TITLE:

AN EXTRACTIVE FERMENTATION PROCESS FOR ENHANCED ACETATE PRODUCTION FROM BIOMASS

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INTRODUCTION

Conventional fermentation technologies are limited due to low reactor productivity, low product yield, and low product concentration mainly due to product inhibition. There are several ways of improving the overall behavior of a biotechnological process. The cells can be modified so that the product is excreted prior to reaching inhibiting concentrations. Furthermore, a selective mutation of the cells can be employed to eliminate points of metabolic pathways sensitive to feedback inhibition. Also, the concentration of an enzyme that is of critical importance to the whole metabolic pathway can be amplified. Another approach to improving the productivity of a process is to increase the catalyst density in the reactor by immobilization or catalyst recycling. Because of the high cell density, the microorganism has a high acid tolerance, thus, the conversion of a substrate to a major product is still possible even up to a very high acid end product concentration accumulated in the fermentation broth.

Different fermentation routes, specifically for acetic acid, could be employed. The most widely established is the aerobic fermentation of sugar with about 10% product concentration but the productivity is very low and the energy requirement is also high because of aeration. Anaerobic fermentation can also be employed with a theoretical yield of almost 100% and high reactor productivity but the product concentration is low because of end-product inhibition. Furthermore, the energy requirement for anaerobic process is very low compared to aerobic process.

Currently, organic acids from fermentation broth are being recovered by conventional recovery process including distillation, precipitation, solvent extraction and evaporation. However, most of these separation techniques are energy intensive because the concentration of the organic acid is very low, usually less than 4%, as in the case of distillation and evaporation, aside from producing a lot of wastes, as in the case of citric acid recovery using precipitation (King, 1992). Solvent extraction is sometimes used in the recovery of fermentation product. However, most of the conventional organic solvents, such as oxygen-bearing and long-chain alcohol do not have high distribution coefficients, K_d, therefore, large amount of extractant is needed.

Amine-based extraction is receiving a lot of research interests because of the high distribution coefficient of amine solvents ranging from 2 to 40 for organic acid aside from the fact that amine can react easily with organic acid to form complexes. Furthermore, amine solvent can be easily regenerated by back extraction with an alkaline solution because K_d of amine for organic acids approaches zero at pH 7.

A solvent extraction with Alamine 336, a tertiary amine, in hexane as the extractant and hot water for back extraction and regeneration was reportedly successful for citric acid fermentation. However, similar process did not work well for lactic acid fermentation because the fermentation process requires high pH, therefore, the fermentation broth needs to be acidified by adding a strong inorganic acid before extraction could be carried out. The extraction efficiency was found to drop dramatically to a very low level due to anion interference. Except for citric acid, amine-based extraction technique has not been used in the industry in recovering fermentation product. Recently, Jin (1997) successfully demonstrated an amine-based extraction of propionic acid in the fermentation broth using free cells. Adogen 283 in oleyl alcohol was used as extractant. A 6N sodium hydroxide was employed for back extraction and regeneration of solvent.

Many works have been published for alcohol extractive fermentation because of the reported increase in reactor productivity. However, very few have been reported for organic acid extractive fermentation, especially for acetic acid, although the same concept used for alcohol was supposed to work for organic acid. Like in alcohol extractive fermentation, the major problem in developing an organic acid extractive fermentation is the solvent toxicity. However, it seems more difficult to find a nontoxic, biocompatible solvent for organic acid than for alcohol because solvents that have better K_d values for organic acids tend to be also more toxic to cells. Another problem in developing organic acid extractive fermentation is the pH requirement. Generally, fermentation favors a pH around 7 but amine-based extraction can work efficiently only at pH below 4.5 or even lower than the acid pK_a.

RATIONALE / SIGNIFICANCE:

No prior work has been done on acetic acid extractive fermentation of lactose via a two-step fermentation process. This research was conducted to solve the end-product inhibition which is the main barrier in obtaining a high reactor productivity and a highly concentrated product. The designed process, with the proper modifications, could also be employed in other organic acid extractive fermentation.

