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Use of Glucose Feeding to Produce Concentrated Yeast Cells

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Abstract

A defined medium and fed-batch feeding process for the production of a yeast biocatalyst, developed at the 23-L scale, was scaled up to the 600-L pilot scale. Presterilized 100-L-vol plastic bags were implemented for the pilot-scale nutrient feeding. Medium of increased concentration Ôqs implemented at the pilot scale, and equivalent dry cell weights were reached with a medium 80% more concentrated than that used at the laboratory scale. The higher medium concentration was believed to be necessary at the pilot scale owing to the additional heat stresses on key components (e.g., complexing of magnesium sulfate with phosphate), increased dilution during sterilization, lower evaporation rate owing to the lower vessel volume per minute air flow rate, and increased dilution owing to nutrient feeding or shot additions. Peak cell density was found to be somewhat insensitive to variations in residual glucose levels. These results suggest that defined medium developed at the laboratory scale may need to be further optimized at the pilot scale for equivalent performance.

Index Entries: Fed batch; disposable sterile bag; yeast; cell mass; defined medium.

Introduction

Chiral drugs clearly are preferred over racemic mixtures for pharmaceutical development *(1)*. Bioconversions, either mediated by enzymes or by whole cells, are important in chiral molecule production *(2,3)*. Productivity and yield achievements often can be attractive when compared with available synthetic routes to prepare these chemical structures in large quantities.

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Baker's yeast has been demonstrated to catalyze a wide variety of bioconversion reduction reactions, making it a highly synthetically useful biocatalyst *(4,5)*. Some specific examples include the reduction of simple ketones *(6–8)*, the asymmetric reduction of unsaturated ketones and carbonyl compounds *(9–11)*, the conjugate reduction of enones *(6,12,13)*, and the reduction of alkenoates *(14)*.

The enzyme activity of a variety of yeasts has been optimized for best bioconversion performance. Examples of such work include the production of the cellobiohydrolase II enzyme of *Trichoderma reesei* using recombinant *Saccharomyces cerevisiae (15)*, production of lipase from *Candida rugosa (16)* or *Yarrowia lipolytica (17)*, as well as production of extracellular glucose oxidase from recombinant *S. cerevisiae (18)*. Peak enzyme activities need to be optimized on both a per cell and a per unit volume basis for best evaluation of improvements in productivity.

The fact that yeast is relatively simple to cultivate and grows relatively well on defined medium makes it an ideal source of bioconversion catalysts for whole-cell or enzyme bioconversions. In most cases, enzyme reaction rates are first order with respect to biocatalyst concentration over the range of enzyme concentrations selected for the process. This relationship can translate into a requirement for high cell density. It thus becomes important to develop methods for the reproducible and robust cultivation of yeast biocatalysts to high cell densities in excess of 50 g/L dry cell weight.

Fed-batch cultivations, one reliable method to produce high cell mass, have been described in the literature for *Escherichia coli (19,20)*, *Y. lipolytica (21)*, and other cultures *(22)*. The use of defined culture media for both the growth and bioconversion portions of the process results in simpler downstream isolation and improved analysis of nutrient requirements during development *(23)*. The ability to cultivate this specific yeast species *Candida sorphophila* to high cell densities on defined medium using a fed-batch process already has been demonstrated at the 23-L scale. This yeast has been used to perform an asymmetric bioreduction, and details of the strain and its origin may be found elsewhere *(24)*. The purpose of the present study was to document the experiences on scale up of this fed-batch process to the 600-L pilot scale.

Materials and Methods

Seed and Production Medium

Seed medium for the seed flasks and tanks was DM9 medium. DM9 medium contained 10.0 g/L of monosodium glutamate, 4.8 g/L of ammonium sulfate, 1.0 g/L of potassium phosphate dibasic, 1.0 mL/L of polypropylene glycol antifoam (P2000; Dow, Freeport, TX), 0.58 g/L of magnesium sulfate heptahydrate, 2.0 mL/L of trace element solution, 1.0 g/L of calcium carbonate (added after the presterilization pH adjustment), 44.0 g/L of dextrose (added poststerilization), and 1.0 mL/L of vitamin solution (added poststerilization). The trace element solution contained 5.0 g/L of

Table 1
Composition of Various Concentrations of 2XSYN9 Medium

Component	1.2X (expt. 1)	1.5X (expt. 2) with 1.2X trace elements	1.5X (expt. 3)	1.8X (expt. 3)	1.8X (expt. 4)
Monosodium glutamate (g/L)	45.4	56.8	56.8	68.2	68.2
Ammonium sulfate (g/L)	41.0	51.2	51.2	61.4	61.4
Potassium phosphate dibasic (g/L)	4.6	5.7	5.7	6.8	6.8
Calcium carbonate (g/L)	2.25	2.8	2.8	3.2	3.2
Magnesium sulfate heptahydrate (g/L)	1.8	2.3	2.3	2.8	2.8
P2000 antifoam (mL/L)	1.8	2.3	2.3	2.8	2.8
Trace elements (mL/L)	7.2	9.0	9.0	10.8	10.8
Composition					
$ZnSO_4 \cdot 7H_2O$ (mg/L)	5.0	4.0	5.0	5.0	5.0
$CuSO_4 \cdot 5H_2O$ (mg/L)	0.5	0.4	0.5	0.5	0.5
$FeSO_4 \cdot 7H_2O$ (mg/L)	5.0	4.0	5.0	5.0	5.0
$MnSO_4 \cdot H_2O$ (mg/L)	1.0	0.8	1.0	1.0	1.0
$CoCl_2 \cdot 6H_2O$ (mg/L)	0.4	0.32	0.4	0.4	0.4
Initial dextrose (g/L)	23	15.7	15.7	15.7	15.7
Vitamins (mL/L)	2.4	3.0	3.0	3.0	3.0
Composition					
Biotin (mg/L)	100	100	100	120	120
Pyridoxin HCl (mg/L)	100	100	100	120	120
Thiamine (mg/L)	100	100	100	120	120

