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REVIEW ARTICLE

Effects and applications of sub-lethal ultrasound, electroporation and UV radiations in bioprocessing

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Abstract Advances in bioprocess technology involving microbial cells have led to increased and improved production of beneficial new products and bioactive compounds. However, the semipermeable barrier of the cell membrane often retards the efficient productivity or reaction rate of the cells. Physical treatments such as ultrasound, electroporation and UV radiation provide an efficient approach to increase membrane permeability, leading to enhanced productivity of microbial cells. It is important to note that extensive membrane permeabilization by these physical treatments could be detrimental to cell viability leading to lower yield. An appropriate selection of sublethal dosage and intensity of these physical treatments are critical to preserve the viability of cells and at the same time maintain their bioprocess applications. Despite the promising applications of these physical treatments, safety issues related to possible genotoxicity or mutation of cells upon treatments have been raised. This genotoxic effect of physical treatments could be prevented if appropriate measures are taken, without compromising their bioprocess potentials. The current review highlights the effect of sublethal physical treatments such as ultrasound, electroporation and UV radiation on the viability of cells, their potential bioprocess applications, and the possibility of mutations.

Keywords Ultrasound · Electroporation · Ultraviolet radiation · Bioprocess

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Introduction

Bioprocess technology involving the use of microbial cells such as biocatalysis and fermentation has received great attention in recent years. Such processes lead to the production of various useful and functional products and compounds. The efficiency of these processes is often restricted by the barrier function of the cellular membrane. Ideally, for a whole-cell process, substrates should be transported into the cells without hindrance and the transformed products be easily released out of the cells (Chen 2007). Physical treatments such as ultrasound, electroporation and ultraviolet (UV) radiation have been demonstrated to serve as promising techniques for the elimination of the membrane barrier. However, alterations of the membrane induced by these treatments may impose viability inhibition.

In the past, the application of such physical treatments has been claimed to be an 'all or nothing' process, where the effect was either lethal and entirely killed the cells or non-lethal which the cells survived intact (Simpson et al. 1999). However, in recent years, promising evidence has been documented that challenges this view (Tryfona and Bustard 2008). Various studies have reported the occurrence of intermediate lethality conditions upon physical treatments, better known as sublethal effects. Sublethal injury refers to conditions where cells exhibit an extended lag time due to cellular membrane alteration, but are able to resume viability upon cessation of the external physical treatments (Berney et al. 2007).

The lethal and sublethal effects of physical treatments on microbial cells are strongly influenced by the intensity, dosage and duration applied. Under appropriate parameters, physical treatments will only cause sublethal effects on the cells. Cells have been reported to repair damage on the cytoplasmic membrane after physical treatments, and this usually occurs within a short duration of time (Hayer 2010). Such repair is vital to preserve the viability of cells and thus allow efficient bioprocesses.

Ultrasound

Ultrasound is a form of physical energy generated by sound wave frequencies above the normal range of human hearing. The mechanism of action for ultrasound is rather complex and predominantly caused by the cavitation effect (Hayer 2010). Cavitation refers to the viability, oscillation, and collapse of microbubbles in an acoustic field. Upon ultrasound, microbubbles grow in volume and reach a stage where the size of the bubbles is close to the resonant size for the applied frequency. At this stage, the bubbles oscillate nonlinearly and eventually collapse, resulting in a shock wave that produces extremely high temperatures and pressures (reaching up to 5,500 °C and 50,000 kPa) which in turn fragment water and other molecules into free radicals (Piyasena et al. 2003). These free radicals readily react with fatty acids of the membrane resulting in peroxidation and deterioration of membrane phospholipid composition and subsequently increase the membrane ratio of cholesterol: phospholipids (Lye et al. 2012; Tang et al. 2008). We have previously demonstrated that the alteration induced by ultrasound occurred at the acyl chain, polar head, and interface region of probiotic (lactobacilli and bifidobacteria) membrane phospholipid bilayers. This alteration eventually leads to the localized rupture and pore formation on the membrane lipid bilayers. These pores create a temporary 'opening', allowing transport of macromolecules across the semipermeable membrane (Hayer 2010). Such an alteration of the membrane bilayer has been reported to affect cellular functions such as nutrient transport, enzymes activities, and cell proliferation.

