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Supercritical fluids technology in bioprocess industries: A review

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Abstract

This article reviews the fundamentals of supercritical fluid (SCF) science and moves on to the biotechnological applications of them e.g. removal of biostatic agents from fermentation broths, SCF disruption of microorganisms, destruction of industrial waste, the gas-antisolvent crystallization and micron-size particle formation. Also gaining ground is enzymatic catalysis in supercritical carbon dioxide (SC-CO_2), which offers the possibility of integrated synthesis product recovery processes. The paper is structured as follows: first, the effects of dense SC-CO_2 on the extraction of biomaterials and disruption of cells are thoroughly reported and discussed. Then the application of SC-CO_2 in particle formation and modifications of biopolymers and enzymes are described. In general, the article is focused on potential bio-industrial applications and future research needs of the SCF technology.

Keywords: Biotechnology, Supercritical Fluids (SCF), disruption, inactivation, extraction, downstream, particle formation

Introduction

In recent years, supercritical fluids (SCFs) have been of interest in the biotechnological processes (Williams et al 2002). They provide solutions to drastic problems related to bacterial (Dillow et al. 1999; Enomoto et al. 1997; Spilimbergo et al. 2003), enzyme (Hong and Pyun 2001), viral (Fages et al. 1998) and yeast inactivation (Hashizume et al. 1995), as well as permeabilization (Aaltonen and Rantakyla 1991; Mesiano et al. 1999) and extraction of fermentation products (Bruno et al. 1993; Cocks et al. 1995; Cygnarowicz-Provost et al. 1999; Hampson and Ashby 1999; Isenschmid et al. 1995; Saykhedkar and Singhal 2004; Shishikura et al. 1992). Application of SCF is inexpensive and non injurious to the enzymes (Lin et al. 1993) and proteins (Juhasz et al. 2003; Kamat et al. 1995; Zheng and Tsao 1996).

A SCF uniquely displays a wide spectrum of solvation power as its density is strongly dependent to upon temperature and pressure. Temperature change of ten of degrees or pressure changes of ten of atmosphere can change a compound's solubility in a SCF by an order of magnitude or more. The selectivity of nonpolar SCF can also be enhanced by addition of modifiers (entrainers or cosolvents), which are typically polar organic solvents e.g. acetone, ethanol, methanol, methylene chloride or ethyl acetate. Varying the proportion of modifier allows wide latitude in the variation of solvent power. The unusual physicochemical properties of SCF in relation to their engineering applications in polymer and waste processing have been discussed (Van-eijs et al. 1983). Advantages and disadvantages of SCE especially for the biotechnology industries have been discussed elsewhere (Van-eijs et al. 1983).

Supercritical-carbon dioxide (SC-CO_2) is the most commonly used fluid and the low critical temperature (31.1°C) and pressure (73 bar) of which make it an ideal medium for processing volatile products (Goodarznia and Eikani 1998) and other new developemnets (Marr and Gamse 2000). Non-toxicity, non-flammability as well as the selectivity of the process and the ease of recovery are the most important features (Wells and DeSimone 2001). Also SC-CO_2 was used as an antisolvent for the preparation of PHB microspheres as drug delivery systems (Bleich and Mueller 1996; Breitenbach et al. 2000; Bustami et al 2000). Applications of SCF in polymer processing have been reviewed thoroughly (Kazerian 2000). Khosravi et al. have reported the equilibrium solubility of poly(hydroxyl butyrate) (ultra-molecular weight) in SC-CO_2 at temperatures ranging from 30 to 70°C and pressures ranging from 122 to 355 bar (Khosravi-Darani et al. 2003). Khosravi et al. also have reviewed many aspects of the SCF extraction in the downstream processing of bioscience (Khosravi-Darani and Vasheghani-Farahani 2005).

The advantage of SC-CO_2 extraction fit well to those of biotechnological production processes are the mild processing temperature. CO_2 is also naturally occurring and readily available

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and is a by-product from the production of ammonia, ethanol, hydrogen and natural gases (Wells and DeSimone 2001). Processes that use CO_2 do not add directly to the green house effect but rather aid in the reduction of emitted CO_2. Wells et al. has discussed the technology platform that allows CO_2 to be an environmental problem solving tool for sustainable economic growth (Wells and DeSimone 2001).