zinc sulfate heptahydrate, 0.5 g/L of cupric sulfate pentahydrate, 5.0 g/L of ferrous sulfate heptahydrate, 1.0 g/L of manganese sulfate monohydrate, and 0.4 g/L of cobalt chloride hexahydrate; HCl was added to dissolve the trace elements. The vitamin solution contained 100 mg/L of biotin, 100 mg/L of pyridoxin HCl, and 100 mg/L of thiamine HCl and was stored at 2–8°C until added to the cultivation poststerilization.

Production medium was 2XSYN9 medium prepared at various concentrations (Table 1). The 2XSYN9 medium at 1.2X concentration contained 45.4 g/L of monosodium glutamate, 41.0 g/L of ammonium sulfate, 4.6 g/L of potassium phosphate dibasic, 1.8 mL/L of P2000 antifoam, 1.8 g/L of magnesium sulfate heptahydrate, 7.2 mL/L of trace element solution, 2.25 g/L of calcium carbonate (added after the presterilization pH adjustment), 23.0 g/L of dextrose (added poststerilization), and 2.4 mL/L of vitamin solution (added poststerilization). The trace element and vitamin solutions were identical to those just described.

The presterilization pH was adjusted to 6.0 for both the seed and production media. Seed tanks were sterilized for 40 min at 122–124°C and production tanks were sterilized for 45 min at 122–124°C. For Experiment 1, the production tank batching volume was 300 L with a poststerilization dextrose shot addition of 15 L. For subsequent experiments, the batching volume was 450 L with a poststerilization concentrated dextrose (500 g/L) shot addition of 15 L, decreasing the dextrose concentration from 23.0 to 15.7 g/L. The total empty tank production volume was 800 L. Details of the tank and impeller configurations are described elsewhere *(25)*.

Cultivation Conditions

Seed flasks, seed tanks, and production tanks were cultivated at 34°C. Production tanks utilized a constant back pressure of 0.7 kg/cm^2, an airflow rate of 100–500 L/min, and an agitation rate of 100–350 rpm. Airflow and agitation rate were cascaded to control dissolved oxygen at 50% of air saturation at ambient pressure. Broth pH was controlled between 5.8 and 6.4 using 25% sulfuric acid or 25% sodium hydroxide. Cultivation conditions for the 23-L fermentation were similar to those used for the pilot-scale fermentations. Fermentations were done as described in each experiment with two production tanks being run together for comparison.

Preparation of Inoculum

C. sorphophila inoculum *(24)* was prepared by inoculating 1.5 mL of frozen seed into 50 mL of seed medium in a 250-mL nonbaffled Erlenmeyer flask. This flask was incubated for 24 h followed by transfer of 5 mL into 500 mL of seed medium in a 2-L Erlenmeyer flask. After a second 24-h incubation period, six flasks were pooled to obtain 3 L to inoculate the seed fermentor.

The seed fermentor was permitted to grow to an oxygen uptake rate (OUR) of 30–60 mmol/(L·h), and then 10 L of this seed was transferred to

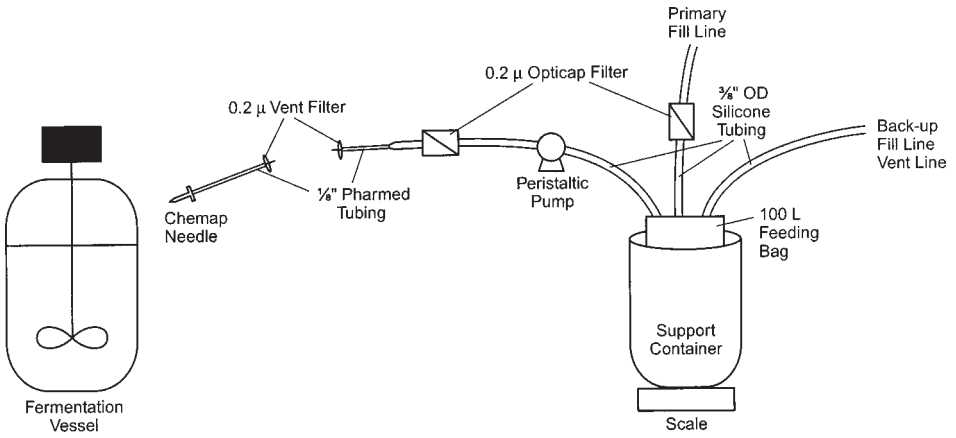


Fig. 1. Schematic of a nutrient feeding bag.

the production tank. Since the seed tanks were not transferred to production until an age of 18–22 h, adequate time was available for a preliminary evaluation of the previous day's culture purity results.