Viability

Ultrasound can cause inactivation of microorganisms, predominantly due to thinning/alteration of cell membranes, localized heating, and production of free radicals (Butz and Tauscher 2002). Such an effect has also been reported to be strain-dependent. Monsen et al. (2009) investigated the effect of ultrasound on several types of Gram-positive and Gram-negative bacteria (initial cell counts of 1×10^3 CFU/ml) and found that ultrasound had a stronger inhibitory effect on Gram-negative bacteria compared to Gram-positive bacteria. Gram-negative bacteria such as *E. coli* and *Haemophilus influenzae* were almost eliminated after 5 min of sonication (40 kHz; 350 W) at 35 °C in phosphate buffer saline, while Gram-positive bacteria including *Staphylococcus aureus* and *Enterococcus faecalis* were found to be resistant at the same intensity and duration of treatment. This difference was probably attributed to the fact that Gram-positive bacteria possess a thicker and more robust cell wall due to cross-linking of peptidoglycan and teichoic acid, resulted in these bacteria being less susceptible to ultrasound. In fact, ultrasound (40 kHz; 350 W; 5–10 min) has been reported to promote the viability of the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* (Monsen et al. 2009). This is due to a split of aggregates and chains by the action of ultrasound. This effect is not seen among Gram-negative bacteria, which normally do not form aggregates or chains.

The impact of ultrasound on microbial viability is also highly influenced by the intensities of ultrasound treatment. Higher intensities (>3 W/cm^2) reportedly caused disruption of microbial cells of *Aspergillus terreus*, while lower intensities (<2 W/cm^2) improved the productivity of whole cells without causing excessive damage to them (Herran et al. 2008). Scherba et al. (1991) demonstrated that a high intensity of 3 W/cm^2 significantly decreased the viability of *Pseudomonas aeruginosa* in aqueous suspension by more than 70 % compared to the initial cell counts. In another study, Pitt and Ross (2003) demonstrated that ultrasound at lower intensities of less than 2 W/cm^2 enhanced the viability of *Pseudomonas aeruginosa* which had been attached to high density polyethylene rods. Most authors attributed this to reversible membrane permeabilization formed upon treatment at lower intensities, which increases transport of substances such as nutrients into the cells that alleviate cell metabolism, and subsequently enhances their viability (Pitt and Ross 2003). Considering this, appropriate selections of intensities are crucial to maintain the viability of cells, and at the same time, enhance their viability and production of bioactive metabolites.

Bioprocess applications and benefits

Ultrasound has been widely used in various fields and commonly applied for disintegration of biological cell walls to liberate intracellular contents of cells. In recent years, the applications of ultrasound are being expanded beyond disintegration of cells to include enhancement of genetic technology without causing damage to the cells. Mehier-Humbert et al. (2004) reported that ultrasound caused transient pores on the cell membrane of rat mammary carcinoma cells (MAT B III), which allowed the delivery of therapeutically beneficial compounds such as drugs and genes into the targeted cells (Fig. 1). The cells with an initial concentration of 1×10^6 cell/ml in MacCoy 5A medium was exposed to ultrasound using a 2.25-MHz focused transducer for 10 s. The pores formed lasted for only a short duration (milliseconds to seconds) and thus could preserve the survivability of the cells. Such an advance serves as an efficient approach for genetic therapy against various diseases and genetic transformation of microbial cells.

In addition, ultrasound has been reported to promote the biocatalysis and bioprocesses of microbial cells. Wu et al. (2000) has previously demonstrated that the application of ultrasound (450 W for 6 min; 20 kHz; probe was immersed into the yogurt by approximately 2.5 cm depth from the sample surface) on starter cultures such as *Streptococcus*

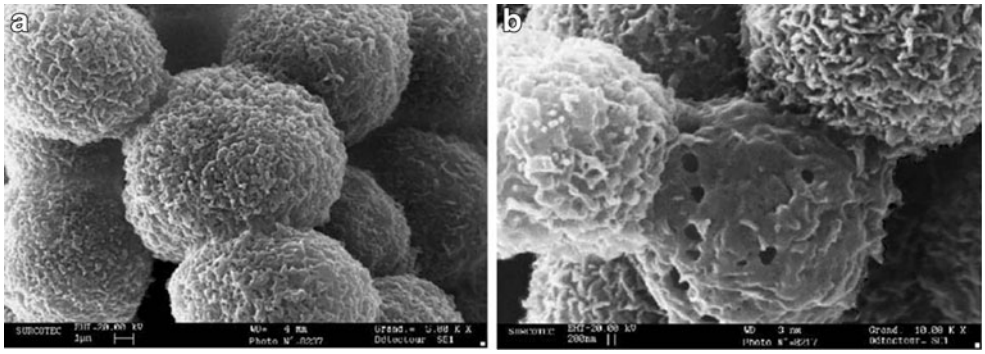

Fig. 1 SEM micrographs of cells before and after insonification MAT B III cells (1×10^6 cells/mL) were fixed with glutaraldehyde **a** before, and **b** after exposition to ultrasound using a focused transducer (225 MHz), at a peak negative pressure of 570 kPa, in the presence of UCA (25 particles/cell). Cells were observed with a scanning electron microscope after gold sputtering. Reprinted from Mehier-Humbert et al. (2004), with permission from Elsevier (License number: 2798561098496)

