Biotechnology Letters Vol 9 No 6 425-430 (1987)
Received May 19

EXTRACTIVE FERMENTATION - INTEGRATED REACTION AND PRODUCT RECOVERY

A.J. Daugulis*, D.E. Swaine, F. Kollerup and C.A. Groom

Department of Chemical Engineering
Queen's University
Kingston, Ontario
Canada K7L 3N6

SUMMARY

Refinements have been made to a prototype process for the production of ethanol by extractive fermentation. The process is characterized by the *in situ* extraction of ethanol from a 7 L continuous stirred tank fermenter and the thermal recovery of ethanol from the extracting solvent, which is circulated in a closed loop through the process. Data are provided to show the efficacy and stability of the process under various operating conditions, and the near complete (> 96%) continuous conversion of a 300 g/L glucose feed.

INTRODUCTION

The productivity of conventional ethanol fermentations is limited because high ethanol concentrations in the fermentation broth decrease the ethanol production rate. This is a well known feature of many fermentation processes and is commonly referred to as end-product inhibition. Extractive product recovery is of particular interest in such cases since it may allow for the *in situ* removal of inhibitory product(s) by solvent extraction. In a Continuous Stirred Tank Fermenter (CSTF), the continuous contact between a water-immiscible organic extractant and the aqueous fermentation broth will result in a lower and less inhibitory product concentration in the broth, thus keeping reaction rates high. In addition, such a process may (i) allow for the fermentation of more concentrated substrate feeds, and (ii) decrease product recovery costs (Maiorella *et al.*, 1984).

In work prior to this project, a computer model simulating the extractive fermentation of ethanol was derived (Kollerup and Daugulis, 1985a). It provided a convenient means of establishing a sensitivity analysis of the effect on the fermentation process of changing aqueous and solvent dilution rates, ethanol distribution coefficients and substrate feed concentrations. An extensive solvent screening program was then undertaken in order to screen over 1,400 solvents in terms of their predicted physical and chemical properties (Kollerup and Daugulis, 1985b), and based on the theoretical screening, 70 solvents were subsequently investigated experimentally. An operational prototype system was developed for extractive ethanol fermentation in a 1.3 L CSTF using a commercially available chemical, comprised largely of oleyl alcohol, as the extractive solvent (Kollerup and Daugulis, 1986; Kollerup and Daugulis, 1987). Operation of the prototype system obtained near complete (96%) conversion of a 535 g/L glucose feed and demonstrated the kinetic advantage of extractive fermentation. The

use of a number of other extraction solvents as well as various advantageous process features have been previously detailed (Kollerup and Daugulis, 1987c).

The present work describes results obtained from a 7.0 L CSTF system which has been designed using the results from the smaller scale. Besides scale, the major difference between the two systems is the inclusion of a flash vaporization unit in the current system to thermally recover ethanol from the solvent stream leaving the fermenter, thereby regenerating the solvent for repeated use. This unit replaces a packed bed water extraction column that had been used to strip ethanol from the solvent in the 1.3 L system (Kollerup and Daugulis, 1987).

MATERIALS AND METHODS

The composition of the medium for the initial batch phase of fermenter operation was: glucose, 150 g/L; yeast extract, 10.0 g/L; $(NH_4)_2SO_4$, 11.6 g/L; KH_2PO_4 , 2.7 g/L; $MgSO_4 7H_2O$, 0.75 g/L; pH adjusted to 4.0. During the continuous operation stages of fermentation, a 300 g/L glucose feed was used with the salts and yeast extract concentrations being increased proportionally from the above formulation. Medium sterilization of the 150 g/L medium was accomplished by autoclaving *in situ* for 30 minutes at 121°C . In the preparation of the 300 g/L glucose feeds autoclaving was not feasible due to the problem of caramelization of the glucose in the medium, which is exacerbated at high glucose concentrations, prolonged heat treatment, and high temperature. Therefore, instead of autoclaving, oxygen-free nitrogen was flushed through the concentrated media for 1/2 hour, and the head space in the medium reservoir was filled with nitrogen throughout the fermentation in order to minimize the occurrence of surface growth of aerobic contaminants. The yeast strain employed was *Saccharomyces cerevisiae* NCYC 716.