Bag Feeding

Presterilized gamma-irradiated 100-L-vol bags were designed and purchased from Stedim (Concord, CA), as shown in Fig. 1. Bags were designed with three ports. The first port consisted of one 10-in. hydrophilic 0.2-µ Opticap filter (cat. no. KVGLG1HH1; Millipore, Bedford, MA) and 3/8-in. od silicon tubing line for filling the bag. The second port consisted of one 10-in. hydrophilic 0.2-µ Opticap filter attached to a length of 1/8-in. Pharmed tubing (adapted to a 0.2-µ vent filter, Aervent 50, cat. no. MTGR8500; Millipore). The vent filter was used to maintain sterility prior to attachment of Pharmed tubing to the tank feeding line via a sterile tubing welder (Terumo SCD; Terumo Medical, Somerville, NJ) for feeding out from the bag. The third port consisted of a length of 3/8-in. od silicone tubing without a filter for filling the bag in cases when prefiltration was not considered necessary or for bleeding out excess air. The empty bag was staged inside a cylindrical support container and placed on top of a 150-kg floor scale. Presterilized nutrient (500 g/L of dextrose) was transferred from a holding tank, maintained at 35°C, into the bag via the inlet Opticap filter using air overpressure. Care was taken not to overpressurize the filling line or the bag. After filling the bag, excess air was bled out of the bag, if necessary, via the third line so that air bubbles settling at the bag outlet were minimized.

By using the sterile tubing welder, the feeding line containing the Opticap filter was welded to the tank feeding line consisting of an autoclaved Chemap needle connected to a length of 1/8-in. Pharmed tubing and a 0.2-µ vent filter. After ensuring that the two other lines to the bag were securely clamped shut, the outlet feeding filter was then primed and feed-

ing into the fermentor began. The outlet filter was placed in a vertical position to aid in removing air from the filter. It was critical to ensure that enough tubing was available so that as the bag collapsed, it would not exert pressure on the attached tubing and tear the bag. The bag was refilled prior to complete exhaustion to minimize the effort of repriming the pump.

The initial bag attachment design placed the feeding line components in the order of bag, filter, pump, and tank. This sequence was found to work less than efficiently with the filter losing its prime quite easily. A revised bag attachment design then was implemented in the order of bag, pump, filter, and tank (Fig. 1). These bags were evaluated as an alternative to Nalgene plastic carboys as a means for staging small-volume liquid feeds. Hard-piped systems are difficult to use at the low flow rates of 0.5–5 L/h desired.

Dry Cell Weights

Samples were taken every 2–4 h over the course of the 48- to 60-h cultivation. Dry cell weight was analyzed by pipetting 2 mL of broth onto preweighed, then presaturated with water, filter paper and vacuum filtering the sample. The filter paper containing the cells was dried using a microwave and the residue was weighed.

Glucose Monitoring

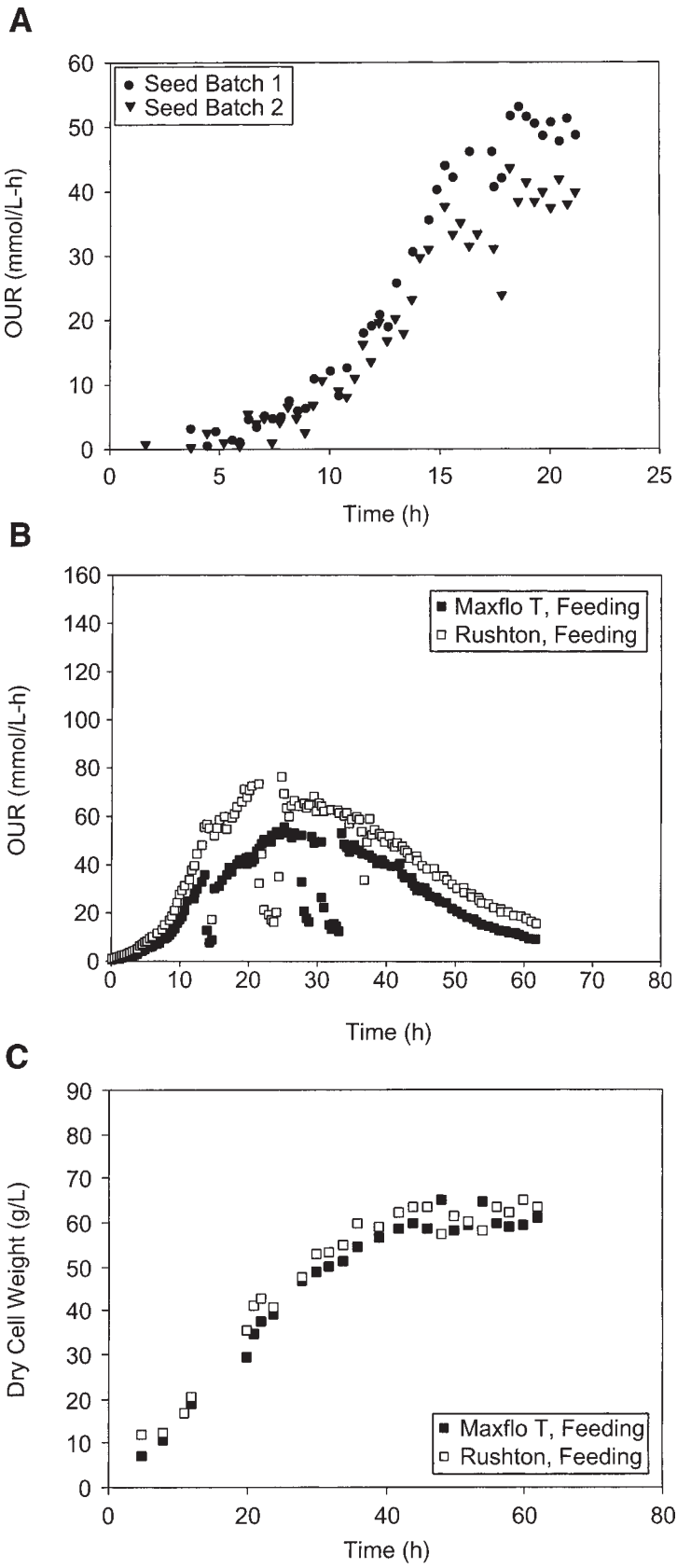
Samples were taken every 2–4 h, and monitoring for glucose was conducted using a glucose oxidase colorimetric test paper assay (Diastix Reagent strips for urinalysis #2806; Bayer, Elkhart, IN). Dextrose feed rate was adjusted manually to maintain a target residual of about 0–3 g/L in the broth. Feeding typically was started at about 12 h postinoculation. In some cases, in which feeding inadvertently was interrupted, the OUR declined rapidly, but it always returned to its previous value on reestablishment of sufficient glucose in the broth. This speed of recovery may not be the case for other cultures and processes.