SC-CO_2 introduced in a liquid decrease the overall viscosity and thus facilitates the handling of the broth and enhances mass transfer from the liquid to SC phase (Van-eijs et al. 1983). If the microorganism is inhibited by its own products, like in ethanol fermentation by yeasts and in acetone/butanol/ethanol fermentations by Clostridium, it is advantageous to extract the product in situ. If cells are resistance to SC-CO_2, the extraction may be carried out using the whole broth. The extracted fermentation broth can be recirculated to the fermenter. Other non biological applications of SCF includes extraction of organic solvents from aqueous solution, removal of chlorinated compounds from water and other water pollutants, treatment of lignocellulosic materials, recovery and purification of biological products and fractionation of cod liver oil. Other application of SCFs including non-thermal inactivation of microorganisms, dispersion media for biocatalysis and the treatment of wastes of food industries have been introduced (Khosravi-Darani 2007).

Separation of biological products from fermentation medium at high quality, efficiency and low energy cost is required. SC extraction (SCE) has some lab, pilot and industrial scale applications in downstream of fermentation. For recovery of intracellular metabolites, the cell disruption is necessary. A variety of disruption techniques have been developed (Harrison et al. 1990) and some are commercially (Tamer et al. 1998). Although the mechanical methods are favored due to economic advantages, but some times several non-mechanical methods, particularly enzymatic lysis, have attracted great attention. SCF has an important application for cell disruption which will be explained more in a separate part.
Many reviews dealt with the stability of proteins (Heremans and Smeller 1998) and enzymes (Athes et al. 1998; Degraeve and Lemay 1997; Degraeve et al. 1996; Knutson et al. 1999; Mozhaev et al. 1994) as a function of pressure. The use of hyperthermophilic enzymes (Noel Marie et al. 2000) and the characterization of deep-sea microorganisms have been also reviewed (Adams and Kelly 1998). Studies have been done to explain the properties of thermozymes.

Product recovery

Complexity of product recovery from a fermentation broth is more than chemical processes, due to product concentration, broth viscosity and biomaterial properties. SCE may provide solutions to some special limitations e.g. low product concentration. SCE by CO_2 can be carried out at fermentation temperature thus avoiding possible thermal damage of the product and saving energy costs. Table I shows a list of extracted bioproducts from fermentation. Application of SCFs in extraction of biomaterials from biomass has been widely reviewed (Khosravi-Darani and Vasheghani-Farahani 2005) and classified as following: post fermentation extraction of products; In situ extraction from the biomass of microbial fermentation; Fractionation of cellular biomass; Removal of biostatic agent from fermentation broth. It has been reported that furfural, a growth inhibitory byproducts was successfully removed during fermentation of Clostridium on sugars by introducing liquefied CO_2 into the aqueous solution at room temperature and 5.9 MPa. Such an approach was explored with several SCF including ethane, CO_2, Xe, and halogenated refrigerants during fermentations of *Propionibacterium freudenreichii* and *E. coli* (Tomasko and Chon 1997). Refrigerant 13 ($CClF_3$) or R116 (CF_3CF_3) did not affect the growth of broth *P. freudenrechii* and *E. coli*. Reverse micelle formation is presented as a new strategy for improving the extraction of polar species with SC-CO_2. The addition of a reverse-micelle forming reagent prior to SFE accelerates the quantitative extraction of the analyte. (Jimenez-Carmona et al. 1998).

Table 1: Application of SCF in downstream of biotechnological processes

Product	Microorganism	Process Condition	Reference
Ethanol	*Saccharomyces cerevisiae*	Pilot scale, continuous countercurrent	Van-eijs 1983
Acetic/Propanoic/Butanoic acid	*Clostridium thermoaceticum*	Laboratory scale, batch	Van-eijs 1983
Aceton/Butanol/Ethanol	*Clostridium acetobutylicum*	Pilot scale, batch	Van-eijs 1983
Substituted thiophenes	*Tagetus patula* cells	-	Van-eijs 1983
Complex lipids: (acylglycerols, phosphoglycerides, sphingolipids, waxes)	Animal source material	-	Kamarei 1983
Simple lipids (terpenes, pigments, steroids, sterols, prostaglandins)	Animal source material	-	Kamarei 1983
Glycolipids, lipoproteins, cell membrane supramolecular complexes	Animal source material	-	Kamarei 1983