Ethanol concentrations were determined with a gas chromatograph equipped with a flame ionization detector and connected to a Hewlett-Packard 3390 A Integrator. Isobutanol was employed as an internal standard. D-glucose was measured at 640 nm with a colorimeter, using the dinitrosalicylic acid method (Miller, 1959) Cell dry weights were obtained by drying at 105°C to constant weight and cell viabilities were determined by a modified methylene blue staining procedure (Lee *et al.*, 1981).

SYSTEM OPERATION

Figure 1 presents a process flowsheet for the experimental extractive fermentation system; Tables 1 and 2 list the legend for the flowsheet and the flowsheet's components respectively. The fermenter ($\mathbf{V_8}$) is a MBR laboratory chemostat with a working volume of 5.5 L. During system operation, the volume of the aqueous phase in the fermenter was 4.75 L. The solvent is introduced at the bottom of the fermenter through a ring sparger. Due to the difference in densities between the solvent and water, the former rises to form a second phase at the top of the fermenter; the volume of the solvent phase during operation was 0.75 L. The level of the interface between the two phases is set by the position of the inlet to the fermentation broth effluent pump ($\mathbf{P_3}$). The degree of contact between the two phases can be independently controlled by a solvent recirculation loop; a pump ($\mathbf{P_5}$) withdraws solvent from the top phase and returns it to the bottom of the fermenter.

An agitation rate of 150 rpm has been found to provide adequate mixing while maintaining a relatively stagnant solvent layer in the upper part of the fermenter, thereby allowing the withdrawal of primarily only solvent by the the overflow pump ($\mathbf{P_7}$). The outlet of this pump is the feed to the staged separation column ($\mathbf{V_9}$). which returns water and cells in this stream back to the fermenter. Filters ($\mathbf{V_{11}} - \mathbf{V_{12}}$) following the staged column serve to remove any remaining yeast cells before the ethanol-ladened solvent enters the flash unit. The flash

