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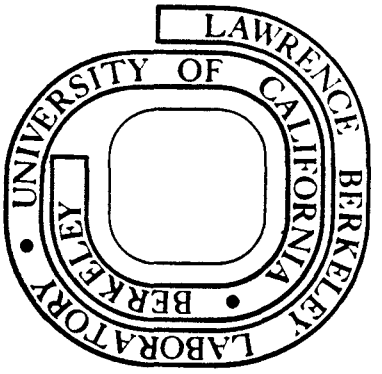

DISCLAIMER

RAPID ETHANOL FERMENTATIONS USING VACUUM AND CELL RECYCLE

by

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ABSTRACT

A cell recycle and vacuum fermentation system were developed for continuous ethanol production. Cell recycle was employed in both atmospheric pressure and vacuum fermentations to achieve high cell densities and rapid ethanol fermentation rates. Studies were conducted with Saccharomyces cerevisiae (ATCC #4126) at a fermentation temperature of 35°C. Employing a 10% glucose feed, a cell density of 50 g dry wt./ℓ was obtained in atmospheric-cell recycle fermentations which produced a fermentor ethanol productivity of 29.0 g/ℓ-hr. The vacuum fermentor eliminated ethanol inhibition by boiling away ethanol from the fermenting beer as it was formed. This permitted rapid and complete fermentation of concentrated sugar solutions. At a total pressure of 50 mmHg and using a 33.4% glucose feed, ethanol productivities of 82 g/ℓ-hr and 40 g/ℓ-hr were achieved with the vacuum system with and without cell recycle respectively. Fermentor ethanol productivities were thus increased as much as 12 fold over conventional continuous fermentations. In order to maintain a viable yeast culture in the vacuum fermentor a bleed of fermented broth had to be continuously withdrawn to remove non-volatile compounds. It was also necessary to sparge the vacuum fermentor with pure oxygen to satisfy the trace oxygen requirement of the fermenting yeast.

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Introduction. A major constraint of conventional alcohol fermentation processes is ethanol or end product inhibition. When a concentrated sugar solution is fermented and the ethanol concentration of the fermentation broth increases above 7% to 10% the specific ethanol production rate and the specific growth rate of the yeast is severely suppressed (1)(2). Ethanol inhibition produces many economic implications when considering industrial ethanol fermentations. In order to maintain the ethanol concentration at the optimal level for ethanol production concentrated sugar solutions, such as molasses, must be diluted to 10% to 20% sugar. The additional water used to dilute the substrate must then be carried through the fermentation process, increasing the size and cost of pumps, mixing and storage tanks, heat exchangers and distillation columns. Also, because the cell mass concentration is a direct function of substrate concentration, lower cell densities will be experienced with diluted substrates. This results in lower fermentation rates per unit volume and hence, dictates that larger fermentors must be employed in the fermentation process. However, by use of a cell recycle arrangement the cell mass and the fermentor productivity may be increased. A portion of the cells in the effluent broth from the fermentor is separated from the beer by either sedimentation or centrifugation and recycled back to the fermentor. By this means the cell mass concentration in the fermentor is increased which produces higher fermentation rates per unit volume.

To circumvent the problem of ethanol inhibition, ethanol must be removed from the fermenting beer as it is formed. One means of accomplishing this task is to take advantage of ethanol's high volatility and to boil off the ethanol as it is formed. Vacuum operation is, of course, necessary to achieve boiling of the fermentation broth at temperatures compatible with the yeast. Besides eliminating ethanol inhibition, vacuum operation produces an

increase in fermentor productivity because the yeast cell mass concentration may be maintained at a high level. Only ethanol and water are boiled away from the fermentor while most of the yeast remains in the fermentor. The one disadvantage of vacuum fermentation is that carbon dioxide produced during fermentation must be compressed up to atmospheric pressure.

This work was then undertaken to develop a cell recycle system and a vacuum fermentor for the continuous production of ethanol. The main emphasis of this study was to assess the advantages of cell recycle and vacuum ethanol fermentations and demonstrate the high ethanol productivities obtainable with each mode of operation.

EXPERIMENTAL PROCEDURES

The organism used in the fermentation studies was Saccharomyces cerevisiae, ATCC #4126. The standard media employed in all vacuum fermentations is listed in Table 1. When the glucose concentration of the media was decreased to 100 g/ℓ for the growth of inocula or atmospheric pressure operation, all other components were decreased by the same ratio. The media was sterilized by dissolving the glucose in an amount of water equivalent to 67% of the desired medium volume and by dissolving the salts and yeast extract in the remaining 33% of the water. After steam sterilization at 121°C for 30 minutes in separate containers the solutions were allowed to cool to ambient temperature and mixed. Separate sterilization of the glucose and minerals was necessary to avoid carmelization of the glucose which, while not affecting ethanol or cell mass production, did interfere with the optical determination of cell mass concentrations.

Table 1

Base Medium for Vacuum Fermentation

Component*	Per Liter
Glucose (anhydrous)	334 g
Yeast Extract (Difco)	28.4 g
NH_4Cl	4.4 g
$MgSO_4 \cdot 7H_2O$	0.4 g
$CaCl_2$	0.2 g
Anti-Foam (General Electric AF60)	0.6 ml
Tap Water to	1 liter

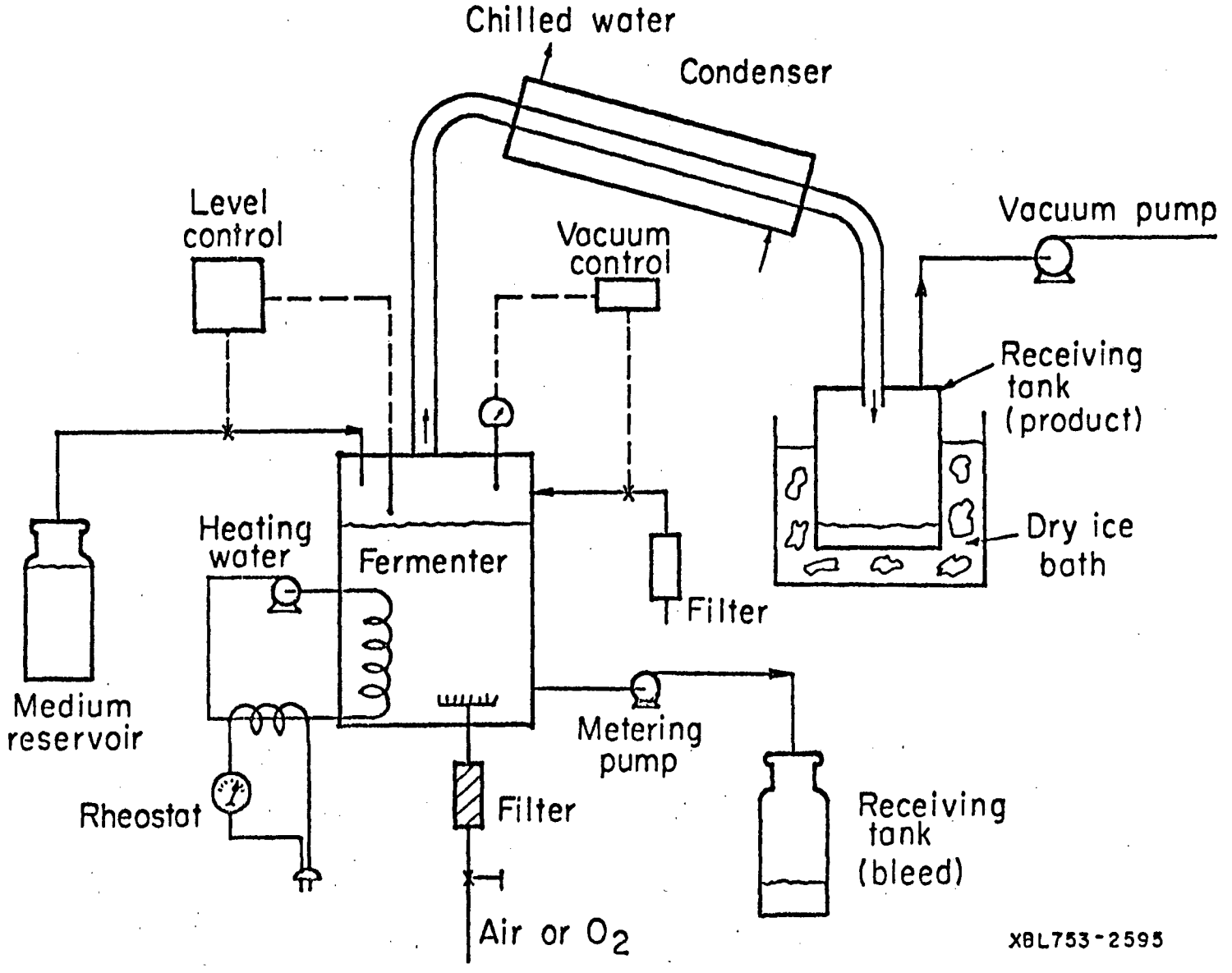
*All salts and glucose reagent grade.