Detecting the presence of a microorganism

Another interesting application of SCF in biotechnology is detecting the presence of a microorganism in a sample. In this strategy, nucleic acid of pathogen will isolate after exposure to SCF. Then, detecting of contamination to a particular sequence will be conducted by hybridization and PCR method (Nivens and Applegate 1996). Importantly, this method can be used the presence of pathogenic microorganisms in water supplies. A major advantage of this method is its application for all microorganisms (preferably, a bacterium; alternatively, a protozoan, parasite, or virus.). Even if partial or preferential lysis occurs, the extracted DNA can be representative of contamination in sample, rapidly. DNA appears very resistance to hydrostatic pressure. Structural integrity of calf thymus or salmon sperm DNA remained unchanged when pressures of up to 10000 at were applied for 60 min at 25-40°C.

A SCF extraction procedure and a chromatographic separation/detection method were developed for the detection of earth-based microorganisms. After microbes in a sand or soil sample were hydrolyzed in a diluted NH_4OH/acetone solution, several redox compounds could be effectively extracted from bacteria (trimethylamine- SC-CO_2 at 35°C and 300 at). The analytical results demonstrated the feasibility of using the reported techniques to detect the chemical signature of life in barren desert sand samples (Lang et al. 2002).

Inactivation of microorganisms

Application of supercritical is a promising alternative method for the pasteurization and sterilization of foodstuff (particularly in the liquid phase), as well as chemically, thermally and hydrolytically

sensitive materials in biomedical applications. The development of this effective alternative method and its lethal action of high hydrostatic pressure CO_2 on microorganisms, has been reviewed by Khosravi (2007).

Cell disruption

The potential use of the intracellular storage products such as poly(β-hydroxybutyrate) (PHB) and recombinant metabolites have led to the increased interest in efficient and cost effective cell disruption to enable the recovery of intracellular microbial products in intact form (Lee et al. 1979). Mechanical disruption methods are favored due to economic advantages. Homogenization after a suitable pretreatment is a proven recovery method, although only relatively dilute biomass slurries can be satisfactorily processed (Tamer et al. 1998). Harrison has shown that chemical (addition of an anionic detergent and monovalent cation) or lysozyme pretreatment can weaken the cell wall sufficiently to decrease energy consumption and allows for operation at lower pressures or fewer passes with the homogenizer (Harrison et al. 1990). Also bead mill disruption is recommended for PHB recovery due to low power consumption and robustness (Heremans and Smeller 1998). In this method the agitation speed will affect the amount of energy consumption but when rotation was increased to a critical level, only a marginal increase was found with the excess energy being converted to heat (Schutte et al. 1983).

The cell disruption operation affects the physical properties of the cell slurry such as viscosity (Mosqueira et al. 1981), density, particle size, and settle ability of suspension (Lee et al. 1979). The necessity of harvesting the producing cells, in order subsequently to extract an internal constituent, is a major economic disadvantage, and, in part, results in the present preoccupation with the manufacture of products of very high value (Chisti and Moo-Young 1986). Because of the high capital and operating costs for large-scale isolation of intracellular products and the requirement of sizeable teams of scientists and technical staff to obtain meaningful biochemical engineering data, few studies on this subject have been published (Chisti and Moo-Young 1986).

Application of SCF in cell disruption (SC disruption) has been confined to yeasts (Castor and Hong 1995, Lin et al. 1991; Nakamura et al. 1994) and a few bacteria (Foster et al. 1962; Fraser 1951; Juhasz et al. 2003). The process involves a sudden release of the applied SC-CO_2 pressure that will results in its penetration into the cells. After expansion of gas within the cells, flash discharge of pressure forces the cell wall and causes cell disruption. The technique is relatively simple and can easily be scaled up (Juhasz et al. 2003). Also, the cells are exposed to minimal shear forces and there is no heat generation, which would otherwise affect the yield of temperature sensitive and labile material recovery adversely.

Disruption of microbial cells by sequential pressurization and explosive decompression was first reported by Fraser (Fraser 1951) and then developed by several researcherss to recover intracellular enzymes and recombinant-DNA proteins (Castor et al. 1996; Foster et al. 1962; Lin and Chen 1994; Lin et al. 1991; 1992; 1993; Nakamura et al. 1994). Several studies have been reported on cell inactivation by SCF (Khosravi-Darani and Vasheghani-Farahani 2005).