OBJECTIVES:

The main goal of this research was to design a process for acetic acid extractive fermentation from lactose via a two-step fermentation process. The process was expected to have a higher reactor productivity, a higher selectivity for acetic acid over lactic acid, and a highly concentrated product. To achieve this goal the following specific objectives were set:

a.) To study the fermentation kinetics of the two microorganisms, *Lactococcus lactis* and *Clostridium formicoaceticum*, to be used in the proposed process
b.) To select an extractant and a diluent with high distribution coefficients and biocompatibility with the two bacteria
c.) To determine a favorable operating condition for fermentation, extraction, and extractive fermentation processes

METHODOLOGY / RESEARCH DESIGN:

Acetic acid extractive fermentation process was designed and demonstrated in this research. Two hollow-fiber membrane contactors were used, one for the recovery of acetate from a mixture of lactate and acetate in the fermentation broth from two-step fermentation process of lactose and the other one for stripping and regeneration of solvent. Amine-based extraction was applied using 7% Adogen 283 in 2-octanol as solvent. Fermentation kinetic study was also conducted for the two microorganisms, *Lactococcus lactis* and *Clostridium formicoaceticum*, to find a good operating condition for biological conversion that would be used in the extractive fermentation process.

In this work, an extractive fermentation process was developed for production of acetate to overcome the above-mentioned problems. A two-step fermentation process was employed, i.e., converting lactose, from low-value agricultural commodities or any low grade biomass, to lactic acid using *Lactococcus lactis* and then lactic acid to acetic acid using *Clostridium formicoaceticum.* These two bacteria were immobilized in the fibrous bed using cotton matrix supported by stainless steel mesh.

A hollow-fiber membrane extractor was used to extract acetic acid from a very dilute fermentation broth. Another unit was used for back-extraction to strip off the acid product while simultaneously regenerating the solvent for reuse. Because of *in-situ* recovery, product inhibition was reduced, at least, if not totally eliminated. Adogen 283 and 2-octanol were used as extractant and diluent respectively. In the experiment using suspended cells, 7% Adogen 283 in 2-octanol was found to be not toxic to any of the two bacteria. Beyond this concentration, the cells cannot any more survive and fermentation did not proceed. Therefore, to reduce toxicity caused by the water-soluble part of the solvent and an accidental breakthrough in the membrane, this solvent concentration is also used in extractive fermentation experiment. The diluent was found to enhance the extraction ability of the extractant by improving the stability of the acid-amine complex in organic phase, by providing solvation, and at the same time reduce the toxicity caused by pure extractant. Since extraction works better at lower pH, lactic acid produced from the first fermentation step was used to acidify acetic acid in the second step.

Membrane-based phase contact used the static phase interfaces that are immobilized by the micropores of the membrane as the mass transfer surface instead of the dynamic phase interfaces in mixing and settling. The high volumetric specific surface of the hollow-fiber membrane cartridge can give much higher mass transfer efficiency in this type of phase contact. Also, hollow-fiber membrane contactor is dispersion-free. Solvent toxicity was mitigated not only because the cells were immobilized but also because there was no direct contact between the cells and the solvent since the design of the contactor provided the aqueous feed to be contained in the shell side while the solvent flows through the tube side.