A five liter "Micro Ferm" fermentor (Fermentation Design Model MA501) was used in the atmospheric pressure cell recycle experiments. Details of the operation and arrangement of the continuous fermentor have been previously given (3). The effluent from the fermentor was passed to a jacketed settler, as described below, and a cell concentrate stream was returned to the fermentor. Tubing pumps (Sigmamotor Model TM-2.0-2) were used to control the flow of fermented beer from the fermentor to the settler and the cell recycle stream.

The heart of the vacuum fermentor was also the five liter "Micro Ferm" fermentor. A schematic diagram of the complete vacuum system is shown in Figure 1. In order to achieve the required boil-up rate of ethanol and water a 1500 watt heater was added to the temperature control loop of the fermentor. The heater was constructed of four 10-in. diameter coils of ½-in. copper tubing wrapped with electrical heating tape. The heat input was controlled by adjusting either of two variable autotransformers (Superior Electric Company Type 3PN1168). A one inch stainless steel pipe connected to the fermentor inoculation port led to two shell and tube condensers (American Standard No. 47M200-8A2) arranged in series. The vapor generated in the fermentor was condensed on the shell side of the exchangers by a 10% methanol-water solution chilled to -4.0% by a Haws Model HR4-24W water cooler. The condensate was then collected for analysis in a 40 liter stainless steel tank which was set in a dry ice bath.

The vacuum system was connected to a Kinney Model K2-8 vacuum pump. The vacuum pump ran continuously and the pressure was controlled by a "Manowatch" Model MW-1 controller (Instruments for Research and Industry, Inc.) which activated a solenoid valve allowing filtered air to be bled into the system when the pressure became too low. Although the fermentor pressure fluctuated 1-2 mmHg with this method of pressure control, it was found super-

Fig. 1. Vacuum Fermentor



ior to placing the solenoid valve in line with the vacuum pump, as recommended by the manufacturer, because the small pressure fluctuations helped to control foaming in the fermentor and allowed better liquid level control. The absolute pressure in the fermentor was measured with a Zimmerli gauge.

As the liquid level in the vacuum fermentor dropped due to boil-off of vapor and the bleed-off of the fermented broth, a liquid level controller (Cole Palmer Model 7186) opened a solenoid valve connected to the medium reservoir, and sterile broth was sucked into the fermentor to maintain a 2ℓ fermentor working volume. The feed rate of fresh medium was thus determined by the boil-up rate and the bleed rate of fermented broth. A liquid level probe for the Cole Palmer controller was constructed of a ¼-inch stainless steel rod which was forced down ½-inch Teflon tubing so that both ends were exposed for electrical contacts. The Teflon coating was necessary because its high hydrophobic surface properties did not allow a condensate film to form on the probe. A liquid film (water) short circuits the probe with the fermentor head plate and causes the controller to sense a high liquid level. However, during long term experiments the anti-foam and protein constituents of the medium adsorbed onto the Teflon changing the surface properties and producing a short circuit. This was corrected by wrapping the length of probe above the head plate with heating tape to boil off any surface water on the probe below the head plate.

A bleed of fermented broth and cells was withdrawn from the vacuum fermentor by a tubing pump [Sigamamotors Model (TM-20-2)] into a 4 liter jar which was maintained at the same pressure as the fermentor. The cell bleed rate was adjusted by changing the speed of the pump, and measured by emptying the 4 liter jar at timed intervals and measuring the volume.

Cell recycle experiments were run with both atmospheric pressure and vacuum fermentations using a jacketed settler vessel. A diagram of the settler arrangement is shown in Figure 2. The pressure in the settler and receiver flask was equalized enabling the clarified liquid to overflow by gravity to the receiver flask. The clarified liquid overflow rate was controlled by adjusting the difference between the pumping rate of the feed to the settler from the fermentor and pumping rate of the cell concentrate recycle stream.

A solution of methanol and water chilled to 4.0°C was circulated through the jacket to slow fermentation in the settler. The settler system was operated at a total pressure of 250 mmHg in the vacuum system and at atmospheric pressure in the atmospheric fermentation system. Both cooling the settler and operating at a pressure higher than the vacuum fermentation pressure of 50 mmHg was necessary to minimize mixing effects of CO_2 evolved during fermentation in the settler.

The vacuum fermentor was sterilized in place by filling the fermentor with 300 ml of a 70 vol % ethanol-water solution and boiling the solution under 250 mmHg total pressure (house vacuum) for eight hours. The system was then flushed with air (3 liter/min) for 4 hours to remove the last traces of the sterilizing solution. The fermentor was filled with three liters of 10% glucose medium, brought to 35°C and inoculated. An air rate of 0.5 liters/min was maintained during batch growth. At the end of batch growth (12 to 16 hours) the air flow was stopped and 0.12 vvm (240 mls/min at STP) of oxygen was sparged through the fermentor. The pressure in the fermentor was slowly decreased, 25 mmHg/min, until the fermentation broth began boiling at 35°C. As the ethanol in the fermentation broth boiled off, the pressure was further lowered to 50 mmHg to maintain boiling.