thermophilus, *Lactobacillus bulgaricus*, *Bifidobacterium* and *Lactobacillus acidophilus* effectively reduced the fermentation time by 0.5 h compared to unsonicated control. This was associated with the increased utilization of the substrate and the production of organic acids as metabolites in yogurt by treated starter cultures. Increased production of organic acids allows the desired pH 4.2 to be achieved in a shorter duration. In addition, the authors also reported that treatment with ultrasound significantly reduced syneresis of yogurt compared to the control. Thus, the application of ultrasound in fermentation may present a significant improvement for cost reduction in the fermentation industry.

Ultrasound has also shown promising effects in promoting the enzymatic biotransformation of microbial cells. *L. bulgaricus* treated with ultrasound (200 kHz; 17.2 kW/m^2) substantially increased the hydrolysis of lactose to produce simple sugars such as glucose in milk (Wang et al. 1995). The elimination of lactose from milk is important, particularly in the development of lactose-free dairy products for lactose-intolerant consumers. Membrane permeabilization upon treatment can increase the released of intracellular β-galactosidase from lactic acid bacteria cells into the milk medium, resulting in increased lactose hydrolysis activity. The alteration of sugar profiles in milk medium could alter the metabolism of starter culture/probiotics. Nguyen et al. (2011) has reported that ultrasound (20 kHz; 100 W for 7–30 min) stimulated the production of lactic acid and reduced the ratio of acetic:lactic acids in milk fermented by *Bifidobacterium breve* ATCC 15700, *B. infantis*, *B. animalis* subsp. *lactis* BB-12, and *B. longum* BB-46. This subsequently enhances the acceptability of fermented milk as high production of acetic acids could lead to unfavourable vinegary taste.

In addition, we have also previously demonstrated that ultrasound (20–100 W; 1–3 min) caused membrane permeabilization of lactobacilli and bifidobacteria, leading to increased release of β-glucosidase enzyme in prebiotic soymilk. Release of this enzyme subsequently increased the biotransformation of isoflavone glucosides to bioactive aglycones in prebiotic soymilk (Table 1). In general, isoflavones occur predominantly in unfermented soy products such as glucoside conjugates which are less bioavailable and bioactive. Hydrolysis of glucosides by the β-glucosidase enzyme can lead to production of aglycones that are absorbed more efficiently and rapidly by the human intestine. Aglycones have been well documented as primary isoflavones with proven health-promoting effects on humans including regulation of post-menopausal disorders, and prevention of osteoporosis, and breast and prostate cancers (Setchell et al. 2002). Thus, this shows that the application of ultrasound is useful for the enhancement of microbial biotransformation, leading to production of functional food products with enhanced bioactivity.

Ultrasound is also useful in promoting the production of bioactives by whole cells. Treatment with ultrasound at 24 kHz on *Eremothecium ashbyii* enhanced the viability and productivity of riboflavin, a water-soluble vitamin, by

Table 1 Concentrations of isoflavones in prebiotic soymilk fermented by untreated and treated lactobacilli and bifidobacteria

Prebiotic-Soymilk	Isoflavones (μg/mL)		% Bioactivity[d]
	Glucosides	Aglycones	
Unfermented	20.83	4.52	17.8
Fermented	6.65–13.70	9.44–12.62	47.9–58.7
Fermented with ultrasound-treated cells[a]	3.64–12.63	9.61–18.06	58.5–72.5
Fermented with electroporated cells[b]	4.65–13.49	9.64–22.36	62.4–67.5
Fermented with UV-treated cells[c]	6.01–13.17	10.92–16.10	55.0–64.5

[a] Cells were treated with ultrasound at 20–100 W for 1–3 min

[b] Cells were treated with electroporation at 2.5–7.5 kV/cm for 3–4 ms

[c] Cells were treated with UVA, UVB and UVC at 30–90 J/m^2

[d] % Bioactivity = (Aglycones/Total Isoflavones) × 100%

five times compared to the control groups (Chuanyun et al. 2003). The alteration of cellular membrane upon treatment with 24 kHz ultrasound had accelerated nutrient transport across cell membrane, leading to increased cell metabolism, viability and biosynthesis of riboflavin. In addition, the membrane alteration also promoted the release of riboflavin extracellularly. In agreement, Yang et al. (2010) also demonstrated that alteration of membrane integrity upon ultrasonication (20 kHz, 200 W/cm^2) significantly increased the yield of cholesterol oxidase by *Brevibacterium* sp. The authors reported that transmission electron microscopy revealed the formation of pores in the cytoplasmic region of the bacterial cells, which facilitated the excretion of the enzyme.