Results and Discussion

Initial Trial of Defined Medium (1.2X) with Continuous Dextose Feeding: Experiment 1

Seed tanks were permitted to attain an OUR of 60 mmol/(L·h) (Fig. 2A) prior to transfer to two production tanks containing 1.2X concentrated 2XSYN9 defined medium. The initial starting point for pilot-scale development was 1.2X concentrated medium based on prior experience translating

Fig. 2. *(opposite page)* **(A)** OUR vs time for seed tank cultivation (experiment 1); **(B)** OUR vs time for continuous feeding for Rushton and Maxflo T impeller designs for 1.2X concentrated medium (experiment 1); **(C)** dry cell weight vs time for continuous feeding for Rushton and Maxflo T impeller designs for 1.2X concentrated medium (experiment 1).


A
Seed Batch 1
Seed Batch 2
OUR (mmol/L-h)
Time (h)
B
Maxflo T, Feeding
Rushton, Feeding
OUR (mmol/L-h)
Time (h)
C
Dry Cell Weight (g/L)
Maxflo T, Feeding
Rushton, Feeding
Time (h)

23-L fermentation results to the pilot scale. Dextrose was fed to both tanks and residuals were generally below 2.5 g/L. Broth pH rose initially over the first 10 h of cultivation from about 6.0–6.1 to about 6.4–6.5, after which it declined to about 6.2 for the duration of the fermentation. This behavior suggested an initial metabolism of nitrogen-containing amino acids in the medium followed by a rise in glucose metabolism. Similarly, the respiratory quotient (RQ) declined from about 1.3 to about 0.8–0.9 during the first 3 to 4 h, followed by a rise to about 1.1 over the next 3 to 4 h. During the remainder of the cultivation (ending at 60 h), it declined slowly to about 0.8, a pattern that suggested metabolism of nutrients with oxygen to carbon atomic ratios lower than glucose such as ethanol.

Cell growth increased similarly in both tanks up to a dry cell weight of 60–65 g/L at 45 h, after which it remained steady for an additional 15–18 h (Fig. 2B). There were some slight differences between the Rushton impeller (radial flow) and the Maxflo T impeller (axial flow). (Impeller details are summarized elsewhere *[25]*.) For the Rushton impeller batch, the OUR increased up to 56 mmol/(L·h) by 25 h and then began to decline starting at about 35 h. For the Maxflo T impeller batch, the OUR increased to 72 mmol/(L·h) by 22 h, which corresponded to the slightly earlier peak in dry cell weight for this cultivation (Fig. 2C). There was no appreciable foam observed in either cultivation.