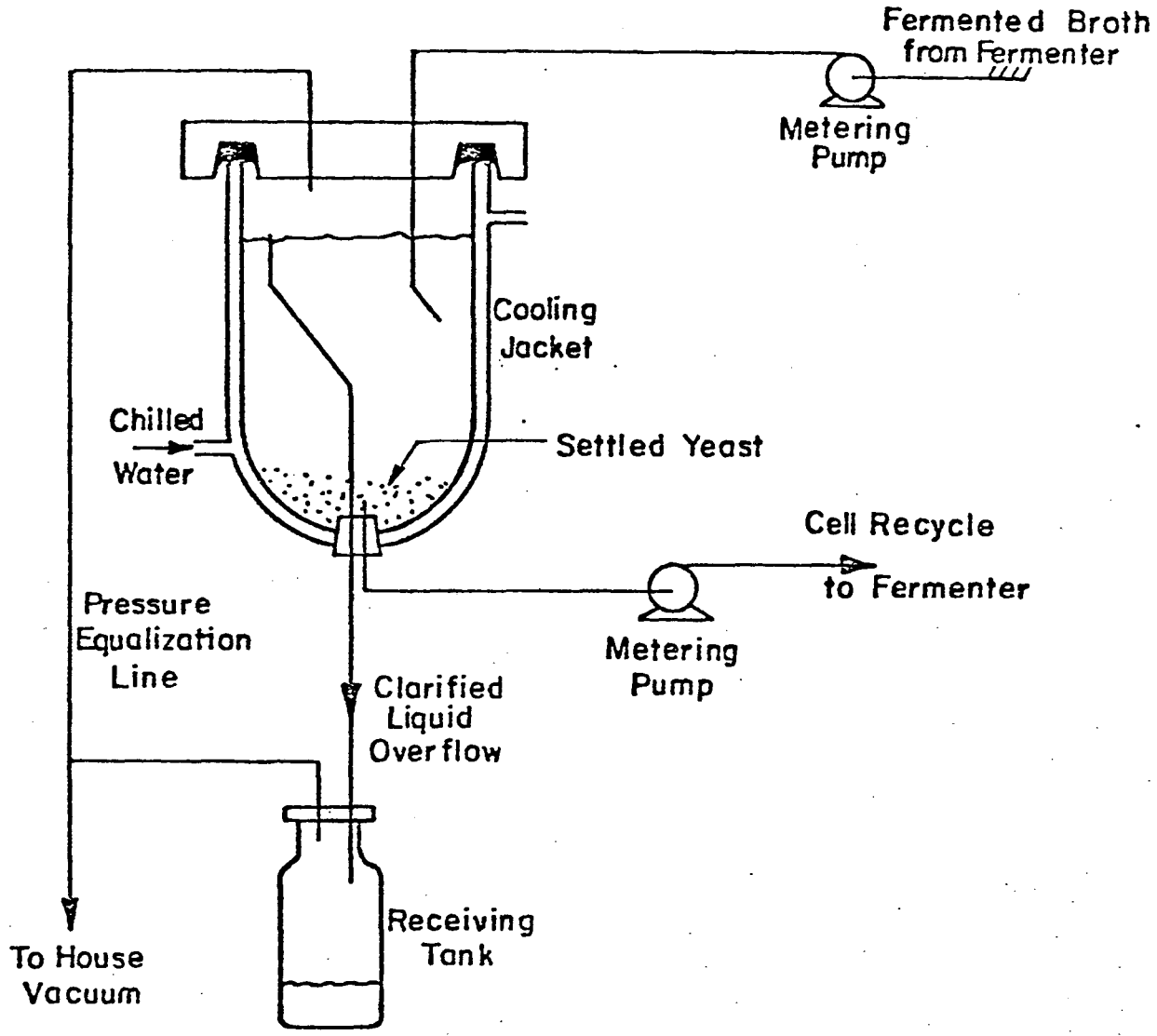


Fig. 2.

Settler Used for Cell Recycle Experiments

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In all vacuum experiments the total pressure was 50 mmHg. At this pressure the boiling point of the fermentation broth containing 1% ethanol was 35°C, the optimum fermentation temperature of the yeast (3). The pH of the fermentation broth during vacuum operation was maintained between 4.0 and 3.5 by the buffering capacity of the medium. Unless otherwise stated, pure oxygen was sparged into the vacuum fermentor at a rate of 0.12 vvm at S.T.P. and an agitation rate of 500 RPM was used to supply adequate oxygen to the yeast.

Assay Procedures

Ethanol Concentrations. Ethanol was measured by gas chromatography using an Aerograph 1520 G-L Chromatograph. A 6 foot ¼-inch column packed with Chromosorb-W acid wash type 60-80 mesh was used with a flame ionization detector. The injector and detector temperatures were 175°C and the column oven operated isothermally at 105°C.

Cell Mass. The cell mass concentrations were measured optically using a Fisher Electrophotometer with a 650 mμ filter.

Glucose Concentration. Glucose was determined by the dinitrosalicylic acid (DNS) method (4).

Yeast Viability. The percentage of viable yeast cells was determined using a methylene blue stain as described by Townsend (5).

RESULTS AND DISCUSSION

Semi-Continuous Vacuum Operation. Figure 3 illustrates the performance of the vacuum system during semi-continuous operation. Fresh medium was continually fed to the fermentor to maintain a constant volume as ethanol and water were boiled away. A bleed stream of fermented broth was not removed from the

fermentor. This allowed the rapid accumulation of cell mass within the fermentor. However, components in the medium which were not metabolized by the yeast also accumulated in the fermentor under this mode of operation.

The step like appearance of the ethanol productivity curve in Figure 3 reflects that the productivity (boil-up rate times the ethanol concentration in the condensed product) was increased by manually increasing the boil-up rate and hence the feed rate to the fermentor. The boil-up rate, was always adjusted so that the yeast was able to ferment almost all the glucose in the feed. By this means the glucose concentration in the fermentor was held between 2 to 5 g/l.

The results shown in Figure 3 were obtained using a 33.4% glucose feed. No ethanol inhibition was detected and the cell concentration and ethanol productivity steadily increased with time for 48 hours. A maximum ethanol productivity and cell mass of 44 g/l-hr and 68 g/l, respectively, were obtained. However, after 48 hours of fermentation the yeast cell mass concentration began to decline and the feed rate, or boil-up rate, had to be sharply reduced to obtain complete fermentation of the glucose, and, as shown, the ethanol productivity correspondingly decreased.

The sharp decrease in cell mass after 2 days of semi-continuous operation indicated that non-volatile components were accumulating in the fermentor and killing the yeast. Only 60% of the yeast were found viable by the methylene blue stain method after 55 hours of operation. This required a bleed stream of fermented broth be continually withdrawn from the fermentor to keep the concentration of non-volatiles at a level which did not inhibit yeast growth or ethanol production.

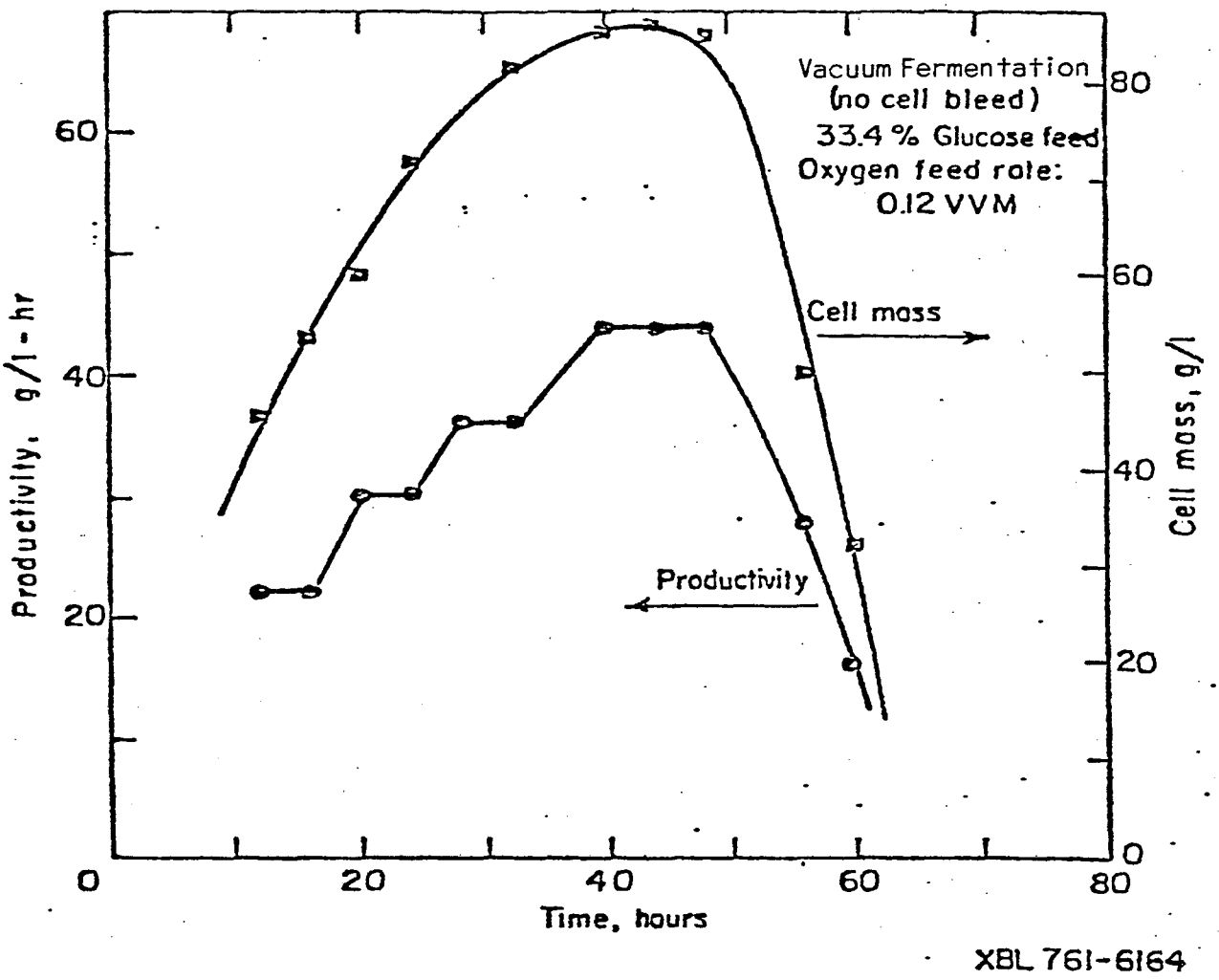

Fig. 3. Vacuum fermentation without cell bleed of a 33.4% glucose feed.