A technique to exploit SC-CO_2 disruption of *R. eutropha* cells has been developed (Hejazi et al. 2003). The effects of different variables such as exposure time, pressure, temperature and volume of methanol (as a modifier) on SC disruption of suspension of bacterium were investigated. Khosravi et al. extended this work to obtain maximum recovery with minimum energy consumption (Khosravi-Darani et al. 2004). The variables affecting cell disruption such as drying strategy, type of modifier and cultivation time, as well as operating pressure, temperature and repeated release of supercritical-CO_2 pressure have been studied. Also a series of pretreatment experiments were arranged to alter cell wall strength prior to disruption and to minimize repetition of pressure release. The effect of cultivation time on the PHB recovery was also studied. On the result of this study, the optimum conditions for the recovery of PHB using SC-CO_2 were 200 bar of pressure, 30°C temperature and 1% (v/v) of toluene with two repeated release of SC-CO_2 pressure. Pretreatment with a minimum of 0.4% (w/w) NaOH was necessary to enable complete disruption with two repetitions of pressure release. Salt pretreatment was less effective; however, disruption was improved by the application of alkaline shock (Khosravi-Darani et al. 2004).

It has been reported that young cells are more susceptible to CO_2 treatment than mature ones due to synthesis of new proteins, which protect cells against a variety of adverse conditions such as high temperature, oxidative stress, high salt concentration and pressure (Kashket 1987; Mackey et al. 1995). Similar results have been reported for maximum efficiency in yeast disruption requiring log phase cells (Castor and Hong 1995). Consequently, biomass produced after 30 h cultivation was used to save energy and time for PHB recovery (Khosravi-Darani et al. 2004).

Table 2: Application of SCF for disruption of different cells

Microorganism	SCF	Disruption Condition			Criteria of disruption	Reference
		P(atm)	T(°C)	Time(h)		
Yeasts, bacteria, water plant, fungi,	N_2, air, CO_2	80-200	n r[a]	n r	Release of Protein, nucleic acids, enzymes, physiological active substances	Grigorian *et al*. 1982
Saccharomyces cerevisiea	CO_2	70-215	25-35	0-10	Release of Protein	Lin et al. 1992
Saccharomyces cerevisiea	CO_2	34-340	10-85	1-15	Release of Protein	Lin and Chen 1994
E. coli	N_2 N_2O Ar CO_2	34-61 17-51 34-61 34	37-38	0.08	Agar plate count & direct observation by electron microscopy	Fraser 1951
Serratia marcescens	N_2	51-118	n r	n r	direct observation by electron Microscopic	Foster et al. 1962
Saccharomyces cerevisiea	N_2, CO_2	40 at	40	3	Agar plate count/ direct observation by electron microscopy	Nakamura et al. 1994
Saccharomyces cerevisiea, E. coli, Bacillus subtilis	N_2, N_2O, CO_2	Near critical	Near critical	n.r	Released protein and nucleic acid	Castor and Hong 1995
Ralstonia eutropha	CO_2	SC	SC	0.33	Released protein and Poly(hydroxybutyrate)	Khosravi et al. 2004

[a] not reported

In another report *Saccharomyces cerevisiae* and the spore cells of *Bacillus megaterium* were selected as model microorganisms and the lethal effect of pressurization and subsequent flash decompression treatments with CO_2, N_2O, N_2 or Ar under various conditions of pressure, temperature, and time were examined. In all reports, during pressurization with CO_2, the absorbed gas may cause the inactivation of key enzymes related to the essential metabolic process, probably due to decreased pH value inside the cells and/or the solubilization of intracellular substances such as hydrophobic compounds in the cell wall and cytoplasmic membrane (Juhasz et al. 2003). Also cells may be broken by the pressurization process, and not in the decompression stage. Yeasts predominantly killed by SCF due to physiological damage of and mechanical rupture. Different experimental data have been also reported to support the lethal action of rapid discharge by the explosive decompression system, as already mentioned (Enomoto et al. 1997; Fraser 1951; Hejazi et al. 2003; Khosravi-Darani et al. 2004). It has reported that higher decompression rates up to 48 at/min could lead to about a two-order-higher reduction of the remaining cells when 10^9 cells/mL of yeast had been treated with CO_2 at 40 at and 40 °C for 4 h (Enomoto et al. 1997). Table II shows some of the reported disruption process using flash discharge of SCF.

