clones with supplementation of the nucleosides cytidine, hypoxanthine, uridine, and thymidine to the culturing media at the optimal concentration of 100 μ M of each nucleoside. We investigated supplementing the same combination of nucleosides to seed bioreactors and production fed batch bioreactors. In the seed bioreactors, growth rate and harvest density were improved. However, in the production fed batch bioreactors, no improvements in growth rate or peak viable cell density were observed. Cell cycle analysis of the passaging cells provides evidence that nucleosides can affect the cell cycle. It is not clear from our work how the nucleosides impact the cell cycle regulatory pathways. Overall, nucleoside supplementation in cell culture media is an effective approach for improving growth rate in passaging and seed bioreactors of certain CHO cells.

Keywords CHO · Growth rate · Media supplementation · Nucleosides · Seed train

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Introduction

Over the past 30 years, increases in productivity for human therapeutic proteins have been driven by improvements in cell culture technology. Chinese Hamster Ovary (CHO) cells are the mammalian expression system that has become the industry standard for recombinant protein production. Media formulations for CHO cell culture have been continually optimized over the past several decades to improve performance and productivity. Early cell culture formulations used complex raw materials such as fetal bovine serum. Cell culture media optimization transformed the industry from complex media to serum-free media to chemically defined media. During this process, many components were evaluated for function and necessity. Amino acids, carbon sources, vitamins, lipids, trace elements, salts, and a buffering system are the components that are necessary to meet demands of the mammalian cells (Landauer 2014).

In this paper, we observed clones with low growth rates and we found it necessary to re-examine media formulations to improve these growth rates. This reassessment involved evaluation of nucleosides, which were found in several of the original commercial media formulation such as Duplecco's Modified Eagle's Medium, Medium 199, Minimum Essential Medium Eagle ("Media Formulations" 2019). These formulations have nucleosides as well as other related components such as purine bases, pyrimidine bases, and nucleotides. Since CHO cells can produce these

chemical building blocks (Lane and Fan 2015), often the components were removed in the development of chemically defined media formulations.

Nucleosides are glycosylamines, combining a nitrogenous base with a sugar. The sugar is either a ribose or a deoxyribose. The nitrogenous base is either a purine or pyrimidine with a variety of types in each category. Adenine, guanine, and hypoxanthine are purine nitrogenous bases. Thymine, uracil, and cytosine are pyrimidine nitrogenous bases. Although nucleosides are commonly supplemented in media formulations, just the nitrogenous base can be supplemented. Hypoxanthine is a commonly supplemented nitrogenous base. In our work, we will refer to hypoxanthine as part of the nucleoside combination, however it should be noted that it is technically a nitrogenous base and not a nucleoside. Figure 1 shows a diagram of the different nitrogenous bases and nucleoside forms.

Nucleosides are non-essential but when phosphorylated they become nucleotides, the backbone of DNA and RNA. Nucleotides are synthesized *de novo* from sugar based molecules and the *de novo* pathway does not incorporate nucleosides. However, nucleosides are metabolized into nucleotides through the nucleotide salvage pathway (Austin et al. 2012). The nucleotide salvage pathway gives cells a unique avenue for nucleotide synthesis other than the *de novo* pathway. This pathway is especially advantageous because it uses less energy than *de novo* synthesis to create nucleotides (Nyhan 2005). Preventing nucleotide deficiency is important. Austin et al. (2012) showed that nucleotide depletion can lead to replication stress causing cells to arrest in early S phase.

As mentioned above, nucleosides are known as potential media supplements for cell culture. Some recent work has examined nucleosides in CHO cells. Takagi et al. (2017) examined the addition of pyrimidine nucleosides to achieve higher peak cell density in fed batch cell culture. The team examined the main pyrimidine nucleosides in the deoxy form (deoxyuridine, deoxycytidine, thymidine) and found that adding a combination of all of the pyrimidine nucleosides achieved the best results. Tang et al. (2017) determined that supplementing nucleosides during selection of GS/MSX selection system reduced sequence variance. In this present work, we

investigate the impact of nucleoside supplementation on cell growth during cell expansion and production.

Materials and methods

Cell line development

CHO cells for this work were derived from the CHO K1 cell line (ATCC, Manassas, VA). Modifications were made to create new host cell lines and a monoclonal antibody (mAb) was transfected into these new host cell lines. The recombinant clones A and B were isolated and selected from two of the host cell lines.