Continuous Vacuum Operation. Figure 4 illustrates the effect of removing a bleed of fermented broth. The data in Figure 4 were taken at steady state operation of the vacuum fermentor using a 33.4% glucose feed. The cell yield factor, $Y_{X/S}$, and cell concentration are plotted against a concentration factor. The concentration factor, c, is defined as

$$c = \frac{F}{B} (S_o)/100$$

where,

F = volumetric feed rate, l/hr

B = volumetric bleed rate, l/hr

S_o = initial glucose concentration, g/l

A decrease in bleed rate, holding the feed rate constant, increases the concentration factor and also increases the concentration of non-volatiles in the fermentor. The concentration factor in Figure 4 was increased by lowering the bleed rate. Thus, as the concentration factor increased the cell mass concentration rose because fewer cells were removed in the bleed stream. But when the concentration factor reached 8.5 the cell concentration and cell yield factor dropped. At this concentration factor the bleed stream was not sufficient and the concentration of non-volatiles reached a critical level which began to inhibit yeast growth. Further increases in the concentration factor had a deleterious affect on yeast growth. The results of Figure 4 show that to sustain stable operation of the continuous vacuum fermentation a bleed of fermented broth had to be removed so that the concentration factor did not rise above 8.5.

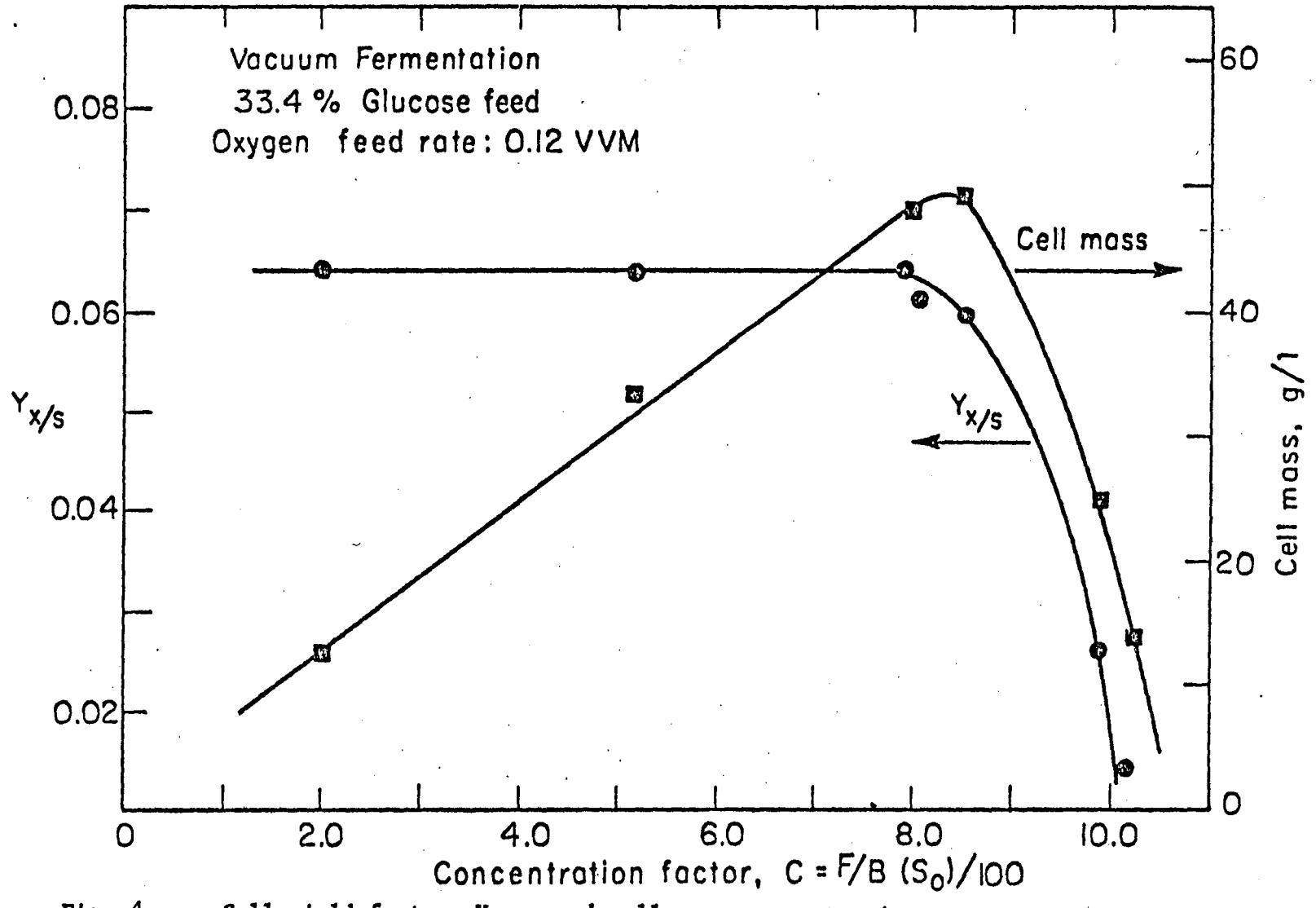

Fig. 4. Cell yield factor, $Y_{x/s}$, and cell mass concentration as a function of concentration factor in vacuum fermentation.

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The cell yield factor remained constant at 0.064 during this vacuum fermentation at concentration factors lower than 8.0. However, cell yield factors typically ranged from 0.055 to 0.066 for continuous vacuum fermentations operated at concentration factors below 8.0. The reason for the variation in cell yield factors between consecutive vacuum experiments is not apparent at this point. But the cell yield factors obtained during vacuum operation were always about 50% lower than the yield factors of 0.1 to 0.12 experienced during atmospheric pressure fermentations (2)(3). The lower yield factors may be a direct result of increased maintenance energy requirements for yeast growth under vacuum. A lower cell yield factor was the only discernible difference between vacuum and atmospheric pressure fermentations.

The results of a long term continuous vacuum fermentation are shown in Figure 5 for a 33.4% glucose feed. A constant bleed of fermented broth was withdrawn to maintain a concentration factor of 7.7. The cell mass concentration remained stable at 50 g dry wt/l for over 13 days of continuous operation, at which point the experiment was terminated. With this concentration of yeast the 33.4% glucose feed was fermented to less than 0.4% residual sugar in a mean fermentor residence time of 3.8 hours. This corresponded to an ethanol productivity of 40 g/l-hr. With conventional continuous fermentation at atmospheric pressure using optimal conditions (pH = 4.0 T = 35°C, 10% glucose feed) the maximum ethanol productivity obtained with this yeast and similar fermentation media was 7.0 g/ℓ-hr (3). Thus, the vacuum system produced almost a 6-fold increase in ethanol productivity compared to conventional continuous operation.