Mutation

Despite promising applications of ultrasound in bioprocessing, safety issues related to possible mutation of cells upon ultrasound have been raised. Continuous ultrasound treatment at 1.2 MHz has been shown to cause breakage of single-strand and double-strand DNA (Kondo et al. 1985). This detrimental effect is predominantly attributed to the impact of cavitation during ultrasound. Cavitation involves both a mechanical effect of hydrodynamic shearing stress arising from oscillation of microbubbles and a chemical effect of radical species produced by the collapse of microbubbles. Kondo et al. (1985) reported that the mechanical effect of cavitation exclusively induced the breakage of double-strand DNA while the chemical effect is accounted for the breakage of single strands.

However, under varying conditions and dosages, ultrasound can exert a complex biological and biophysical effect where mutation can be prevented. Ritenour et al. (1991) previously demonstrated that pulsed ultrasound at 1 kHz was not mutagenic, when human–hamster hybrid cells treated at such a dosage did not show an increase in the mutant fraction of the cells. In another study, Hirst (1991) also demonstrated that kHz ultrasound did not increase the mutation rate of *Salmonella typhimurium* TA102. These findings indicate that an appropriate selection of frequencies and dosage of treatment is critical to prevent the incidence of cell mutation while maintaining the beneficial applications of ultrasound.

The changes of genetic material upon ultrasound could possibly induce changes of the physiological properties of the cell in subsequent generations. Hence, the absence of mutations upon ultrasound treatment are often evaluated via changes in physiological properties of descendant cells compared to their parent cells. Lanchun et al. (2003) demonstrated that ultrasound treatment on *Saccharomyces cerevisiae* at 24 kHz and 2 W enhanced the fermentation strength and proteinase activity of parent cells. However, such an effect was not inherited by the subsequent generation of cells, indicating that ultrasound did not alter the genetic substance of the cells. Additionally, we have demonstrated that the effect of ultrasound (30 kHz; 60 W for 3 min) on the viability and biotransformation of isoflavones was solely observed in parent *Lactobacillus casei* FTDC 2113, without inheritance by subsequent passages (first, second and third passage) of the cells. In our study, the viability of ultrasound-treated parent *Lactobacillus* cells in fermented soymilk (37 °C for 24 h) increased by 5.0 % compared to the untreated control. This subsequently increased β-glucosidase activity and enhanced biotransformation of isoflavone glucosides to bioactive aglycones in soymilk fermented by parent-treated cells (2.2–44.2 % higher compared to that of control). However, this beneficial effect of ultrasound was not observed in subsequent passages of cells and thus suggests that physiological changes induced by ultrasound are reversible and do not permanently alter the genetic substances of cells. Although temporary, ultrasound serves as a beneficial technology for enhancement of bioprocessing.

Electroporation

Electroporation is a physical technique involving short pulses of strong electric fields to permeabilize the cell membrane (Prasanna and Panda 1997). The application of external electric field pulses induces a local increase of transmembrane potential difference on living cells, leading to local membrane rearrangement involving a new orientation of the phospholipid head groups. In agreement, our previous study also demonstrated that the phospholipid head group of lactobacilli and bifidobacteria was altered upon electroporation (2.5–7.5 kV/cm for 3–4 ms) as evaluated by fluorescence anisotropy. This local structural modification causes the cellular membrane to be transiently permeable and allows the transport of molecules across the membrane (Fig. 2). Molecular transport generally occurs in the period after pulse application until the resealing of the cell membrane (Berney et al. 2007; Gabriel and Teissie 1999). The resealing ability is influenced by the external electric field magnitude and duration. If these parameters exceed their optimal values, the cells would lose the ability to reseal which strongly affects their viability and physiological properties.

Viability

Various effects of electroporation on microbial viability have been previously documented. Tryfona and Bustard (2008) reported that treating *Corynebacterium glutamicum* with electroporation at 6 kV/cm and 25 μF for between 2 and 5 times for 1–3 ms led to membrane permeabilization and unchanged viability. However, the delivery of multiple pulses with a 35-min resting gap reportedly increased cell viability, as the resting gap allowed resealing and the restoration of cellular membrane integrity. Increased cell viability