Cell culture

Initial passaging work was done in CD CHO media (Thermo Fisher Scientific, Waltham, MA). An evaluation of the supplementation in CD CHO media in passaging was also completed. All other experiments were conducted using proprietary chemical defined media formulations designed internally. The experiments used the proprietary passaging media, basal media, and feed media formulations. Passaging cultures were executed in passaging media in duplicate for one 3-day passage (seeded at 0.3×10^6 cells/ml) and one 4-day passage (seeded at 0.2×10^6 cells/ml). At the start of the passage and on the final day of the passage, cell density was measured by a Nova Bioprofile FLEX Analyzer (Nova Biomedical, Waltham, MA). The Nova Bioprofile FLEX Analyzer measured total cell density, viable cell density, viability, and cell diameter. Growth rates were calculated from the viable cell density. The growth rates were averaged from the two passages of duplicates to give an $n = 4$ for most experimental condition. For the no nucleoside condition, data was accumulated from multiple experiments to give an $n = 22$. For the combination of adenosine, guanosine, hypoxanthine, thymidine, cytidine, and uridine (Combo AGHTCU) and the combination of hypoxanthine, thymidine, cytidine, and uridine (CHUT), data from two experiments was used to give an $n = 8$.

For the seed bioreactor experiment, the cells were grown in the same passaging media as the passaging experiments. Two 4-day cultures were conducted in bioreactors, seeded at 0.5×10^6 cells/ml. The

Fig. 1 Depiction of different forms for nitrogenous bases and nucleosides

Nitrogenous Base	Ribonucleoside	Deoxyribonucleoside
Adenine	Adenosine	Deoxyadenosine
Guanine	Guanosine	Deoxyguanosine
Hypoxanthine	Inosine	Deoxyinosine
Thymine	5-Methyluridine	Thymidine
Uracil	Uridine	Deoxyuridine
Cytosine	Cytidine	Deoxycytidine

experiment compared using the passaging media to passaging media with the added optimized nucleoside combination (100 μ M of each cytidine, hypoxanthine,

uridine, and thymidine). Both cultures used standard bioreactor parameters for temperature (36.5 °C), pH (7.20 $\pm$ 0.1), culture volume (1L), agitation rate (186

RPM), and dissolved oxygen (40% of air saturation). Each day of the culture, measurements were taken with the Nova Bioprofile FLEX Analyzer.

The production fed batch bioreactors were compared with and without the optimized nucleoside combination in the basal media. Two production fed batch bioreactor processes were used, Process 1 and Process 2. Both processes had the same parameters for target seed density (1.5×10^6 cells/ml), temperature (36.5 °C), culture volume (1L), agitation rate (260 RPM), and dissolved oxygen (40% of air saturation). The major difference between the processes was the feeding strategy. Process 1 feeding strategy was to add a fixed rate of feed starting on Day 3. Process 2 feeding strategy was to use the glucose restricted fed batch process (hereafter called HIPDOG) developed by Gagnon et al. (2011). In the HIPDOG process, an initial amount of glucose is in the basal media and as that is being consumed lactic acid is produced by the cells. Once the glucose reaches a sufficiently low level, the lactic acid begins to also be consumed, which causes a slight rise in pH that triggers the addition of the feed media which contains glucose. The HIPDOG feeding scheme was used through Day 6 of the culture and then a fixed rate of feed was initiated. Other minor differences between Process 1 and Process 2 are that the two processes had slightly different basal media compositions and the pH for Process 1 was 7.20 ± 0.10 while the pH for Process 2 was 7.20 ± 0.025 during HIPDOG feeding and 7.20 ± 0.15 after the end of the HIPDOG feeding (Day 6). Viable cell density and other metabolites were measured daily a Nova Bioprofile FLEX Analyzer. Spent media samples were collected on various days for titer analysis performed by protein A HPLC (model 1100 HPLC, Agilent Technologies, Inc., Santa Clara, CA, protein A column model 2-1001-00, Applied Biosystems, Foster City, CA).

Nucleoside media preparation

Adenosine (Cat. No. A9251), guanosine (Cat. No. G6752), cytidine (Cat. No. C4654), hypoxanthine (Cat. No. H9377), uridine (Cat. No. U6381), and thymidine (Cat. No. T9250) were all obtained from Sigma-Aldrich (St. Louis, MO). Nucleosides were added to proprietary media prior to passaging to target a specific nucleoside concentration. The nucleoside concentration added to media is reported but it is

acknowledged that in the process of passaging, the concentration will be slightly lower.