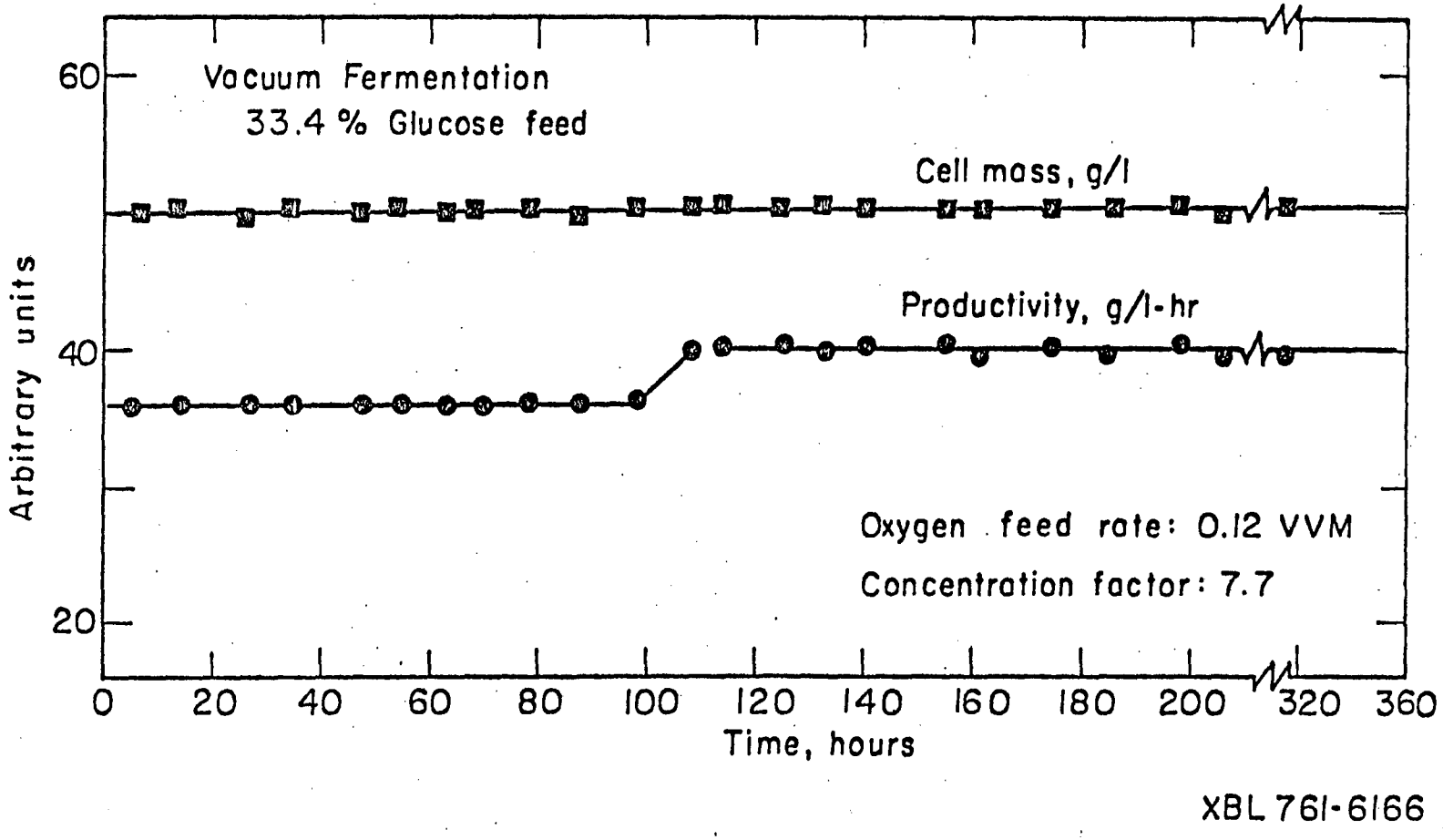

Fig. 5. Long term continuous vacuum fermentation.

The specific ethanol productivity in the vacuum fermentor was 0.8 hr^{-1}. This is 38% higher than obtained for conventional continuous fermentations at optimal conditions (3). The increase in specific productivity experienced in the vacuum system seems to be a direct result of lowering ethanol inhibition. The ethanol concentration in the fermentor was always below 10 g/l, however, during atmospheric continuous operation the enthanol concentration was 46 g/l for the optimal feed sugar concentration of 10%. If the effluent ethanol concentration was reduced from 46 g/l to 10 g/l in the atmospheric fermentations, the specific productivity was increased from 0.58 hr^{-1} to 0.8 hr^{-1} (3). This is in direct support of the finding in the vacuum system. It should be remembered however, that the primary evidence of eliminating ethanol inhibition is the ability to completely ferment a 33.4% glucose feed in the vacuum fermentor. This was not possible in atmospheric fermentations because of ethanol inhibition.

The increase in ethanol productivity shown in Figure 5, after 100 hours of fermentation, was achieved by simultaneously increasing the fermentor bleed and feed rate, thus keeping the concentration factor at 7.7. The productivity could not be increased above 40 g/l-hr and still maintain stable operation. To further increase the productivity an increase in feed rate was necessary. But from the above discussion, a corresponding increase in bleed rate had to be made to keep the concentration of non-volatile components at a level compatible with the yeast. Simultaneously increasing the bleed rate and feed rate to maintain a constant concentration factor dictates a constant cell mass concentration within the fermentor. This fact may be predicted from a simple mass balance and is borne out by experimental results. Since the fermentor ethanol productivity is the product of the specific cell ethanol productivity and the cell mass concentration, the fermentor productivity is

limited by the cell mass concentration obtainable at any given concentration factor. Thus, for a concentration factor of 7.7, the maximum fermentor ethanol productivity is 40 g/ℓ-hr corresponding to a cell mass concentration of 50 g dry wt/ℓ and a specific ethanol productivity of 0.8 hr^{-1} ($Y_{x/s}$ in this experiment was 0.055).

Cell Recycle in Vacuum Fermentation. In order to remove inhibitory substances and increase the cell concentration, a settler was used in conjunction with the vacuum system. The bleed stream from the fermentor was passed through the settler and the settled cells returned to the fermentor. In this manner, a high concentration of cells was maintained in the fermentor at high bleed rates.

The settler was not 100% efficient and some cells were lost in the overflow of clarified product. At steady state the amount of cells lost in the overflow was equal to the amount of cells produced during fermentation. The cell concentration was adjusted by changing the pumping rate of the recycle stream.

The results of the settler-vacuum system are shown in Figure 6. A final cell mass of 124 g dry wt/ℓ was achieved resulting in an ethanol productivity of 82 g/l-hr. This is almost a 12-fold increase in productivity over that obtained in conventional continuous operation (3). The specific productivity of the yeast decreased from 0.8 hr^{-1} to 0.66 hr^{-1} when cell recycle was used in the vacuum system. This, no doubt, reflects that some of the yeast died during the extensive recycling. The mean residence time of the yeast in the fermentor was 10 times that in conventional continuous operation.