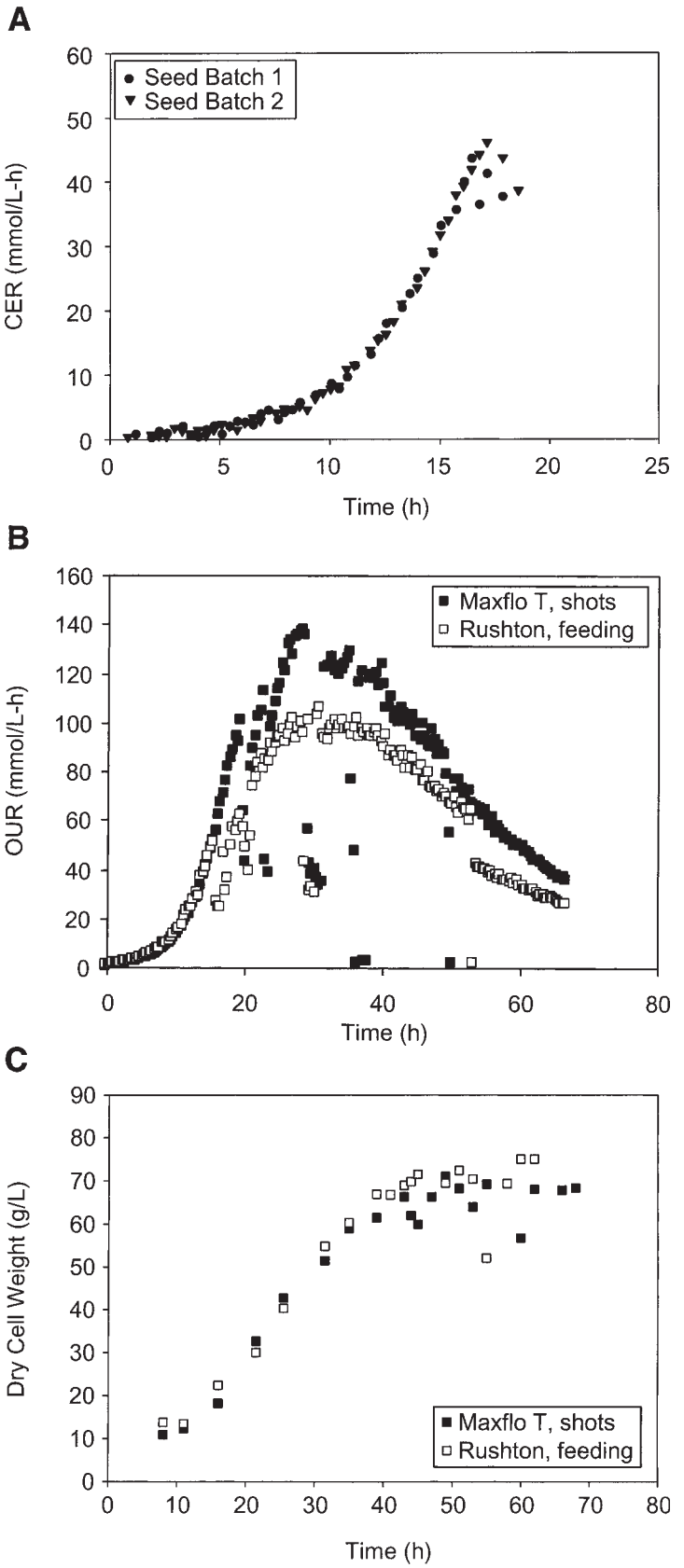
Comparison of Continuous Dextrose Feeding with Dextrose Shots Using Defined Medium (1.5X): Experiment 2

Seed tanks were inoculated into production at an OUR of 45 mmol/(L·h). A graph of the carbon dioxide evolution rate (CER) vs time for these seed tanks is shown in Fig. 3A, and the trend is generally similar to that of Fig. 2A.

Dextrose feeding (for a fermentor with a Rushton impeller) was compared with dextrose shots (for a tank with a Maxflo T impeller) using 1.5X defined medium with only 1.2X trace elements owing to a miscalculation. Shot additions of 30–50 L of 500 g/L of dextrose were given periodically about 6–14 h apart. Residual glucose levels were under 2.5 g/L, and there was no observed foam for the batch using continuous feeding. Residual glucose levels ranged up to 10 g/L, and P2000 antifoam was used for foam control (along with raising the pressure to 1.2 kg/cm^2) for the batch using shot additions. Some of the batch volume (100–150 L) in this latter cultivation was sewered to maintain an appropriate volume in the tank.

While the pattern of the respiratory quotient variation was similar to that observed in experiment 1, the pH profile was markedly different.

Fig. 3. *(opposite page)* **(A)** CER vs time for seed tank cultivation (experiment 2); **(B)** OUR vs time for batch using continuous feeding vs batch using shot additions for 1.5X concentrated medium with 1.2X concentrated trace elements (experiment 2); **(C)** dry cell weight vs time for batch using continuous feeding vs batch using shot additions for 1.5X concentrated medium with 1.2X concentrated trace elements (experiment 2).


A
Seed Batch 1
Seed Batch 2
CER (mmol/L-h)
Time (h)
B
Maxflo T, shots
Rushton, feeding
OUR (mmol/L-h)
Time (h)
C
Dry Cell Weight (g/L)
Maxflo T, shots
Rushton, feeding
Time (h)

After an initial rise and fall, the broth pH remained at a value of 6.2 for only the next 15 h, after which it fell to 5.8–5.9 for the remainder of the cultivation. This suggests a very strong glucose metabolism for both of these cultivations (i.e., the tank with continuous feeding and the tank with shot additions).

The peak OUR for the batch using continuous feeding reached about 105 mmol/(L·h), while the peak OUR for the batch with shot additions reached 138 mmol/(L·h) (Fig. 3B). This difference was not believed to be owing to the impeller type, as confirmed in experiment 4. Whereas the OUR profiles were notably different between the tank with continuous feeding and the tank with shots, the dry cell weight profiles were similar. Cell growth increased in both tanks up to a higher dry cell weight of 70–72 g/L at 45 h, after which it remained steady for an additional 20–25 h (Fig. 3C). An accidental 150-L dextrose shot to the batch using continuous feeding may have resulted in the lower dry cell weight obtained for the sample taken at 54 h.