Cell cycle analysis

Samples for cell cycle analysis and for measuring growth rate were taken every day of a routine 4-day passage (seeded at 0.2×10^6 cells/ml). Passaging media and passaging media with optimized nucleoside combination were examined, each condition with five replicates. Approximately 1×10^6 viable cells were centrifuged at 1000 RPM for 10 min followed by resuspension in 1 ml ice-cold methanol and storage at -80 °C. On the day of the analysis, a portion of the cells were washed with PBS followed by incubation with Pridium Iodine/RNase Staining Solution (Cell Signaling Technology, 4087S, Danvers, MA) by following the manufacture's instruction. Cell fluorescence was measured on a Fortessa® flow analyzer (BD Scientific, San Jose, CA) using a 488 nM laser and cell cycle analysis was performed using ModFit® software (Verity Software House, Topsham, ME).

Results

Evaluation of nucleoside supplementation to improve growth rate of clone A

Clone A was selected because it had the highest productivity during clone screening experiments. When initial passaging work was performed with clone A, a low growth rate was observed in CD CHO media. Over nine passages, the clone A growth rate in CD CHO media was 0.0245 h^{-1} ($s = 0.00176 \text{ h}^{-1}$), which equates to a doubling time of 28.3 h. Internally, clones with growth rates over 0.0289 h^{-1} (doubling times lower than 24 h) are characterized as having desirable growth rates for supporting manufacturing.

In an effort to understand if this low growth rate was inherent to the clone or caused by the CD CHO passaging media, we evaluated the clone in proprietary passaging media. The proprietary passaging media improved the growth rate but clone A was still characterized as having a low growth rate. Accumulating data from multiple experiments, the growth rate of clone A in the passaging media, described as the “No Nucleosides” condition, was 0.0283 h^{-1} ($s = 0.00119 \text{ h}^{-1}$).

The supplementation of nucleosides was investigated to improve the growth rate to desirable levels that would support manufacturing practices. Based on knowledge from past media formulations and general understanding of nucleosides, six nucleosides were selected and tested in combination: adenosine, guanosine, hypoxanthine, thymidine, cytidine, and uridine, hereafter referred to as Combo AGHTCU. This combination was added to the media at concentrations of 100 μM and 1 mM. Figure 2a shows the comparison of the growth rates of cultures containing the Combo AGHTCU at 100 μM compared to the no nucleosides condition. The 1 mM concentration was shown to inhibit growth and therefore was not investigated further (data not shown). The Combo AGHTCU at 100 μM improved the growth rate of clone A to 0.0305 h^{-1} ($s = 0.00172 \text{ h}^{-1}$).

Assessment of nucleoside combinations and individual nucleoside concentration to improve growth rate of clone A

To understand whether we could further optimize the nucleoside concentrations, we evaluated the impact of

each nucleoside on passaging growth rate. Each individual nucleoside was examined from the Combo AGHTCU at 100 μM (Fig. 2b). Individually, none of the nucleosides improved growth rate as compared to the no nucleosides condition. Surprisingly, the supplementation of both adenosine and guanosine inhibited cell growth significantly.

The nucleosides that did not inhibit growth, hypoxanthine, thymidine, cytidine, and uridine, were tested in all possible combinations for their effect on growth rate. The optimal combination was the combination with all four nucleosides each at 100 μM (hereafter called CHUT). In these experiments, there was not a statistically significant difference between the Combo AGHTCU and CHUT conditions. However because of the observed negative effect of adenosine and guanosine, CHUT was chosen as the optimal combination. The growth rate with CHUT supplementation was 0.0311 h^{-1} ($s = 0.00225 \text{ h}^{-1}$). Figure 2c shows the comparison of the no nucleosides condition versus the condition with CHUT supplementation.