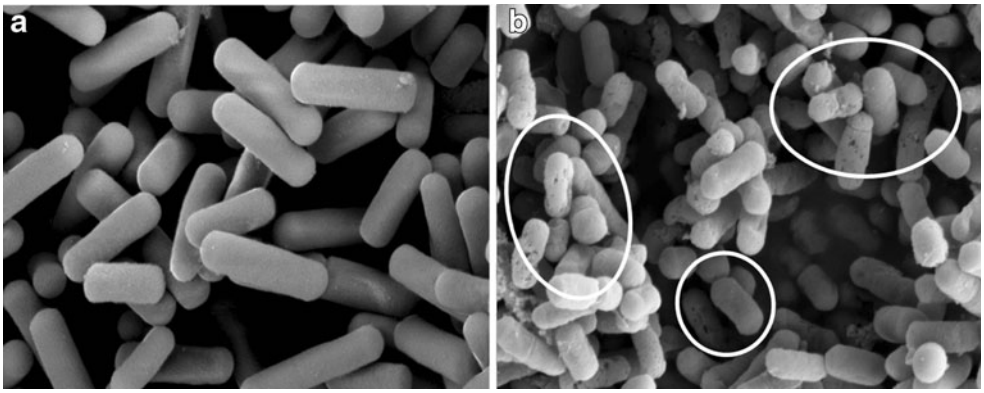

Fig. 2 Scanning electron micrographs of lactobacilli without treatment (**a**) and lactobacilli treated with electroporation at 75 kV/cm for 4 ms (**b**). *Circles* show cells with pores

was attributed to enhanced nutrient availability, where the creation of transient membrane pores facilitated nutrient uptake. In contrast, electroporation of *Lactobacillus plantarum* LA 10–11 (initial inoculum: 10^8CFU/ml) at higher intensity (25 kV/cm for 2.3 μs) caused extensive permeabilization that subsequently led to permanent loss of membrane integrity and death of cells in pH 4.5 and 6.8 phosphate buffer (Wouters et al. 2001). Extensive membrane permeabilization reportedly caused leakage of intracellular ATP that may contribute to substantial decreases in the bacteria viability (Wouters et al. 2001). Thus, it is critical that the selected intensity of electroporation does not exceed the optimal value for reversible membrane permeabilization.

The survivability of cells upon electroporation also strongly depends on the type of microorganism studied. It is widely known that different types of microorganism possess different cell envelopes and thus determine the susceptibility of the microorganism to electroporation. Garcia et al. (2007) demonstrated that Gram-positive microorganisms (*Lactobacillus plantarum* and *Listeria monocytogenes*) were capable of reversing permeabilization and survived better in citrate-phosphate buffer, while the Gram-negative (*Escherichia coli* and *Salmonella* Senftenberg 775 W) cells died upon electroporation at 9–25 kV/cm from 10 to 400 μs. This was due to irreversible damages in cellular membrane and other structures/functions of Gram-negative cells such as mitochondria (regulate cell respiration) and nucleus (DNA integrity and cell signaling).

Additionally, the effect of electroporation on microbial viability has also been associated with growth phases, as culture age strongly affects the composition of the cytoplasmic membrane. Noci et al. (2009) reported that *Listeria innocua* in the stationary phase were more resistant to the impact of electroporation (30 and 40 kV/cm, 50 μs) and retained higher viability in milk compared to cells in the logarithmic phase. In another study, the mid-exponential phase has been demonstrated to be the best viability phase for electroporation-induced permeabilization. Upon electroporation (6 kV/cm for 1 and 3 ms), *Corynebacterium glutamicum* cells at mid- and late exponential phase (48 and 72 h cultures) showed higher membrane permeability and viability compared to cells at other growth phases (Tryfona and Bustard 2008). The cytoplasmic membrane of cells at this phase has been reported to exhibit higher rigidity compared to those at other growth phases, thus allowing efficient permeabilization and post-treatment resealing.

Bioprocess applications and benefits

One of the most promising advances in bioprocessing is the production of recombinant plasmid DNA for vaccine and gene therapy (Li et al. 2008). The process for manufacturing plasmid DNA involves several steps including plasmid construction, cell transformation, cell viability, and extraction and purification of the plasmid. This process is generally tedious and involves critical breakage of cells to allow entry and recovery of plasmid DNA across the membrane. Genomic DNA is often destroyed upon vigorous breakage, while insufficient breakage would reduce the overall yield. In order to overcome this problem, electroporation offers an alternative technique that allows the process to be accomplished efficiently within minutes. This rapid technique involves the application of high voltage electric field and causes the formation of transient pores that are sufficiently large and persist long enough to facilitate the delivery of macromolecules such as plasmid DNA, RNA, or protein into the intracellular spaces (Calvin and Hanawalt 1988). In addition, electroporation (13 kV/cm) has also been shown to have efficient application for the recovery of intact plasmid from *E. coli* K-12 (Calvin and Hanawalt 1988). The simplicity and efficiency of DNA delivery by this technique could encourage successful production of stably transformed microbial cells and contribute to the advancement of genetic manipulation.