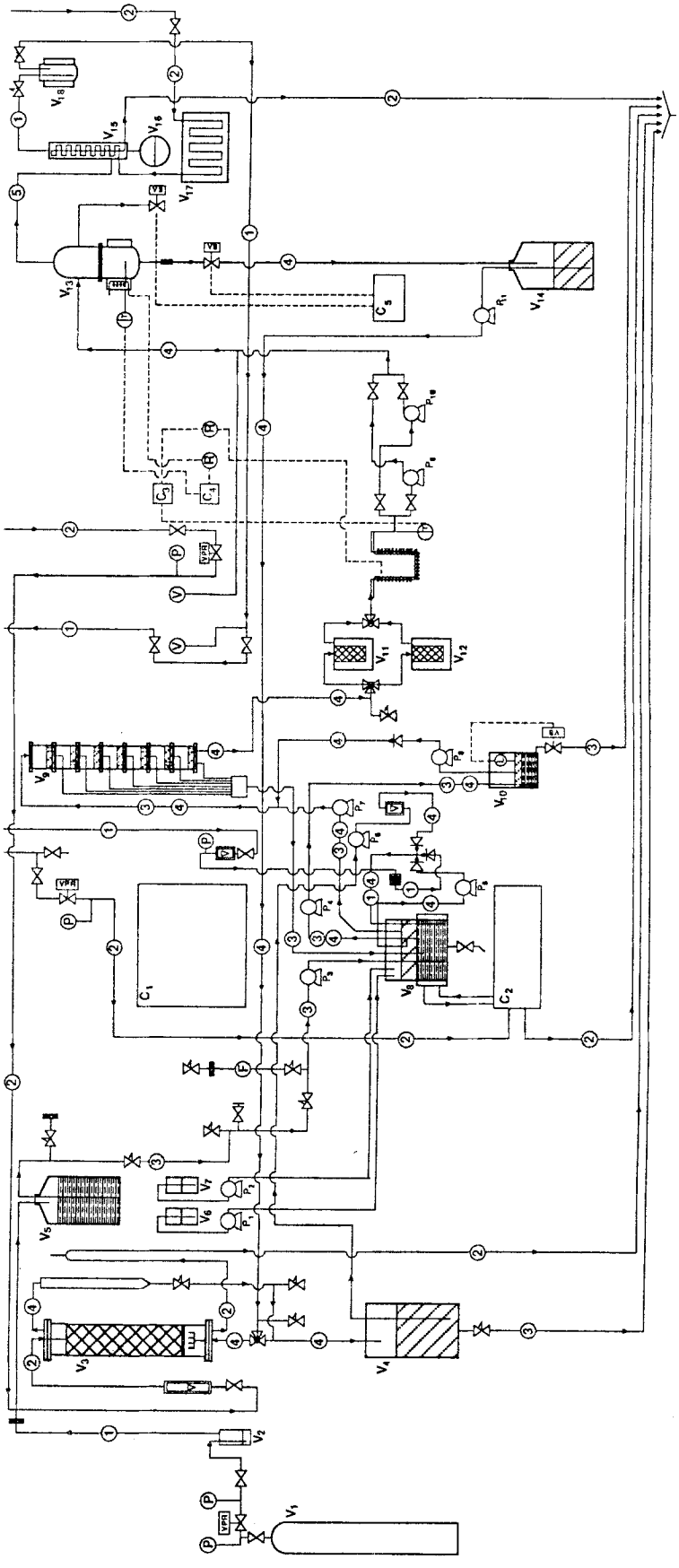

Figure 1: Process flowsheet for the extractive fermentation system.

Table 1: Flowsheet legend.

SYMBOLS:

	VALVE
VPR	PRESSURE REDUCING VALVE
	CHECK VALVE
VS	SOLENOID VALVE
P	PRESSURE GAUGE
V	VACUUM GAUGE
T	THERMOCOUPLE
F	FLOWMETER
R	RHEOSTAT
L	LIQUID LEVEL SENSOR
	ROTAMETER
	FILTER
	AQUEOUS VOLUME
	SOLVENT VOLUME

PHASE WITHIN TUBING:

1	Air, Vacuum, N_2
2	Hot or Cold Water
3	Medium, Aqueous Fermentation Effluent
4	Solvent
5	Ethanol Vapour

Table 2: Flowsheet components.

VESSELS:

V_1	O_2 - FREE NITROGEN CYLINDER
V_2	WATCH GLASS
V_3	SOLVENT REGENERATION COLUMN
V_4	SOLVENT RESERVOIR
V_5	MEDIUM RESERVOIR
$V_6 - V_7$	ACID - BASE RESERVOIRS
V_8	FERMENTER
V_9	STAGED SEPARATION COLUMN
V_{10}	FERMENTER EFFLUENT RESERVOIR
$V_{11} - V_{12}$	FILTER
V_{13}	VACUUM FLASH SEPARATOR
V_{14}	SOLVENT SURGE TANK
V_{15}	CONDENSER COLUMN
V_{16}	CONDENSATE FLASK
V_{17}	REFRIGERATED WATER BATH
V_{18}	VAPOUR TRAP

CONTROLLERS:

C_1	FERMENTER CONTROL
C_2	FERMENTER DRIVE
C_3	VACUUM SEPARATOR FEED TEMP.
C_4	VACUUM SEPARATOR TEMP.
C_5	TIMER

PUMPS:

$P_1 - P_2$	ACID - BASE DOSING
P_3	MEDIUM FEED
P_4	FERMENTATION BROTH EFFLUENT
P_5	SOLVENT RECIRCULATION
P_6	REGENERATED SOLVENT FEED
P_7	SOLVENT EFFLUENT
P_8	EFFLUENT RESERVOIR SOLVENT RETURN
$P_9 - P_{10}$	VACUUM SEPARATOR FEED
P_{11}	REGENERATED SOLVENT RETURN

vaporization unit (V_{13}) is operated under vacuum at *ca.* 75°C , conditions at which essentially all the ethanol is removed from the solvent. The ethanol is recovered in the condenser (V_{15}) while the regenerated solvent is returned to the solvent reservoir for re-use. The packed bed solvent regeneration column (V_3), previously used to strip ethanol from the solvent in the 1.3 L system, is also incorporated into the present system; however, its use has not been necessary.