Enzymatic pretreatment of cells (e. g. beta-glucuronidase, lysozyme, glucanase) is another effective means to reduce the resistance of microorganisms to disruption (Lin et al. 1992). Preferably the enzyme and protein in the cells remains active and native state in the ruptured cell suspension (Lin and Chen 1994).

In some reports continuous disruption of microorganisms by SCF have been reported (Kashket 1987). To effect disruption of yeast, bacteria and fungi, a suspension of cells is presaturated with a compressed gas and then passed through one or more disintegration units containing a plate valve seat and valve member urged toward each other. The disruption unit can be attached to a mechanical vibrator-shaker. There were two thick wall vessels (mixing part) in the inlet of this unit (to effect intermixing of the suspension) and a homogenizing vessel (to maintain the suspension under pressure of 100-120 at). The cell walls are intensively disrupted when the suspension is passed through a throttling slit formed between the valve seat and valve member, due to the internal pressure.

The variables which must be studied in order to obtain efficient cell breakage include temperature, pressure, exposure time, moisture content of the cells, initial rate for gas desorption, microbial age or shape, number of flash discharge of pressure, vessel capacity and shape, as well as fermentation conditions e. g. strain of microorganism, media composition and carbon source (Khosravi-Darani et al. 2004; Hopekins 1991).

Biochemical reactions in SCF

The solubility of substrate in SCF, the stability of proteins (Athes et al. 1998) and enzymes in SCF have been widely studied (Adams and Kelly 1998; Jimenez-Carmona and Luque de Castro 1998; Mozhaev et al. 1994; 1996; Nakamura 1990; Noel Marie et al. 2000). Among the enzymatic reactions in SCF, the use of lipase shows most commercial promise. A SC-CO_2/H_2O mixture may be used as a reaction medium for either hydrolytic or synthetic reactions catalyzed by lipase and other appropriate by hydrolases (Giebauf et al. 1999). In continuous reaction of acidolysis of triolein with stearic acid, the constants of the reaction and mass transfer such as rate constant, solubility, effective diffusivity, mixing diffusivity and mass transfer coefficient depend on temperature, pressure and flow velocity (Nakamura 1990).

Immobilized *Candida antarctica* lipase B was successfully used as catalyst to synthesize butyl butyrate from butyl vinyl ester and 1-butanol in SC-CO_2) with excellent results. A clear enhancement in the synthetic activity and selectivity was observed with the decrease in fluid density for both liquids and SC-CO_2 media. However, all SC conditions assayed enhanced up 84-folds respect to the organic solvents the synthetic activity of the lipase-membrane derivative. For the best SC conditions (60°C, 8 MPa), the enzymatic membrane was assayed by repetitive operational cycles of 6 h/day, showing a 360 cycles half-life time in their synthetic activity (Lozano et al. 2004).

Also a commercial solution of free *Candida antarctica* lipase B (Novozyme 525L) has been immobilized by adsorption onto 12 different silica supports modified with specific side chains (e.g. alkyl, amino, carboxylic, nitrile, etc.). The immobilized derivatives were assayed for the kinetic resolution of *rac*-1-phenylethanol in both ionic liquid/hexane and ionic liquid/SC-CO_2 biphasic media. The best results were obtained for the supports modified with non-functionalized alkyl chains and when the in water activity increased from 0.33 to 0.90 (e.g. the CALB/butyl-silica activity was enhanced up to five times). Immobilized derivatives coated with ionic liquids clearly improved their synthetic activity in SC-CO_2 by up to six times with respect to the hexane medium, which agrees with the "philicity" between alkyl chain lengths of both the silica support and the cation of ionic liquid (Lozano et al. 2007).

Pseudomonas cepacea lipase (PCL) was used to catalyze the trans-esterification reaction between 1-phenylethanol and vinyl acetate in SC-CO_2. The effect of SC-CO_2 pressure on the catalytic efficiency of PCL at a constant temperature was evaluated. The catalytic efficiency of enzymeenhanced by increasing pressure of the reaction medium. Moreover SC sulphur hexafluoride (SCSF6) was used as reaction medium. Results showed high stability of the enzyme in this SC medium and kinetic curve reported point out increased reaction rates in comparison to those achieved in SC-CO_2 (Celia et al. 2005).