Currently, the potential application of electroporation is extended beyond genes transfection to include enhancement of biotransformation and production of functional components by living cells. In general, biotransformation by living cells is often impeded by the membrane that acts as a barrier for efficient transport of substrate and enzymes into and out of the cells. We have previously demonstrated that

electroporation (2.5–7.5 kV/cm; 3–4 ms) efficiently eliminated the membrane barrier of lactobacilli and bifidobacteria, thus allowing transport of substrate (isoflavone glucosides) and enzyme (β-glucosidase) across the membrane. This subsequently led to enhanced biotransformation of isoflavone glucosides to bioactive aglycones in prebiotic soymilk (Table 1). In agreement, Loghavi et al. (2007) also reported that treatment with a moderate electric field (1 V/cm, 60 Hz, for 40 h) created temporary pores on the membrane of *Lactobacillus acidophilus* which promoted the production of bioactive bacteriocins (lacidin A) in culture broth. These temporary pores increased the diffusive permeability of bacteriocins across the cell membrane and, subsequently, bacteriocin activity.

Additionally, electroporation has also enhanced the yield of important microbial enzymes. Considering the selective permeability of membrane, it is often difficult to secrete macromolecules such as enzymes out of the cells, and thus lower yields are obtained. Electroporation serves as an efficient technique for altering membrane permeability and allowing easy recovery of enzymes. Shiina et al. (2004) reported that electroporation (12 kV, 2 Hz) for 30 min promoted the extracellular release of α-amylase by *E. coli* (initial inoculums OD_{660} 0.7) into the culture broth (L-broth). This enzyme is of great significance in bioprocessing with wide applications ranging from food fermentation to the textile, pulp and paper industries (Gupta et al. 2003). In another study, Ohshima et al. (1995) demonstrated that the application of a pulsed electric field at <10 kV/cm on beer *Saccharomyces cerevisiae* allowed the rapid release of invertase and alcohol dehydrogenase without destruction of the cells. Both these enzymes are essential in the beer brewing industry where they play an essential role for the enhancement of ethanol yield from molasses/dextrose (Bajaj and Sharma 2010). In the same study, Ohshima et al. (1995) reported that the intracellular enzymes were selectively released from the cells by controlling the applied pulse field strength. Invertase activity was increased when treated at 6 kV/cm, and ADH activity was increased at 10 kV/cm. The difference of the releasing properties is mainly due to varying pore sizes induced by pulse electric strength where high voltage forms large pores while low voltage forms smaller pores. Generally, invertase exists around the cell membrane while ADH is in a cytoplasm near the center of the cell; thus, invertase requires smaller pores to be released from the cells. This finding suggests that electroporation has the great advantage of inducing a selective release of particular materials such as the target protein.

Mutation

Considering the vast applications of electroporation on living cells, the risks of mutation are constantly being investigated, so that the detrimental effects of electroporation do not outweigh its benefits. Past studies have reported that electroporation could possibly induce genotoxic effects. Genotoxicity or mutation of cells can occur upon electroporation by direct damage to the chromosomes or DNA, and this has been widely investigated on human cell lines. Delimaris et al. (2006) reported that exposure of single cells (human lymphocytes) to electroporation at 400 kV/cm significantly increased the amount of damaged DNA compared to the untreated control. In agreement, Stacey et al. (2003) also reported that application of electroporation at high electric field strength (300 kV/cm) induced DNA damage on human cell lines.

However, such genotoxic effects can be prevented by proper selection of electric field strengths. Electroporation at lower electric field strengths (≤100 kV/cm) has been shown to be safe and pose no risk of genotoxicity. Gusbeth et al. (2009) reported that application of electroporation at a strength of 100 kV/cm on *Pseudomonas putida* showed no visible changes in variable intergenic spacer region of their genome structure. In addition, the phenotypic characteristics of the bacteria cells were also unaltered in their descendant cells upon repetitive treatment. We have also demonstrated that electroporation (7.5 kV/cm; 3.5 ms) solely promoted the viability and bioactivity of parent *B. longum* FTDC 8643 cells, without inheritance by the descendant cells. Upon electroporation, the viability of parent cells increased by 5.4 % compared to that of the untreated control. This treatment also promoted the production of bioactive aglycones by the parent generation of cells in prebiotic soymilk (4.1–34.2 % higher concentration compared to the control). However, this effect was not inherited by their descendant cells. Considering that the descendant cells do not inherit the physiological changes induced by electroporation, it appears that there is no genetic alteration of cells upon electroporation when an appropriate pulsed electric field strength is applied to *Bifidobacterium*.