Comparison of 1.5X and 1.8X Medium Using Defined Medium with Continuous Dextrose Feeding: Experiment 3

The seed tank was transferred to each of two production tanks at an OUR of 36 mmol/(L·h). One production tank contained 1.5X concentrated 2XSYN9 defined medium and utilized a Maxflo T impeller. The second production tank contained 1.8X concentrated medium and utilized a Rushton impeller. Dextrose was fed continuously to both tanks and residuals were generally <2.5 g/L.

As shown in Fig. 4A, the peak OUR reached about 110 mmol/(L·h) for the 1.8X medium (Rushton impeller) and about 85 mmol/(L·h) for the 1.5X medium (Maxflo T impeller). The pattern of the RQ again was similar to that observed in experiment 1. The pH profile was similar to that observed in experiment 2. For the 1.8X medium cultivation, there was an extension of the initial rise in pH (and consequently a delay in its fall) of about 5 h, presumably owing to the increased amount of nitrogen-containing amino acids present initially in the medium. The broth pH remained at 6.2 for an additional 5 h longer for the cultivation using the 1.8X medium concentration compared with that using the 1.5X medium concentration. The fall in pH to 5.8–5.9 for the remainder of the cultivation was delayed about 15 h for the cultivation using the 1.8X medium concentration.

Dry cell weight data were scattered about 60–80 g/L with no clear advantage of 1.8X over 1.5X media concentration, nor impeller type, because of this high amount of scatter (Fig. 4B). Residual glucose was <2.5 g/L and similar amounts were fed to each cultivation. No broth needed to be sewered from the Rushton impeller tank, but two sewerings (50 and 100 L) were necessary from the Maxflo T impeller tank. This suggests that the axial hydrofoil impeller design might have produced more foam and a higher holdup for this process, as seen in previous experiments *(25)*. This behavior was confirmed in experiment 4.

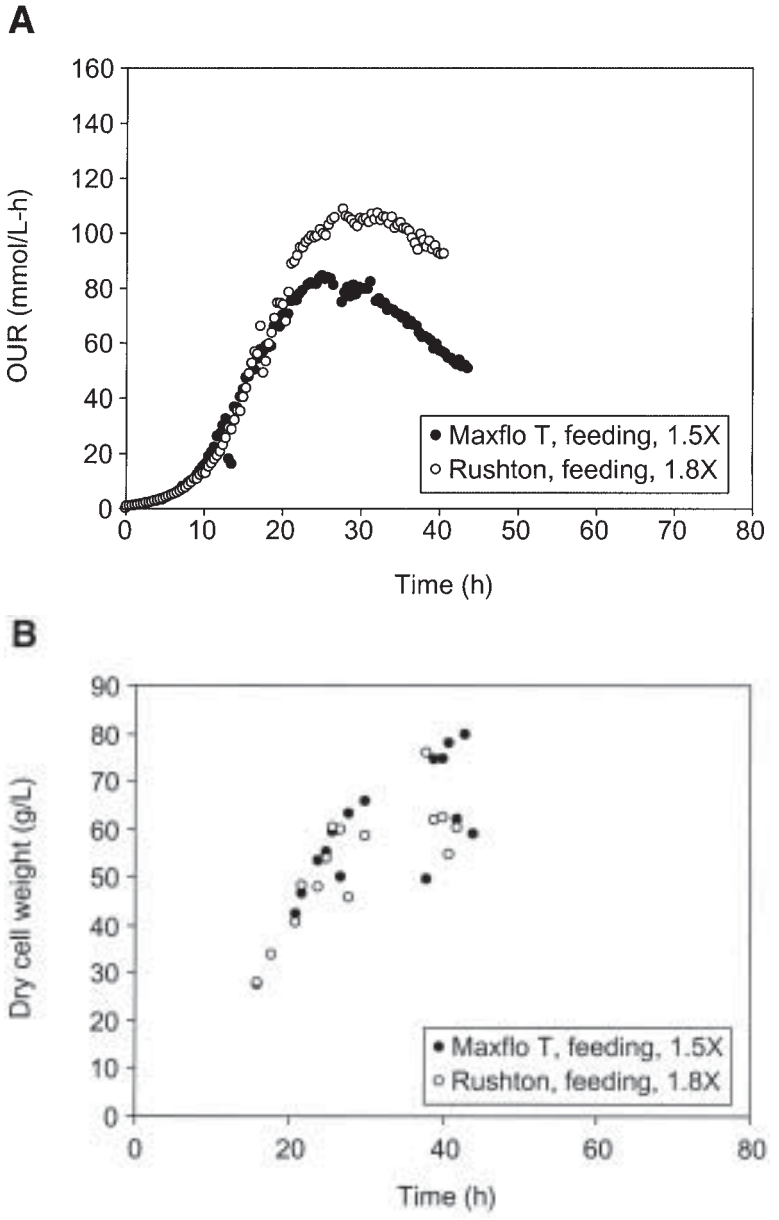


Fig. 4. **(A)** OUR vs time for 1.5X vs 1.8X medium concentration using continuous feeding (experiment 3); **(B)** dry cell weight vs time for 1.5X vs 1.8X medium concentration using continuous feeding (experiment 3).