Proteinase from *Carica papaya* latex was tested on its thermal stability at atmospheric pressure and in SC-CO_2, nearcritical propane and dimethyl-ether. In SC-CO_2 at 300 bar thermal activation of the examined proteinase was improved in the comparison to atmospheric pressure. Activity of the examined proteinase decreased in propane and dimethyl-ether (300 bar). Addition of water in the system increased activity of proteinase from *C.papaya*, which was incubated in SC-CO_2 for 24 h. Optimum amount of water was found to be between 0.5 and 0.7 g/L (Habulin et al. 2005).

Isoamyl acetate was synthesized from isoamyl alcohol in SC-CO_2 by enzymatic catalysis. Among several reactants, including acetic acid and two different acetates, acetic anhydride gave best yields. Two different immobilized lipases (Novozym 435 from *Candida antarctica* and Lipozyme RM-IM from *Rhizomucor miehei*) as biocatalysts were compared. An esterification extent of 100% was obtained in continuous operation using acetic anhydride as acyl donor and Novozym 435 as enzyme. The effect of substrates load in the solvent was investigated. Operating at a CO_2/substrates molar ratio below 7.0, the conversion of alcohol decreased, due to an inhibitory effect of high concentration of acetic anydride or acetic acid on enzyme. Pressure in the range of 8–30 MPa showed no effect on this reaction, while an increase in temperature (over 313 K) led to lower production of isoamyl acetate (Romero et al. 2005).
In another report, cocoa beans had been subjected to various pod storage periods prior to fermentation were analysed for pyrazines. SC-CO_2 extraction was used for the extraction of the compounds

and quantitative and qualitative analyses of the extracts were achieved by using gas chromatography and gas chromatography-mass spectrometry. Pyrazine compounds identified in the extract included pyrazine, 2-methylpyrazine, 2,3-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine and tetramethylpyrazine. The concentration of pyrazine compounds in the cocoa beans, in particular 2,6-dimethylpyrazine and tetramethylpyrazine were found to be largely proportional to pod storage period (Sanagi et al. 1997).

Acidic or basic species can partition into the microenvironment of the enzyme molecules and adversely affect their protonation state. In present of a dilute aqueous phase, these effects may be analyzed in terms of a pH value. At low a_W, it may be more useful to think in terms of direct reaction with protein groups (Halling 1994).

Continuous method with microbubbles of SC-CO2 was applied to enzyme inactivation in a buffer system. Alfa-amylase and acid protease were inactivated by continuous treatment with microbubbles of SC-CO2. Inactivation efficiency of this method was affected by initial pH and the buffer action of samples (Yoshimura et al. 2002a; 2002b).

SCFs: puissant media for the modification of biopolymers

The use of SCFs as media for several types of polymer modification has been demonstrated (Yalpani 1993). Treatment of mixtures of chitosan with glucose or malto-oligosaccharides in SC-CO_2 afforded the corresponding water soluble imine-linked, branched chitosan derivatives with high degrees of conversion. Treatment of starch, maltodextrins, cellulose acetate, poly(vinyl alcohol) and paper with mixtures of SC-CO_2 and O_2 (19:1 v/v) led to the corresponding oxidized materials.

Gasification of straw

Bioconversion of lignocellulosics consists of substrate pretreatment by high pressure steam (for fractionation into cellulose, hemicellulose and lignin components), enzymatic hydrolyz, followed by fermentation of the liberated sugars to ethanol. The various technoeconomic models developed by network members were used to identify probable process schemes and determine technical "bottlenecks" (Saddler 1992).

Waste treatment

Waste treatment is one of the most important and urgent problems in environmental management around the world. SC-water oxidation has attracted attention for the treatment of industrial waste, especially toxic and refractory waste. In a study, SC-water oxidation with hydrogen peroxide was applied as the oxidant to the treatment of a model municipal solid waste containing proteins, fats, vitamins, fiber, and inorganic minerals. The effects of temperature, oxidant concentration, and reaction time on the decomposition of solid waste were investigated in a batch reactor with hydrogen peroxide over the temperature range of 673-823 K. The liquid reaction products were analyzed to determine the total organic carbon, organic acid, and ammonium ion contents. The activation energy was 97.2 kJ/mol for the reduction of total organic carbon and 130.8 kJ/mol for the reduction of ammonium ion, when analyzed by first-order kinetics (Mizuno et al. 2000).