Once this combination was found, we performed an extensive dose curve. This dose curve showed that

Fig. 2 Growth rates for clone A. **a** Growth rate of no nucleosides condition versus Combo AGHTCU (100 μM of each nucleoside). **b** Growth rate of no nucleosides condition versus individual nucleosides adenosine, guanosine, hypoxanthine, thymidine, cytidine, and uridine (each nucleoside at 100 μM). **c** Growth rate of no nucleosides condition versus CHUT (100 μM of each nucleoside). **d** Growth rate of no nucleosides condition versus different concentrations of CHUT (concentration of each nucleoside). Statistical significance on the difference of growth rate between the no nucleosides condition and experimental conditions was assessed. **indicates P value < 0.01

100 μM of each nucleosides was the ideal concentration for the CHUT supplementation (Fig. 2d). Inhibition of growth was not seen even at 800 μM with this combination, even though inhibition was seen at 1 mM with the Combo AGHTCU. We hypothesize that the inhibition of growth at 1 mM concentration with the Combo AGHTCU was likely due to the presence of adenosine and guanosine in the combination.

CHUT supplementation to improve growth rate of clone B

Similar to clone A, clone B was shown to have higher productivity but also a low growth rate in proprietary passing media, 0.0240 h^{-1} ($s = 0.00054 \text{ h}^{-1}$). We found that supplementation of 100 μM CHUT to the passing media could boost the growth rate of clone B significantly to 0.0303 h^{-1} ($s = 0.00176 \text{ h}^{-1}$). Figure 3a shows the comparison of the growth rates with and without CHUT supplementation.

We also examined whether CHUT supplementation could improve the growth rate of this clone in media other than our proprietary passing media. In CD CHO media, clone B's growth rate was 0.0283 h^{-1} ($s = 0.00109 \text{ h}^{-1}$). The growth rate in CD CHO is higher than that of our proprietary passing media for this particular clone. With the addition of the CHUT supplement to the CD CHO media, the growth rate was increased to 0.0306 h^{-1} ($s = 0.00104 \text{ h}^{-1}$). Figure 3b shows the comparison of the growth rates with and without CHUT supplementation in CD CHO media.

Evaluation of CHUT supplementation in seed and production bioreactors

After confirming CHUT's positive effect in passing for clone B, experiments were performed with clone B to determine if CHUT supplementation might also increase growth rate and viable cell densities in seed bioreactors and production fed batch bioreactors. An intensified seed bioreactor allows for robust culture expansion that result in higher harvest cell densities allowing for a higher seeding density inoculation in the production fed batch bioreactor. Production fed batch bioreactors inoculated at higher densities tend to reach peak viable cell density faster thereby producing more product within the same batch duration. It is difficult to reach higher harvest cell densities within the normal seed bioreactor duration of three or four days when the cell line exhibits a low growth rate such as clone A or clone B. We examined the addition of CHUT to the seed bioreactor media to assess the impact to seed bioreactor harvest cell densities.

Clone B was expanded in the passing media (without CHUT) and then run with this same passing media as a seed bioreactor with and without CHUT supplementation. The condition with CHUT supplementation had significantly higher growth rate than the no nucleosides seed bioreactor condition, 0.0319 h^{-1} vs of 0.0270 h^{-1} , and reached a significantly higher viable cell density in the 4-day culture, $9.04 \times 10^6 \text{ cells/ml}$ vs $5.88 \times 10^6 \text{ cells/ml}$ (Fig. 4a). The CHUT supplementation with clone B could support delivering a higher seeding density inoculum for a production fed batch bioreactor. The experiment was repeated

Fig. 3 Growth rates for clone B. **a** Growth rate of no nucleosides condition versus CHUT in proprietary passing media. **b** Growth rate of no nucleosides condition versus CHUT in CD CHO media. Statistical significance on the difference of

growth rate between the no nucleosides condition and experimental conditions was assessed. *indicates P -value < 0.05 and **indicates P -value < 0.01

Fig. 4 Bioreactor data for clone B. **a** Seed bioreactor viable cell density. **b** Production fed batch bioreactor Process 1 (set feeding strategy) viable cell density. **c** Production fed batch bioreactor Process 2 (HIPDOG feeding strategy) viable cell density. **d** Production fed batch bioreactor Process 2 (HIPDOG feeding strategy) normalized titer

with slight variations in the seed bioreactor seed density and similar results were observed.