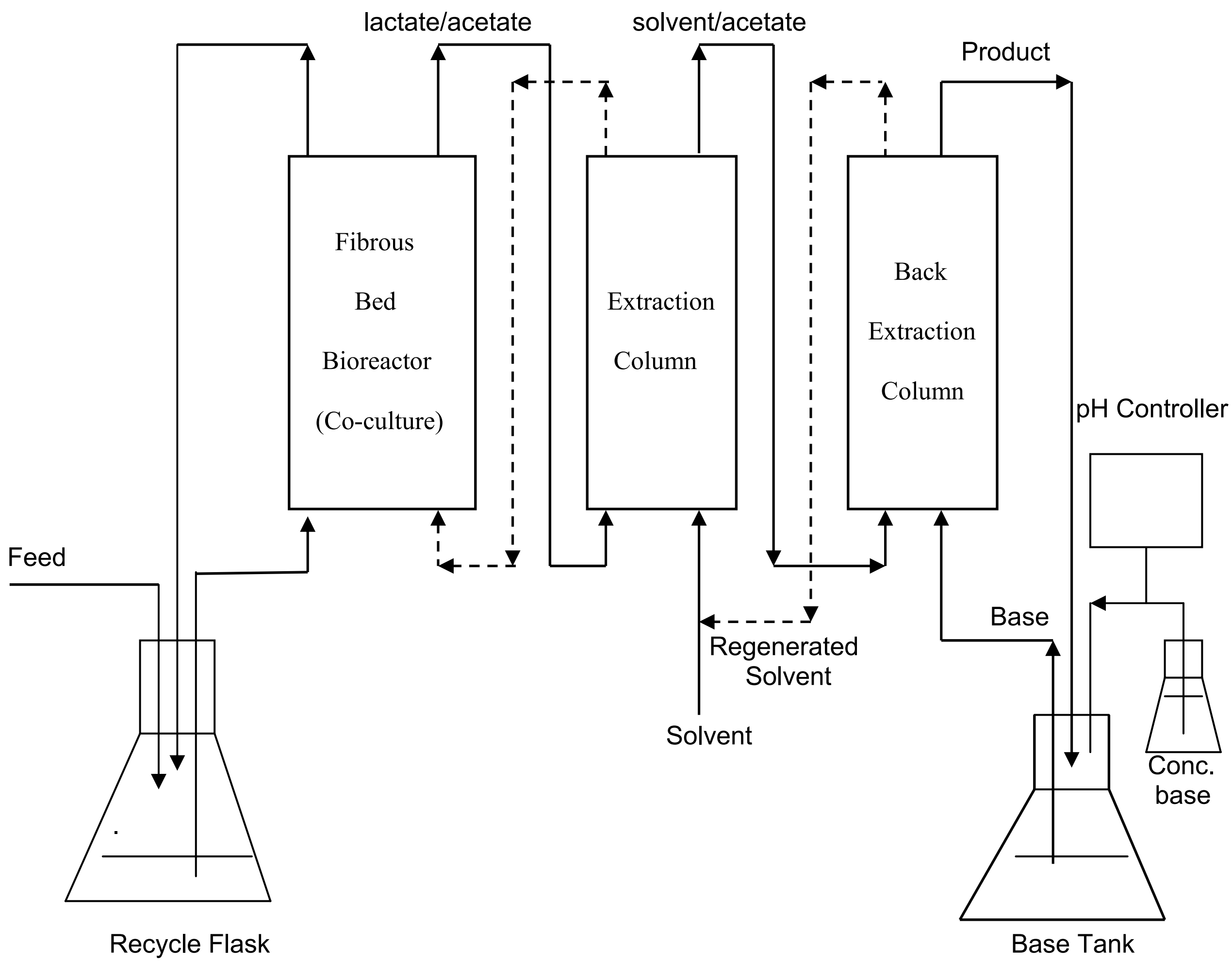

Figure. 1 Schematic diagram of an extractive fermentation set-up

SUMMARY OF KEY RESULTS AND FINDINGS:

From the results obtained, for mixed-culture fermentation without *in situ* extraction, and for extractive fermentation, the reactor productivity increased by about 300% for system containing 1.5 % (w/v) sodium acetate as compared to mixed culture-fermentation without *in situ* extraction. Furthermore, the increase in reactor productivity for the same system was even higher, about seven times, as compared to fed-batch fermentation without extraction. The addition of dissociated acid before *in situ* extraction begun was used as the strategy to promote extraction of the acid product while raising fermentation pH.

CONCLUSIONS AND RECOMMENDATIONS:

Based on the results obtained, the following conclusions were arrived at:

1. The optimum pH for *L.lactis* was ~ 6.5, whereas that for *C.formicoaceticum* was ~ 7.6, as can be deduced from the values of the product yield and reactor productivity obtained for the separate fermentation processes. However, *L.lactis* can still survive at pH ~ 5.0 and *C.formicoaceticum* at pH values above 6.6
2. Based on the hollow-fiber membrane and amine-based solvent extraction system, the extraction was feasible even at a higher pH and there was a good selectivity for acetic acid over lactic acid.
3. The integrated process, i.e. the acetic acid extractive fermentation from lactose using co-culture, was feasible. The addition of a dissociated acid in the medium promoted the extraction of the acid product while raising the pH, thus increasing the product yield and the reactor productivity.

With the results obtained, the following recommendations were made to further enhance the efficiency of the integrated process:

1. Optimization of the process variables, especially those used in the extractive fermentation, would further increase the reactor productivity and product purity.
2. The cell density of *C.formicoaceticum* in the coculture should be increased to reduce the lactate concentration and to increase the acetate concentration in the bioreactor, so as to increase the acetate production rate and selectivity.
3. The acetic acid fermentation rate should almost be the same as the acetic acid extraction rate to avoid the accumulation of this acid in the bioreactor.

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