Destruction of Industrial wastes

Several SCFs have been tested successfully on waste materials on industrial nature. For example SC-water is very reactive, corrosive, and miscible with air and oxygen. An industrial process was describes the use of SC water to treat aqueous solutions containing organic compounds (Haas et al. 1989). A major pharmaceutical company has described the operation of a process based on this technology to treat waste generated from recombinant fermentation (Krishna et al.1986).

Particle formation

The rapid expansion of SCF is a promising new technology for particle formation and distribution of biodegradable polymeric (Debenedetti et al. 1993). Because of the extreme fragility of organic aerogels attempts are made to develop inorganic aerogels. Such microcellular polymers foams can be obtained directly by polymerization in a near critical diluent and SC drying in the same reactor vessel. The diluent is the key factor and Freon-22 is found to be superior to propane for the polymerization of poly(methyl metacrylate-co-ethane glycol-dimethacrylate) (Srinivasan and Elliott 1992).

In polymer industry, polymerization is stopped by adding a termination agent. The polymer solution is contacted with superheated steam to remove unreacted monomer and polymerization solvent (de solvent process). SC-CO_2 extraction can be alternative for the de-solvent process of polymer solutions. In fact SC-CO_2 can reduce the drying process due to its capability of complete recovery by depressurizing. In addition, SC-CO_2 can dissolve the typical polymerization solvents, *n*-hexane or toluene at higher pressures. The design of the de-solvent process requires quantitative information on the distribution of organic solvent between the polymer solution and the SC-CO_2 phase (Inomata et al. 1999).

Production of different morphologies of biocompatible polymers

SC antisolvent method has great potential for processing of pharmaceuticals (Mosqueira et al. 1981; Steckel et al. 1997) and labile compounds such as proteins (Debenedetti et al 1993; Winters et al. 1999; Yeo et al. 1994; Yeo et al. 1993) and to obtain various morphologies of biopolymers (Bleich et al. 1996; Debenedetti et al. 1993; Dixon and Johnstone 1993; Reverchon 1999; Subramanianet al. 1997), such as microspheres (Falk et al. 1997) threads, fibers, networks (Dixon and Johnstone 1993), sponges, foams, and films. One of the advantages of using SCF in polymer processing is the possibility of producing different solid shapes and structures at low temperature with a minimum amount of residual organic solvents. Also the process is environmentally safe and economic (Elvassore et al. 2001). A basic description of these techniques is reported in Bertucco and Pallado (2000). Also SC-CO_2 has been exploited as an antisolvent for processing value added materials e.g. hyaluronic acid-based biopolymers.

Preparation of liposome

Liposomes are non-toxic (mostly) and effective in encapsulation of materials from the environment (Mortazavi et al. 2007). Several studies have indeed shown that liposomes form a basis for controlled release food processes (Mozafari and Khosravi-Darani 2007) such as for the release of enzyme involved in cheese ripening (Kheadr et al. 2003). Liposomes can be prepared in a 50-mL autoclave equipped with a variable speed agitation device. The general protocol started with the injection of predefined quantities of the solute and phospholipids to the reactor. After heating to a specified temperature, solution was pressurized to 30.0 MPa under gentle agitation. After agitation at 100 rpm for 5 minutes, the vessel was depressurized over a period of about 45 minutes. The liposomes were finally isolated and stored at 4°C under nitrogen.

Encapsulation efficiency was independent of solute concentration between 10 and 50 mg/mL and of pressure above the critical pressure. However, significant effects were observed with respect to lipid concentration, temperature and initial volume of aqueous phase (Frederiksen et al. 1997).

Manufacturing of liposome by SCF covers three separate methods including: (i) phospholipids solvation in a near critical fluid, mixture with a protein containing buffered solution (ii) decompression of solvated phospholipids prior to injection to solution, (iii) the critical fluid decompression technique in which phospholipids are first hydrated in an aqueous buffer, mixed with SCF, with the mixture being then submitted to decompression. Several parameters can improve the characteristics of the liposomes prepared with SCF ethane. Optimization studies would be necessary to examine whether liposomes of higher quality can be made using SCF technology. Also, other SCF should be tested (Frederiksen et al. 1997).