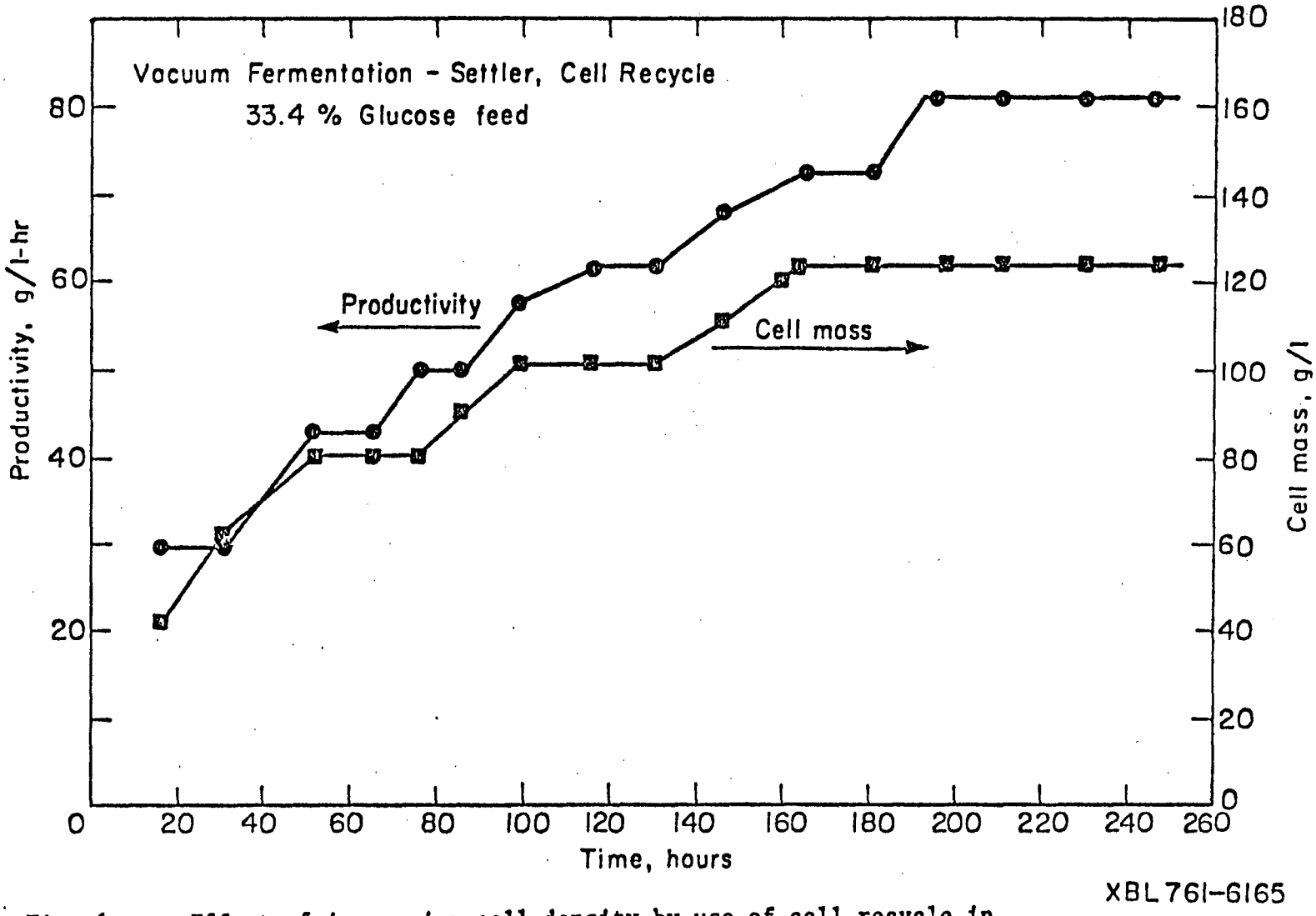

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Fig. 6. Effect of increasing cell density by use of cell recycle in vacuum fermentation.

The maximum ethanol productivity of 82 g/ℓ-hr of the vacuum-recycle system was limited by the capacity of the settler. When an attempt was made to increase the productivity by increasing the fermentor through put the flow velocity in the settler became higher than the settling velocity of the yeast. As a result, more cells were lost in the overflow stream than produced during fermentation and the cell mass concentration in the fermentor and the ethanol productivity rapidly declined.

The extremely high ethanol productivity obtained with the vacuum-cell recycle system is a direct result of the high cell mass concentration achieved with the recycle system. Whereas 11.0 g dry wt./ℓ of yeast cell mass is typically obtained in conventional atmospheric continuous culture, over 120 g dry wt./ℓ of yeast cell mass is obtained in the vacuum-cell recycle system.

The reason such high cell densities were achieved in the vacuum-recycle system was ability of the vacuum system to ferment a concentrated sugar solution. This permitted low flow velocities within the settler because a relatively low feed rate and hence a low bleed rate from the settler was required to achieve high productivities. A clarified liquid bleed rate corresponding to a fermentor dilution rate of only 0.23 hr^{-1} was required to achieve a productivity of 82 g/ℓ-hr when the 33.4% glucose feed was fermented to a concentration of 0.4%. This low flow rate allowed the settler to operate efficiently and produce a concentrated cell recycle stream. As mentioned previously, above this flow rate the settler became inefficient during vacuum operation.

A similar advantage would be experienced in an industrial vacuum-cell recycle system employing a centrifuge rather than a settler. By fermenting a concentrated sugar solution, the through put of the centrifuge would be reduced in a vacuum system which would lower both operating and capitol costs of the centrifuge.

The high productivities obtained in the vacuum system agrees with the recent work of Finn (6) on vacuum fermentations. However, Finn used an ergosterol supplemented growth medium to eliminate the oxygen requirement of the yeast and did not employ cell recycle. The ethanol productivity reported by Finn was 12.5 g/l-hr. This is much lower than the productivities reported here of 82 g/l-hr and 40 g/l-hr for the vacuum system with and without cell recycle, respectively. The lower productivity reported by Finn may be a result of not pushing the vacuum system to its limit. The main emphasis of his work was to demonstrate that a 50% sugar feed could be fermented in a vacuum fermentor.

Effect of Oxygen on Vacuum Fermentation. As noted by numerous workers, trace amounts of oxygen stimulate alcoholic fermentation rates (3)(7)(8). However, there is an optimum oxygen tension above which fermentation rates are suppressed (3)(7). The optimum oxygen tension for the strain of Saccharomyces used in this work was found to be 0.07 mmHg for atmospheric pressure operation after the yeast had been "adapted" to high oxygen tensions (3).

It was not possible to measure the oxygen tension of the medium in the vacuum system, although this would have been very desirable. When an oxygen probe was put in the vacuum fermentor a stable reading could not be obtained because of the intense boiling taking place.

The optimal oxygen sparging rate was, however, determined for the vacuum system. The results are shown in Figure 7 for the fermentation of a 33.4% glucose feed. The data were obtained at a concentration factor of 7.7. The ethanol productivity is plotted against the oxygen feed rate to the fermentor for various agitation rpm's. The highest oxygen feed rate used was 0.37 vvm at S.T.P. This corresponded to 5.6 vvm in the fermentor because of

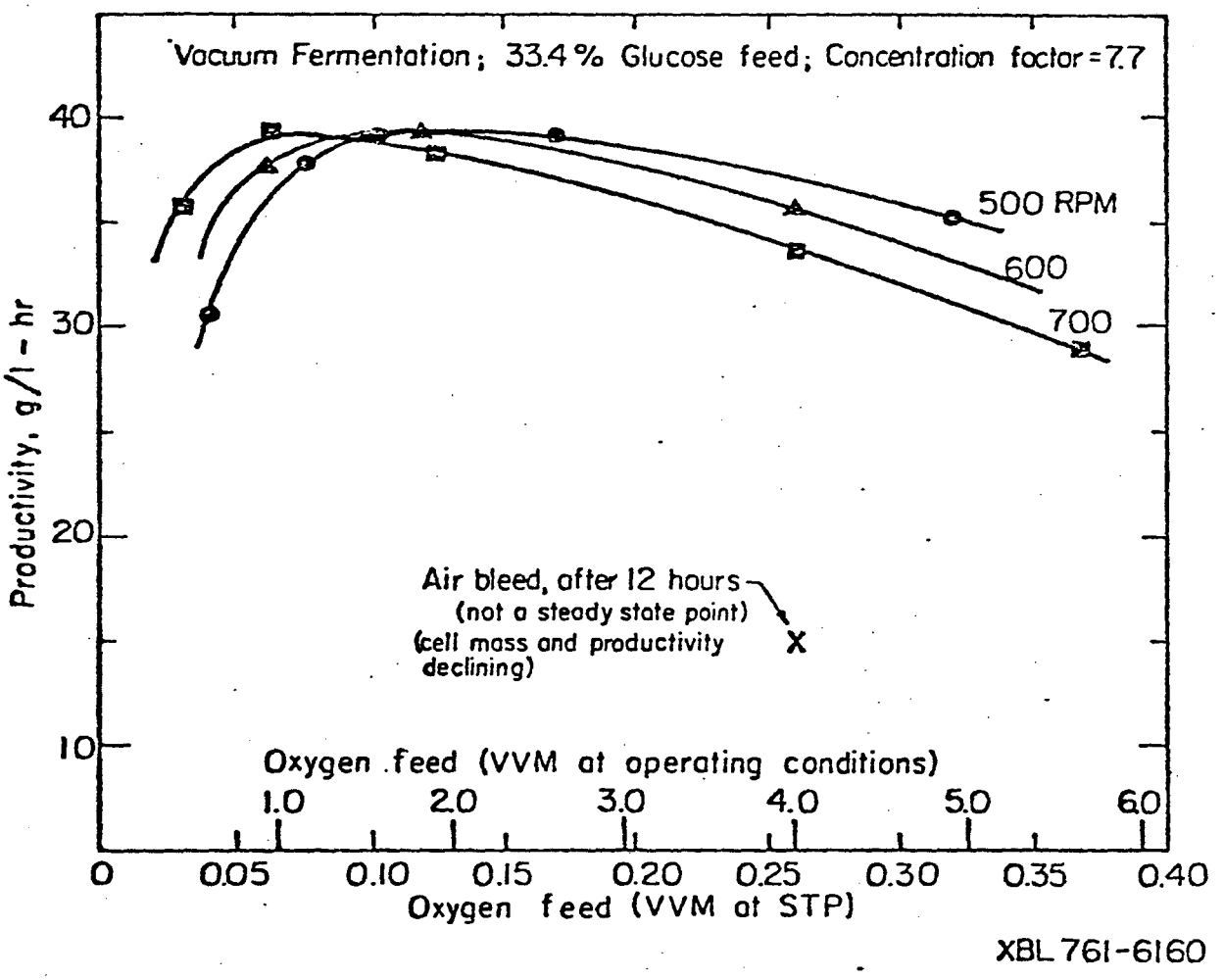

Fig. 7. Ethanol productivity as a function of oxygen feed rate at various agitation rates.