RESULTS AND DISCUSSION

Following extensive testing of the system, an extractive fermentation run of over 400 hours was carried out. The main objectives of this run were (i) to demonstrate the thermal and chemical stability of the solvent through repeated solvent recovery and (ii) to determine the ethanol productivities, based on ethanol recovery from the effluent solvent stream, which could be achieved.

Figure 2 illustrates the time course of this experiment with respect to the glucose, cell and ethanol concentrations in the fermentation broth; **S** , **X** , and **P** respectively. Major changes in operating conditions are indicated by vertical dashed lines. Fermentation conditions that were kept constant were: agitation speed 150 rpm; temperature 30°C ; pH 4.0.

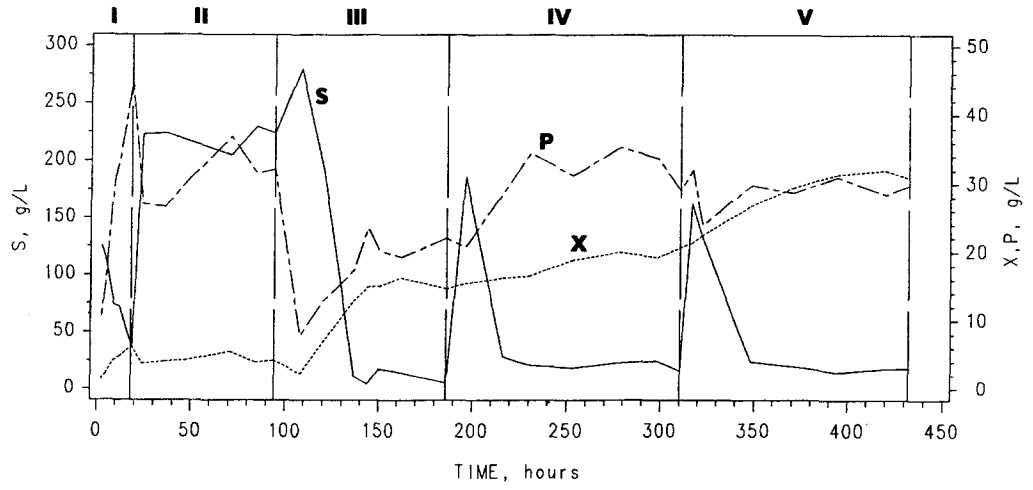

Figure 2: Concentration in the aqueous phase *vs.* time for extractive fermentation of 300 g/L glucose feed.

The different stages of the fermentation were:

I. Conventional Batch Fermentation (0-18 hours).

II. Conventional continuous operation (18-94 hours) with an average aqueous dilution rate of 0.04 h^{-1}.

III. Extractive fermentation (94-186 hours) with an average aqueous dilution rate of 0.04 h^{-1} and an average solvent dilution rate of 1.05 h^{-1}.

IV. Extractive fermentation (186-310 hours) with an average aqueous dilution rate of 0.11 h^{-1} and an average solvent dilution rate of 1.05 h^{-1}.

V. Extractive fermentation (310-432 hours) with an average aqueous dilution rate of 0.15 h^{-1} and an average solvent dilution rate of 2.10 h^{-1}.

Both the aqueous and solvent dilution rates are based on the aqueous volume of the system; the aqueous dilution rate is also based on the medium feedrate which is greater than the aqueous effluent flowrate due to conversion of the substrate to ethanol. During all stages of extractive fermentation, the solvent recirculation rate was 20 L/h.