Ultraviolet

Ultraviolet radiation is a type of electromagnetic energy that occupies a wide band of wavelength in the non-ionising region of the electromagnetic spectrum. It can be subdivided into three spectra with different regions including UVA with wavelength at 320–400 nm, UVB at 280–320 nm, and UVC at 200–280 nm (Bintsis et al. 2000). UV radiation mediates its biological effects on bacteria predominantly via the reaction of reactive oxygen species on the cellular membrane (Smith et al. 2009), leading to the deterioration of the membrane lipid, rearrangement of the phospholipid bilayer, and pore formation. This is further supported by our study where UV radiation (UVA, UVB, and UVC; 30–90 J/m^2) induced membrane lipid peroxidation and permeability of

lactobacilli and bifidobacteria. Such alterations may increase mass transfer across the cellular membrane. However, the effect is transient upon treatment at sublethal doses, where cells undergo reacylation to repair the alteration of the cellular membrane (Sakanashi et al. 1988) (Fig. 3).

Although membrane lipid has been reported as the most prominent site of alteration by physical treatment such as UV radiation, limited study has focused on their effects on cellular membrane fatty acid composition. To our knowledge, one of the most detailed studies was conducted by Smith et al. (2009) who examined the changes of lipid bilayer upon short wavelength UV-induced oxidative stress (14 J/s) by using neutron reflectrometry. The authors demonstrated that unsaturated fatty acids deteriorate at a faster rate compared to saturated fatty acids upon UV-induced oxidative stress. This was due to the lower packing density of unsaturated fatty acids, which accelerates the diffusion rate of reactive oxygen species within the membrane. The presence of double bonds also makes unsaturated fatty acids more susceptible to oxidative attack than saturated lipids. In our previous study, we also demonstrated that UV radiation caused deterioration to the unsaturated lipid on the membrane phospholipid tail. The loss of the unsaturated lipid causes membrane reorganization with subsequent increases in the packing order of the membrane hydrophobic tails, thus increasing membrane rigidity.

Viability

Different types of UV radiation and different doses impose varying effects on the viability of microorganisms. Generally, bacteria cells exposed to UV radiation (regardless of the type of UV radiation) at low doses are subjected to sublethal injuries where the cells exhibit a transient reduction of specific growth rates prior to resuming their growth (Berney et al. 2007). At a higher dose, UV radiation often causes lethality to bacterial cells due to extensive protein oxidation. Such effects may contribute to the inhibition of key cellular enzymes, leading to cellular dysfunction, DNA damage, and, eventually, cell death (Hoerter et al. 2005).

Different types of UV exert different mechanisms of actions on living cells. UVC has been shown to exert greater lethality than UVB and UVA (Bintsis et al. 2000). UVC exerts a strong impact on cells by penetrating through the membrane, leading to extensive membrane alterations and