CHUT supplementation was also evaluated for any benefit at the final step of the cell culture process, the production fed batch bioreactor. The CHUT supplementation was tested with two different production fed batch bioreactor processes, Process 1 which used a set feeding strategy and Process 2 which used a HIPDOG feeding strategy, as shown in Fig. 4b, c, respectively. Both processes compared one bioreactor with no nucleosides and one bioreactor with the CHUT supplementation. For both processes there was no improvement in growth rate or peak viable cell density with the CHUT supplementation. Process 1 had significant lactate accumulation, which could be inhibitory to the cells. The lactate in Process 2 was well controlled as expected with the HIPDOG feeding strategy due to HIPDOG design.

Antibody productivity (titer) and product quality derived from Process 2 were compared between the no nucleosides and CHUT supplementation condition. As seen in Fig. 4d, the addition of CHUT did not yield changes in antibody productivity (titer). Product quality was assessed by comparing the glycan profile of the material generated from the bioreactors. No significant difference in the glycan profile was detected (data not shown). The experiment was repeated with slight variations but the overall results were the same that CHUT supplementation was not able to improve growth rate, peak viable cell density, or titer during the production fed batch bioreactor step. Also we examined conditions with CHUT supplementations of higher than 100 μ M CHUT. These conditions included 200 μ M CHUT in the basal media, 400 μ M CHUT in the basal media, and 200 μ M CHUT in the basal media plus bolus additions of CHUT early in culture. Again, we did not observe positive effect in either growth or productivity.

Impact of nucleoside supplementation on cell cycle

In an effort to understand how nucleoside supplementation increased the growth rate of clone A and B, we examined the cell cycle attributes of our cell culture. We hypothesized that our cells were being slowed down in either the G1 or S phase where nucleotides are needed to support DNA replication.

For both clones A and B, a 4-day passage with and without CHUT supplementation was completed. Samples were taken every day of the passage to measure growth rate and for cell cycle analysis. Figure 5 shows the growth rate per day, percent of cells in G1 phase, and percent of cells in S phase for both clones. Percent of cells in G2 is not shown as it did not vary significantly and was relatively low for all days and conditions. Growth rate per day allows us to examine the dynamic nature of the cell growth. From Day 0 to 1 the growth rate was similar between the conditions. From Day 1 to 2 and Day 2 to 3, there was a significant increase in growth rate for the condition with CHUT supplementation. From Day 3 to 4 the growth rate was lower for the condition with CHUT. It was likely that

by Day 4, other factors influencing cell growth, such as differences in pH or by-product production, are confounding the growth rates. The cell cycle data from Day 1 and Day 2 was considered the most relevant since it might elucidate the cell state during the period of high growth for the condition with CHUT.

On Day 1, both clones for the condition with CHUT had a lower percentage of cells in G1 phase and a higher percentage of cells in S phase compared to the conditions without CHUT. On Day 2, the clones have conflicting results. Clone B has similar results for Day 2 as it does for Day 1. For clone A on Day 2, the percent of cells in G1 phase was slightly higher and the percent of cells in S phase was slightly lower for the

Fig. 5 Cell cycle analysis results for growth rate and cell cycle phase for both clones A and B. **a** Growth rate per day of clone A. **b** Growth rate per day of clone B. **c** Percent G1 Phase per day of clone A. **d** Percent G1 Phase per day of clone B. **e** Percent S Phase per day of clone A. **f** Percent S Phase per day of clone B. Statistical significance on the difference of growth rate, % G1 phase, and % S Phase between the no nucleosides condition and + CHUT conditions was assessed. There was a statistical significance with a P -value < 0.05 for all conditions except for the comparison of the following: growth rate Day 0–1 for clone A, growth rate Day 0–1 for clone B, percent of cells in S phase on Day 3 for clone B, and percent of cells in S phase on Day 3 for clone A

condition with CHUT compared to the no nucleosides condition. This data shows that the nucleosides have a strong impact on the different phases of cell cycle.

Discussion

Nucleosides supplement the precursors for DNA and RNA through the nucleotide salvage pathway (Austin et al. 2012). If cells grow adequately, then nucleoside supplementation may not be necessary. However, it may be helpful to evaluate supplementation of nucleosides for cell lines with low growth rates.