Comparison of 1.8X Medium Using Defined Medium with Continuous Dextrose Feeding: Experiment 4

The seed tank was transferred to each of two production tanks at an OUR of 42 mmol/(L·h). The production tanks contained 1.8X concentrated 2XSYN9 defined medium and utilized either a Maxflo T or a Rushton

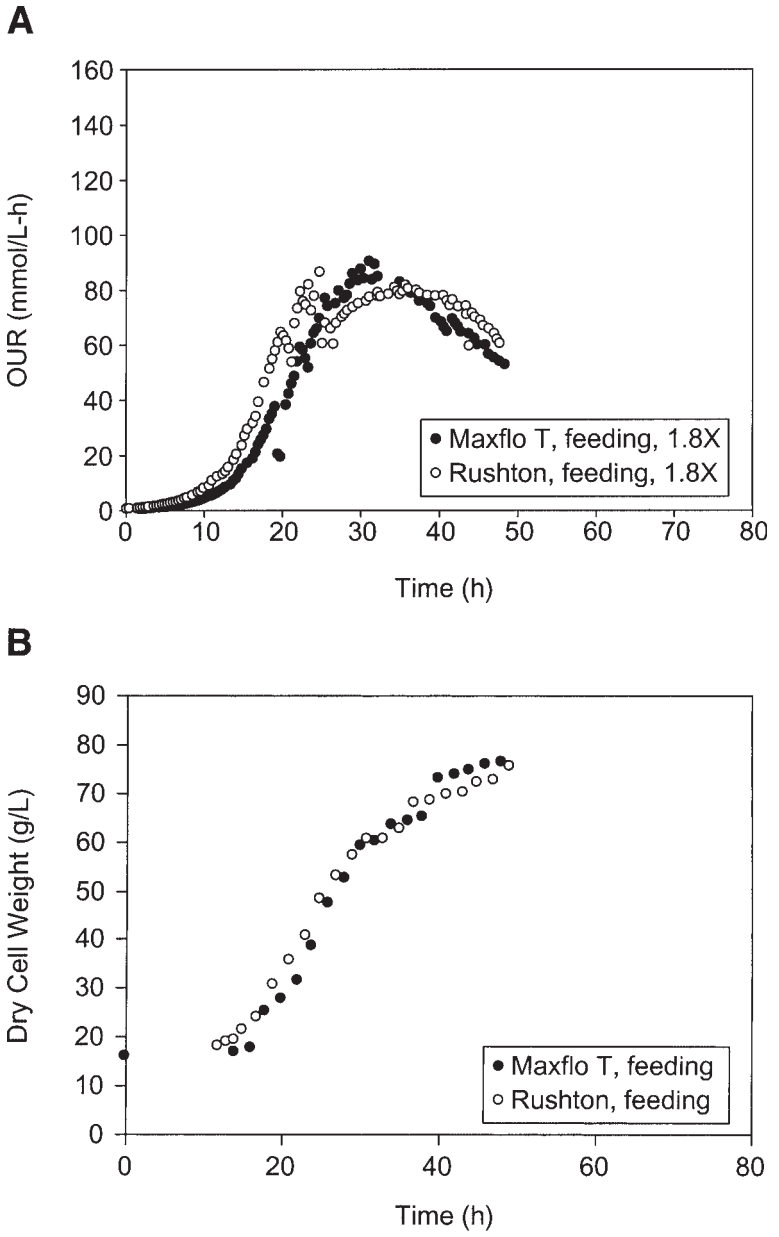


Fig. 5. **(A)** OUR vs time for 1.8X medium concentration for two production tanks using continuous feeding (experiment 4); **(B)** dry cell weight vs time for 1.8X medium concentration for two production tanks using continuous feeding (experiment 4).

impeller. Dextrose was fed continuously to both tanks and residuals were generally <2.5 g/L. The major purpose of this experiment was to compare directly the performance of the two types of impellers used.

The peak OURs reached about 90 mmol/(L·h) for both tanks with the Rushton impeller tank exhibiting slightly faster growth (Fig. 5A). The pat-

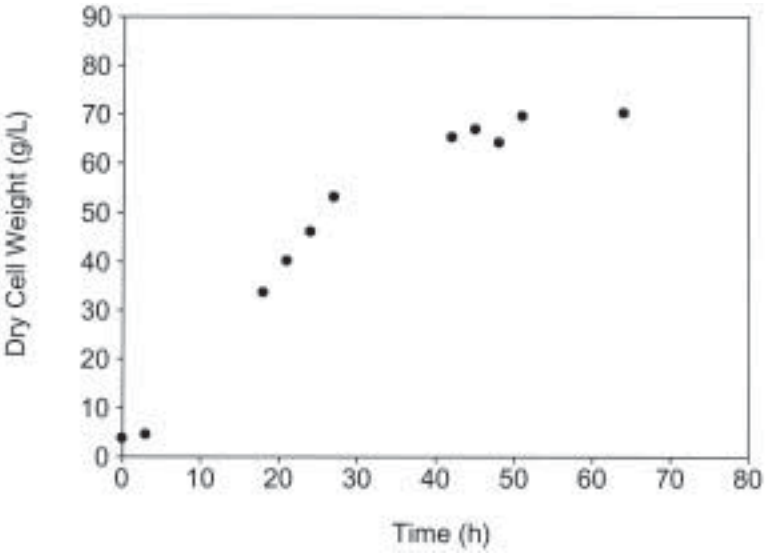


Fig. 6. Dry cell weight vs time for 23-L fermentation for 1.0X medium using continuous feeding.

tern of the RQ again was similar to that observed in experiment 1. The pH profile was similar to that observed in experiment 3 for the cultivation using the 1.8X medium concentration.