Purification of natural active copolymers

Conventional purification methods (e.g. molecular sieving, ion exchange chromatography, etc) are not specific and must be repeated or combined for highly purification. Although, (immuno) affinity-based procedures are rapid and specific; but they are expensive, and reagents from biological origin are needed. Also the interactions involved between the product and the support are often strong and imply the use of rather denaturing reagents (either for the product or the support) to attain an efficient desorption yield (Lemay 2002). SCE has introduced as a more suitable method for purification of natural products. This technique helps to remove trace impurities in the synthetic active biocopolymers from maleic anhydride and pinene (Jarzebski and Malinowski 1995).

In situ studies of protein conformation

The conformation of monomeric enzyme trypsin has been reported in SC-CO_2 (Zagrobelny and Bright 1992). To follow in situ conformation of trypsin (as a function of CO_2 density), steady state fluorescence spectroscopy was used. Zagrobelny showed that protein denaturation can occur during the fluid compression step and that the native trypsin is only slightly more stable (1.2 kcal/mol) than the unfolded form.

Conclusion

From the results of a number of extractions reported in literature can be concluded that by SC-CO_2 application selective extraction of several compounds from aqueous media of fermentation broth is possible. Non polar compounds can be extracted at low energy costs by this procedure. The process is cost effective due to carrying out at fermentation temperature. If whole fermentation broth put in contact with SC-CO_2, may inactivate the microrganisms. The survival rate may increase by buffering the medium and carefully controlled pressure change. These results offer the opportunity of in situ extraction of fermentation products with SC or sub critical (liquid) CO_2. Use of SCF for both the disruption and extraction simplifies the procedure, and minimizes equipment and labor needs, time, contamination and loss of yield. Indeed the entire process can be readily automated. The use of super or near critical fluids allows for easy removal of much of the solvent by depressurization. The use of SCF allows the control of extraction condition by variation of temperature, pressure or modifier solvents. By varying the choice of SCF, experimental conditions and animal source material, one may obtain lipids, proteins, nucleotides, saccharids and other desirable components or remove undesirable components.

The bactericidal effect of the CO_2 treatment on baker's yeast was found to be dramatically enhanced with increasing pressure ranging from 10 to 40 at and temperature range of 20 to 40 °C and these two factors tended to synergistically act with each other.

The finding that fermentation conditions influence the resistance of microbial cells to disruption should be further investigated. Studies of disruption kinetics and of the influence of cell morphology on kinetics of disruption are needed, and not information is available on disruption of mycelial organisms. The effects of thermal deactivation on cell properties and pre-incubation temperature on cell resistance to heat shock have received less attention. Further work is therefore required to characterize this interaction and relate it to changes in cell and broth properties.

Also cell wall of microorganisms can be weakened by a physical, chemical and/or enzymatic pretreatment, or lysogenic bacteriphage; will the enzymes remain active, native and applicable for reuse. Selection of wall-deficient mutants which are triggered to lyse by an increase in temperature, shift in pH, or the like seems attractive. Application of SCF is useful for establishment of a simple, safe and inexpensive sterilization method for heat-sensitive materials.

Biocompatible biopolymers, which have been produced by SC-CO_2 antisolvent techniques, can be used in the design of controlled delivery systems.

In the near future research development of CO_2 treatment should move to retention of vitamins and the modifications of cell enzymes and biopolymers, extraction of fermentation products, cell disruption and, in general, the effect of CO_2 processing on biotechnological materials.

SC disruption of microbial cells is comparable with a mechanical method from efficiency as well as economical points of view. Alkaline shock is recommended as a powerful pretreatment for cell wall weakening, however, especial care is necessary to ensure protection of disrupted homogenate against metabolite degradation by alkaline hydrolysis. The survey presented here clearly shows that the rapid expansion of SCFs has great potentials for application in the several industries, especially for micron sized particle formation. A major disadvantage in SFE application for biomolecules is the difficulty in measurement and prediction of their solubilities in SCF at various pressures and temperatures for process optimization. Also it is difficult to make an accurate of the solubility and separation efficiency of these compounds in SCF because the composition of the equilibrium SCF phase is easily modified shifted by both temperature and pressure on sampling. Much attention should be draw to the measurement in situ at the SC state.

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