The steady-state data from the chemostat and extractive fermentation stages are shown in Table 3. The **extraction efficiency** is defined as the ratio of the amount of ethanol removed in the solvent stream relative to the total amount of ethanol produced. The **ethanol productivity** of the system is based on the aqueous volume of the fermenter and the amount the ethanol recovered from the solvent stream by the flash unit and condenser only. Approximately 90% of the ethanol in this stream was recovered as condensate. The composition of the condensate was between 65-85% (by weight) ethanol with the remainder being water. The major source of this water was water vapour in air entrained in the line from the staged separation column.

Table 3: Steady-state data for conventional and extractive fermentation of 300 g/L glucose feed.

	FERMENTATION STAGE			
PARAMETER	**II**	**III**	**IV**	**V**
Aqueous Dilution Rate, /h	**0.04**	**0.04**	**0.11**	**0.15**
Residual Glucose Conc., g/L	**221.**	**10.8**	**22.0**	**19.0**
Aqueous Ethanol Conc., g/L	**30.7**	**20.5**	**32.1**	**29.4**
Cell Conc., g/L	**4.2**	**14.7**	**18.6**	**30.1**
Cell Viability, %	**95**	**84**	**87**	**81**
Solvent Dilution Rate, /h	**--**	**1.05**	**1.05**	**2.10**
Extraction Efficiency, %	**--**	**89**	**70**	**73**
Ethanol Productivity, g/Lh	**--**	**3.5**	**7.8**	**11.6**

During this experiment, the system was being operated with a reservoir of 40 L of solvent, and with the solvent circulating in a closed loop (no bleed, no make-up). There did not appear to be any accumulation of toxic or inhibitory compounds (fermentation by-products) during the more than 14 days that the system was operated in extraction fermentation mode. Volatile by-products are expected to have been removed from the solvent, along with the ethanol, in the flash unit. It is possible that some non-volatile materials may accumulate in the solvent over the course of more prolonged extractive fermentation operation, although such compounds could be readily removed by the water stripper (V_3) as long as they were water-soluble (the solvent is virtually insoluble in water: < 30 mg/L at 30°C). No deterioration in the physico-chemical properties of the solvent were observed even though the solvent volume passed through the system (including flash unit) more than an estimated 50 times.

In summary, this work continues to show the continuous, stable and long-term operation of the extractive fermentation previously developed in this laboratory. The initial 1.3 L prototype system has been scaled up to a 7 L vessel with more sophisticated ancillary equipment, including a thermal recovery unit to separate the product from the extracting solvent. The solvent appears to have excellent physical/chemical properties in connection with the present application, and has shown no accumulation of inhibitory fermentation by-products in the present process lay-out and duration of operation. Experimental data are currently being used as the basis of a detailed economic evaluation of the process.

ACKNOWLEDGEMENTS

A contract from the Bioenergy Development Program of Energy, Mines and Resources Canada saved this project from being abandoned at a critical stage of the work. This support and the assistance of Dr. W.E. Lowe are gratefully acknowledged.

REFERENCES

Kollerup, F. and Daugulis, A.J. (1985a). *Biotech. Bioeng. 27*, 1335-1346
Kollerup, F. and Daugulis, A.J. (1985b). *Can. J. Chem. Eng., 63*, 919-927
Kollerup, F. and Daugulis, A.J. (1985c). *U.S. Patent Application No. 6-775-791*
Kollerup, F. and Daugulis, A.J. (1986). *Can. J. Chem. Eng., 64*, 598-606
Kollerup, F. and Daugulis, A.J. (1987). *Biochemical Engineering V*, New York: The New York Academy of Sciences (In press).
Lee, S.S., F.M. Robinson, and H.Y. Wang (1981). *Biotechnol. Bioeng. Symp., No. 11*, 641-649
Maiorella, B.L., Blanch, H.W. and Wilke, C.R. (1984). *Biotech. Bioeng., 26*, 1003-1025
Miller, G.N. (1959). *Anal. Chem., 31*, 426-428