We evaluated supplementation of both purine and pyrimidine nucleosides based on growth. When tested as the individual nucleosides, we saw that the purines adenosine and guanosine both inhibited growth. Adenosine and guanosine are well known to be important in cellular signaling so it is possible this concentration of adenosine and guanosine inhibited important metabolic pathways. Extracellular guanosine has been shown to induce S phase cell arrest (Guarnieri et al. 2009) and extracellular adenosine is known to have a negative effect on cells at 100 μM or greater concentrations (Ohana et al. 2001). At the concentrations we tested (up to 800 μM in combination with other nucleosides) the purine hypoxanthine did not inhibit growth. Experiments with hypoxanthine were performed because it is a commonly used nucleoside in commercial media, potentially due to its utility as a precursor to purine nucleotides. Hypoxanthine can be metabolized to adenosine monophosphate or guanosine monophosphate and from there to other RNA and DNA precursors (Kanehisa and Goto 2000). It adds robustness to have both purine and pyrimidine components. Our work corroborates the work by Takagi et al. (2017) that the combination of nucleosides is most effective at improving growth.

Takagi et al. (2017) showed significant improvement in the bioreactor peak viable cell density but the team did not observe significant difference in growth rates with nucleoside supplementation. For passaging, growth rate is more relevant than peak viable cell density because the cells are always in exponential phase and do not peak in the short batch time of passaging. We hypothesized that improved growth rates in passaging would translate to improve growth rates in bioreactors and improved peak viable cell densities. In bioreactors, we observed a significant

improvement in growth rate for seed bioreactors but we did not observe an improvement in growth rate or peak viable cell density in production fed batch bioreactors. This was unexpected as we assumed the improvement in growth rate seen in the cell expansion work including the seed bioreactor work would at the very least translate to faster growth during the early days of the fed batch production bioreactor. Though the reason is unclear, we hypothesized that different operation conditions between seed bioreactors and production bioreactors (basal media composition, feed addition, initial CHUT concentration per cell, cell inoculum, and seed density) may play a role.

The basal media composition is a similar proprietary formula between the seed and production bioreactor, but the basal media is approximately two times richer in amino acids. The addition of feed which occurs only in the production bioreactor could definitely make a difference in the culture, especially for peak viable cell density. However, there is no appreciable difference in growth rate through Day 3 in the production bioreactor by which point no feed had been added for Process 1 and a very minimal amount of feed had been added for Process 2. In our first experiment, we kept the initial CHUT concentration (100 μM) the same from passaging to bioreactors. We thought that perhaps the production bioreactor needed higher concentration of CHUT due to higher initial cell densities. Therefore, we examined adding up to 400 μM CHUT to the basal media and we examined adding a CHUT bolus feed to the bioreactor early in culture. Neither of these conditions resulted in improvement in peak viable cell density but it is possible the concentration could be optimized further. The cell inoculum was different for the seed and production run. The inoculum for the seed bioreactor was at a lower density and a lower pH than the inoculum for the production run. The seed density was also significantly different between the seed and production run. Since most of these differences are inherent to the nature of the runs, we did not investigate these differences further. Each of these differences could have potentially contributed to the lack of effect of CHUT in the production bioreactor.

Cell cycle analysis allowed us to dive deeper into the underlying mechanism of nucleoside supplementation. CHO cells have a complex, highly integrated, and tightly controlled cell proliferation process. The progression of cells through the cell division is

regulated by extracellular and intracellular signals that monitor and coordinate the various processes that take place during different cell cycle phases (Vermeulen et al. 2003). We have shown that nucleoside supplementation significantly affects cell cycle progression for two slow growing clones in passaging. The effect seems to be most prominent early in culture. Recent work in the field of cancer research showed that exogenous nucleoside supplementation increased replication rates for cells with an aberrant Rb-E2F pathway which is involved in S phase entry (Bester et al. 2011). Also it has been shown that exogenous nucleoside supplementation suppressed the expression of markers of senescence in cells that were induced to be senescent (Aird et al. 2013). Based on this literature and our knowledge of the cell cycle, we hypothesize that either the nucleosides are providing substrate or are acting as a signal for faster cell cycle progression. More work is needed to understand how the nucleosides are affecting the cell cycle.

In conclusion, we report that nucleoside supplementation is able to significantly improve passaging growth rate for two different clones. Growth rate and harvest density were also improved in seed bioreactors with the nucleoside supplementation. However, in our work, nucleoside supplementation to basal media did not improve the growth in the production fed batch bioreactors. We have also shown that nucleoside supplementation has a significant impact on the cell cycle but further work is needed to determine potential mechanisms.

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Compliance with ethical standards

Conflict of interest The authors declare no financial or commercial conflict of interest.

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