gas expansion under vacuum. Above this oxygen feed rate foaming was extensive and interferred with the liquid level control system.

The optimum oxygen feed rate for ethanol production was between 0.08 to 0.14 vvm at S.T.P. At high agitation rates the ethanol productivity declined more rapidly as the oxygen sparging rate was increased. Both increasing the agitation and oxygen feed rate increased the mass transfer rate of oxygen into the medium. This undoubtedly increased the oxygen tension in the fermentor. The productivity curves in Figure 7 may then be viewed as analogous to the ethanol productivities obtained for atmospheric operation presented in reference (3). Trace amounts of oxygen stimulated ethanol production but if the oxygen concentration became too high the ethanol productivity decreased.

The result of using an air feed rather than oxygen in the vacuum system is also shown in Figure 7. When air was sparged into the fermentor at a rate of 0.26 vvm at S.T.P., or 4.0 vvm at operating conditions, the ethanol productivity substantially decreased after only 12 hours of operation. The datum at 12 hours shown in Figure 7 does not represent a steady state point. The productivity and cell mass concentration were declining. An oxygen feed was resumed because conditions of fermentor "washout" were feared. This points out the necessity of using pure oxygen instead of air to maintain a high enough oxygen transfer rate under vacuum to support yeast growth.

Figure 8 illustrates the effect of oxygen on yeast viability in the vacuum system. If oxygen was not sparged into the fermentor the viability of the yeast continually dropped. Whereas, an oxygen feed rate of 0.12 vvm at S.T.P. maintained yeast viability above 95%.

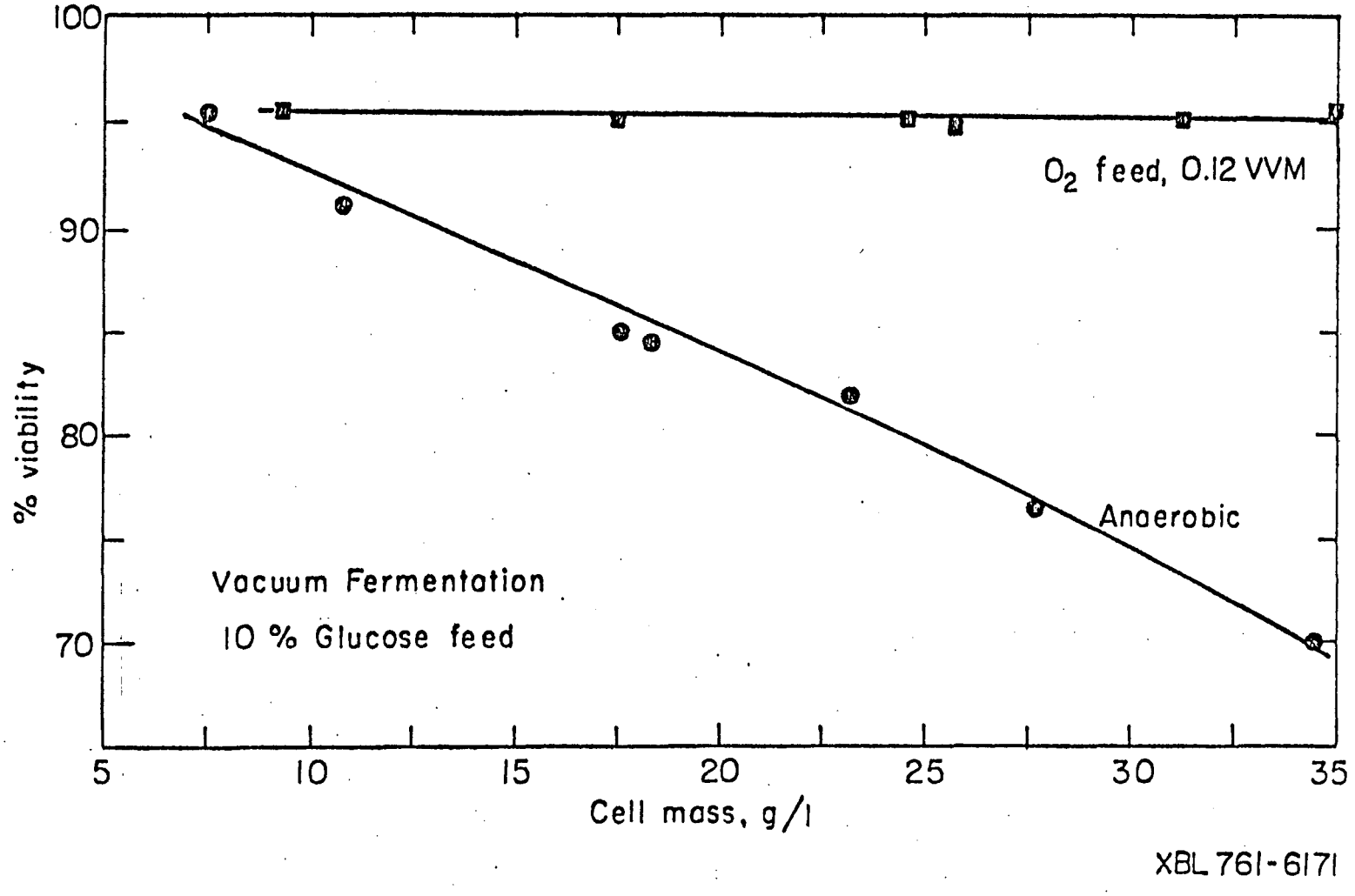

Fig. 8. Effect of an oxygen feed on yeast viability during vacuum fermentation.

Cell Recycle in Atmospheric Pressure Fermentations. The results of a continuous fermentation employing cell recycle are shown in Figure 9. A 10% glucose feed was used in these experiments to avoid ethanol inhibition (1)(3). Ethanol remained in the fermentation broth under atmospheric operation and was not boiled away as in the vacuum system. The oxygen tension in the fermentor was maintained at 0.12 mmHg. The cell concentration was adjusted by changing the pumping rate of the cell recycle stream and the system allowed to reach steady state before samples were withdrawn for analyses.

The data presented in Figure 9 definitely show an increase in ethanol productivity was realized by increasing the cell mass concentration in the fermentor with a recycle system. The maximum specific productivity of the yeast in the recycle system was identical to the specific productivity obtained with conventional continuous operation, 0.58 hr^{-1}, at conditions of complete substrate utilization (3). However, a cell mass concentration of 50 g dry wt./l or 4 times higher than without cell recycle was achieved. The net effect was a fourfold increase in fermentor ethanol productivity in the recycle system over conventional continuous operation.

The yeast did not degenerate or lose viability in the recycle system. This is evident by the same specific productivities obtained with or without cell recycle. Also, yeast viability, as determined by methylene blue stain, remained over 96% for the duration of the 14 day experiment.