Dry cell weight data reached about 75 g/L for both of the 1.8X medium concentration cultivations with minimal scatter (Fig. 5B). There did not appear to be any clear-cut advantage or disadvantage with respect to cell concentration achieved between the Maxflo T and Rushton impellers. These values are comparable to the performance at the 23-L scale using 1.0X medium (Fig. 6). The higher medium concentration was likely necessary to compensate for higher levels of heat stress and possibly greater dilution occurring during sterilization and nutrient feeding at the larger pilot scale. Table 2 summarizes the 600- and 23-L experimental results.

Residual glucose was <2.5 g/L for the cultivation using the Maxflo T impeller. An accidental dextrose shot of 130 L at 26 h was given to the cultivation using the Rushton impeller. As found in experiment 3, no broth needed to be sewered from the Rushton impeller tank, but three sewerings (40, 20, and 40 L) were necessary from the Maxflo T impeller tank. During this experiment, it was specifically observed that the axial hydrofoil impeller design produced more foam.

The similar performance of both tanks with respect to cell density confirms the earlier results of experiment 2 in which dextrose shots were compared with dextrose feeding. These results suggest that upsets to the carbon source feeding to maintain a low residual glucose level <2.5 g/L may be tolerated by the cultivation. Characterization of the range of tolerability is expected to be an important future process development target.

Table 2
Comparison of OUR and Biomass for Various Experiments

Experiment	Impeller type	Medium concentration	Dextrose addition	Biomass (g/L dry cell wt)	OUR (mmol/[L·h])
600-L scale					
1	Rushton	1.2X	Continuous feeding	60–65	56
	Maxflo T	1.2X	Continuous feeding	60–65	72
2	Rushton	1.5X with 1.2X trace elements	Continuous feeding	70–72	105
	Maxflo T	1.5X with 1.2X trace elements	Shot addition	70–72	138
3	Rushton	1.5X	Continuous feeding	60–80	110
	Maxflo T	1.5X	Continuous feeding	60–80	85
4	Rushton	1.8X	Continuous feeding	75	90
	Maxflo T	1.8X	Continuous feeding	75	90
23-L scale	Rushton	1.0X	Continuous feeding	70–72	Not available

Conclusion

A defined medium and fed-batch feeding process for the production of a yeast biocatalyst, developed for the 23-L scale, was scaled up to the 600-L pilot scale. The initial trial resulted in a lower dry cell weight than expected using medium of the same composition as the laboratory scale. Medium of increased concentration then was implemented at the pilot scale. Equivalent dry cell weights were eventually reached with a medium 80% more concentrated than that used at the laboratory scale. The maximum cell density of 75 g/L (dry cell weight) was reached at 45–50 h for both the 23- and 600-L scales. After this time, further increases in cell mass happened slowly and it generally was not worthwhile to continue the cultivation. The higher medium concentration was believed to be necessary at the pilot scale because of the additional heat stresses on key components (e.g., complexing of magnesium sulfate during sterilization), increased dilution during sterilization, lower evaporation rate owing to lower vessel volume per minute airflow rate, and increased dilution owing to nutrient feeding or shots. These factors are examples of four qualitative differences on scale-up that may need to be quantified in future work. These results suggest that defined medium may need to be further optimized at the pilot scale for equivalent performance.

Presterilized 100-L vol plastic bags were implemented for the pilot-scale nutrient feeding and proved to be an attractive alternative to plastic carboys or hard-piped nutrient feed systems for the 0.5–5 L/h nutrient flow rate range desired. By varying the bag and pump size, it appears that these bags might be used for a wide range of nutrient flow rates. Optimal bag attachment and filling and feeding procedures were established for reliable operation. The process was found to be somewhat insensitive to variations in residual glucose levels. In the extreme case of residual glucose fluctuations, in which glucose shot additions were substituted for glucose continuous feeding, equivalent cell densities were obtained.

There was no clear-cut advantage or disadvantage of the Rushton (radial turbine) vs Maxflo T (axial hydrofoil) impeller designs for these high-cell-density yeast fermentations in terms of cell mass production. There may be a slight difference in OUR, but specific experiments aimed at characterizing mass transfer would be needed to investigate this aspect further. Qualitatively, however, the axial hydrofoil impeller design might have produced more foam and a higher holdup for this process.

This work demonstrates the ability to scale up defined medium to the pilot scale and to optimize both the medium and the feeding process to achieve similar cell mass as that obtained on the laboratory scale. The degradation and/or dilution, believed to have occurred with this defined medium on scale-up, needs to be explored further so that future experiments can target specific heat-sensitive medium ingredients or specific sources of nutrient dilution. During this optimization, a qualitative analysis of the sensitivity of the process to variations in residual glucose level

was obtained. The ability to produce yeast biocatalysts at high cell density can directly translate into improved volumetric productivity in subsequent bioreduction reactions.

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