The steep decrease in cell mass and ethanol productivity above a dilution rate of 0.75 hr^{-1} was due, once again, to exceeding the capacity of the settler and not because of a loss of yeast viability. When the dilution rate was increased above 0.75 hr^{-1} the flow velocity in the settler became higher than the settling velocity of the yeast. As a result, more cells were lost in the overflow stream than generated during fermentation and conditions of "washout" were experienced.

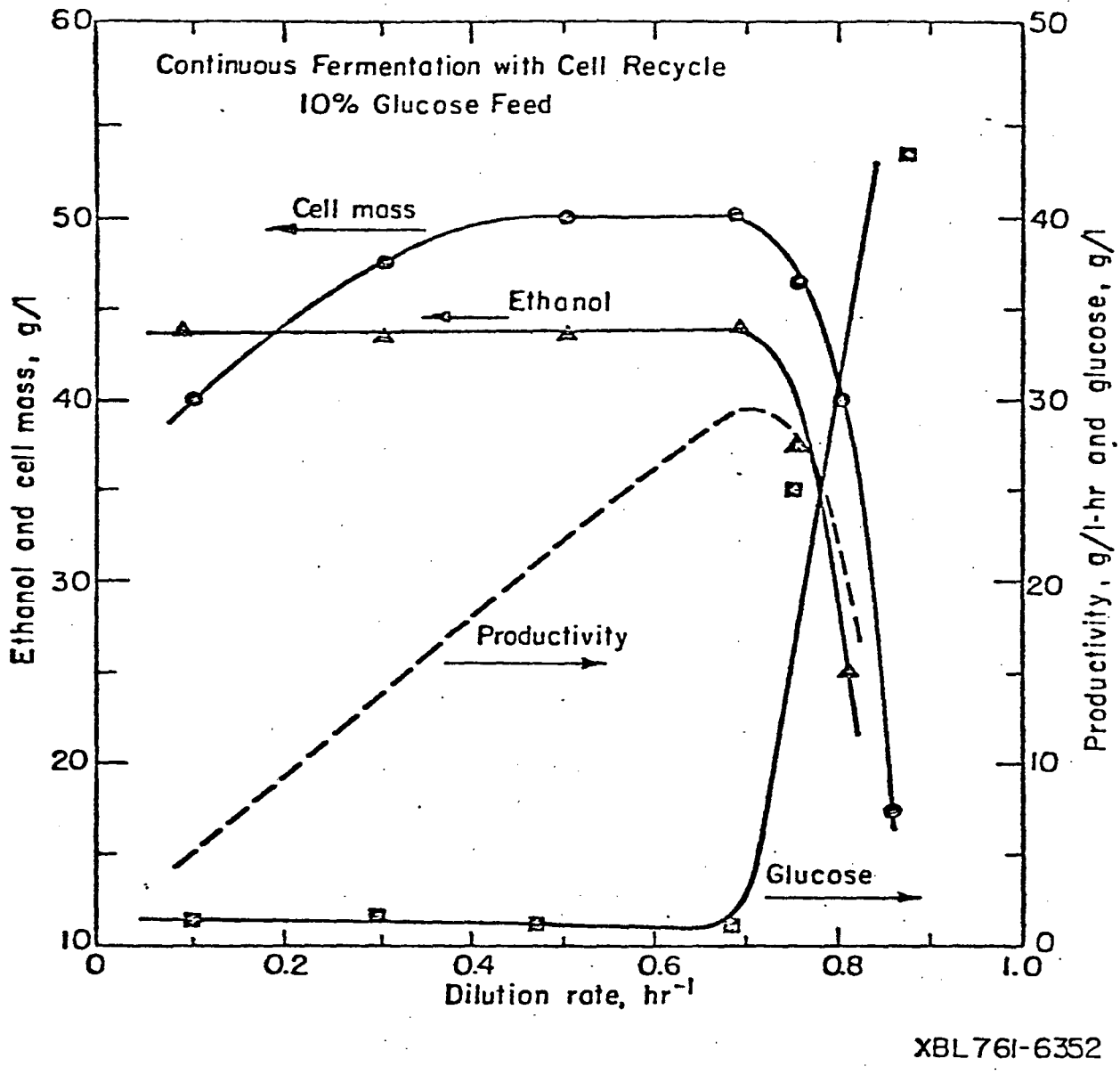

Fig. 9. Effect of increasing cell density by use of cell recycle in continuous fermentation.

The maximum ethanol productivity obtained with atmospheric-cell recycle operation was only about one third that obtained with vacuum operation. A dilute glucose feed, 10%, was used with the atmospheric fermentation to avoid severe ethanol inhibition (1)(3). The low glucose concentration required high dilution rates and hence high flow velocities through the settler be used to achieve high ethanol productivities. The increased load on the settler over the vacuum-recycle fermentation, in which a 33.4% glucose feed was used, did not allow the settler to achieve as effective separation of cells from the fermentation broth. This fact lowered the cell mass concentration in the recycle stream and thus lowered the cell density in the fermentor during atmospheric operation. The end results being lower ethanol productivities for atmospheric operation as compared with vacuum operation when the same size settler was employed.

The recycle experiments were conducted to demonstrate the feasibility and advantages of cell recycle operation for continuous ethanol production. The use of a settler was for experimental convenience only. In an industrial operation a continuous centrifuge would most probably be employed. A centrifuge is not as sensitive to changing flow rates as is a settler and would produce a more stable operation. Also a higher cell mass concentration can be obtained in the recycle stream with a centrifuge. Thus, it may be possible to achieve higher ethanol productivities than shown in Figure 9 or Figure 6 with the use of a centrifuge.

CONCLUSIONS

An overwhelming advantage of the vacuum fermentor is elimination of ethanol inhibition. This permits concentrated sugar solutions to be fermented at extremely fast rates. By use of cell recycle in conjunction with the vacuum system, ethanol productivities of almost 12 times that obtained with conventional fermentations were achieved. The direct consequence of this increased productivity would be a 12 fold reduction in fermentor volume required for an industrial ethanol fermentation.

Another advantage of the vacuum system, owing to the systems ability to utilize highly concentrated sugar solutions, is the production of a concentrated ethanol product (16-20% ethanol). This high concentration of ethanol in the fermentation product will reduce distillation costs for the final recovery of 95% ethanol. In this respect, when comparing the productivities of various fermentation schemes distillation costs should be taken into account.

Atmospheric pressure-cell recycle fermentations produced an increase in ethanol productivity of 4 times over conventional continuous operation. This was about one third the productivity achieved with vacuum operation. The productivity of the atmospheric-cell recycle fermentation was limited by the low feed glucose concentration which had to be employed to avoid severe ethanol inhibition. The low substrate concentration increased the flow rate required through the settler and thus limited the cell density and volumetric fermentation rate in the atmospheric system.

A major constraint of vacuum fermentation is the accumulation of non-volatile components in the fermentor. As a result, a bleed of fermented broth must be continually withdrawn from the fermentor to maintain the concentration of non-volatile components at a level which will not inhibit yeast

growth and ethanol production. The required bleed rate will be set by the concentration of non-volatile components in the fermentation substrate. Thus, experiments should be conducted using industrial fermentation media (i.e. molasses or hydrolysate sugars), before a process design can be finalized.

As in atmospheric pressure fermentation, trace amounts of oxygen were found to be an important supplement for the alcoholic fermentation during vacuum operation. To satisfy the yeast oxygen requirement a low flow rate (0.12 VVM) of pure oxygen had to be sparged through the vacuum fermentor in order to maintain a viable and actively fermenting yeast population. It may, however, be possible to eliminate the pure oxygen requirement by employing an aerobic atmospheric pressure fermentation stage preceding the vacuum fermentor. The aerobically grown yeast would then be fed to an anaerobic vacuum fermentor. The yeast would be able to actively ferment during anaerobic conditions in the vacuum fermentor using the pool of unsaturated fats and lipids stored during aerobic growth (9).

Vacuum and cell recycle alcoholic fermentations represent new approaches to an age old fermentation process. Because fermentation derived ethanol may someday serve as a supplement to or even a replacement for conventional petroleum liquid fuels, new and improved fermentation processes are needed. The economic implications of the vacuum ethanol fermentation will be examined in a later paper.

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