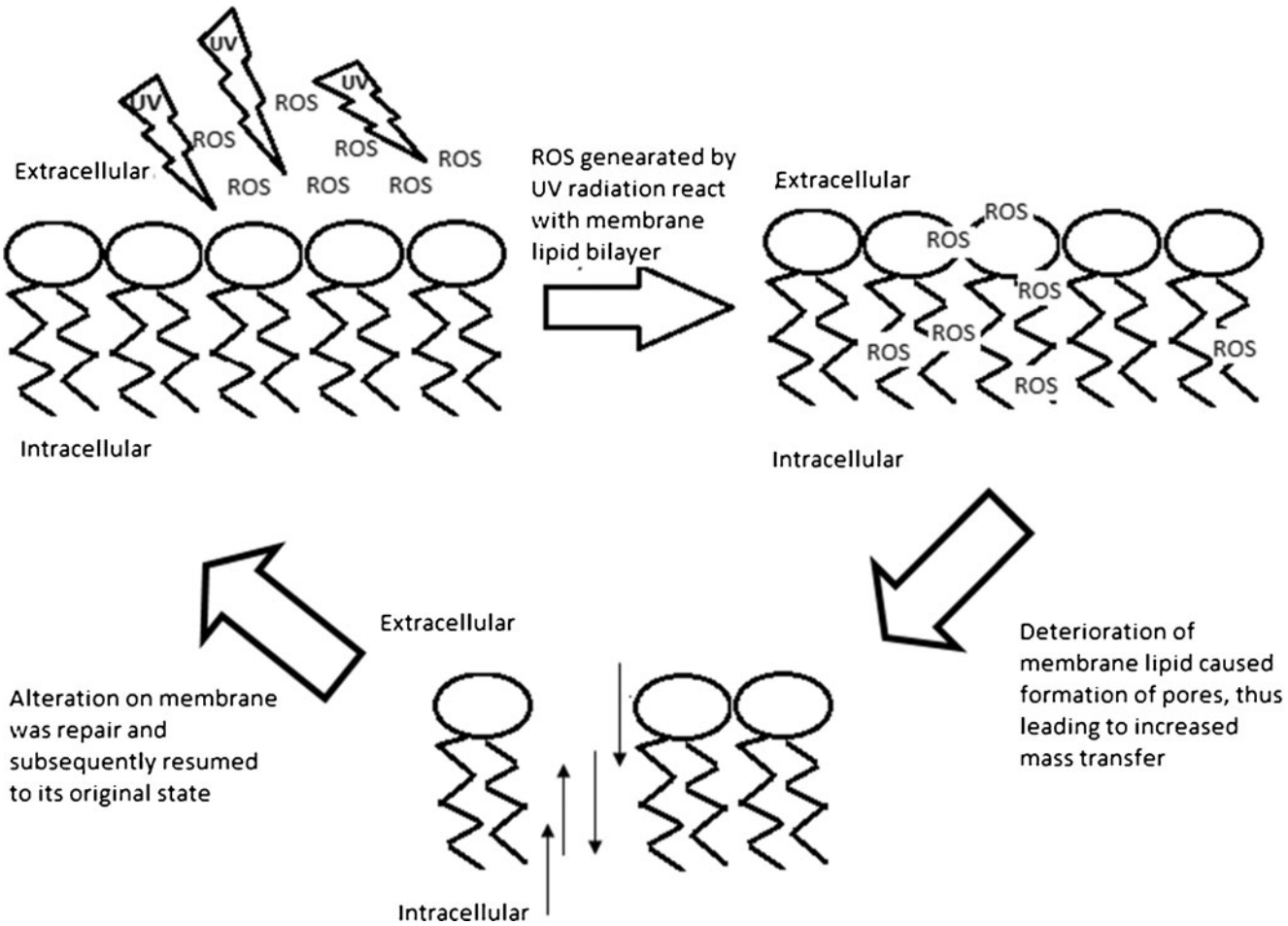

Fig. 3 Mechanism and effect of UV radiation on the cell membrane of living cells

DNA damage. In addition, cells treated with UVC at high doses also show metabolic imbalance and, eventually, cell death (Bintsis et al. 2000). Therefore, UVC has been widely used for sterilization purposes, including disinfection of drinking water, wastewater, and air due to its lethality effect on most cells. A maximum effect has been reported for UVC at 254 nm (Miiller-Niklas et al. 1995), and at a dose exceeding 80 J/m^2 (Rochette et al. 2006).

The lethality of UVC radiation is often reduced by reducing the doses of UV radiation. Radiation at a lower or sublethal dose (<4 J/m^2) does not compromise the viability of cells, owing to sustained metabolic activities. Villarino et al. (2003) has previously demonstrated that *E. coli* treated with UVC (254 nm) at the low dose of 4 J/m^2 remained in a metabolically active state, retaining a high level of glucose transport and capacity to metabolize glucose at a similar rate to those of untreated cells.

Similar to UVC, middle-wavelength UV (UVB) radiation may also exert detrimental biological impacts on living cells via direct induction of protein damage, leading to lethality of cells. However, the penetration ability and protein damaging effect of UVB is less than that of short-wavelength UV (UVC) radiation (Horikawa-Miura et al. 2007). Therefore, the lethality of the UVB irradiation occurs at a much higher dose, exceeding 150 J/m^2, which is twice that of UVC (Kang et al. 2007). Cell damage was observed and the viability of living cells was extensively decreased upon irradiation with UVB at this lethal dose (150 J/m^2). On the other hand, when living cells were irradiated with UVB at a sublethal dose (50–100 J/m^2), the damage to the cells was minimal, with viability exceeding 90 % (Kang et al. 2007).

The bactericidal property of UVA is less prevalent where a lethal effect is observed at a much higher dose. Sublethal effects in bacteria such as *E. coli* reportedly occurred at doses less than 100×10^3 J/m^2 at 366 nm (UVA) (Jagger 1981), while a higher dose of 135×10^3 J/m^2 caused cellular dysfunction and death of bacteria cells (Hoerter et al. 2005). The survivability after UV radiation was probably due to the ability of bacteria to repair the injury on the membrane and eventually resume viability after exposure to UVA radiation at a sublethal dose. Kramer and Ames (1987) investigated the effect of UVA on *Salmonella typhimurium* and reported that cells exposed to UVA radiation at 35 J/s.m^2 for 15 min can recover and resume growth.

Bioprocessing applications and benefits

Past studies have reported that UV radiation promoted bioprocess applications by permeabilization of the cellular membrane and enhanced mass transfer across the cellular membrane without causing cell death. UVC radiation (253.7 nm; 4.7 J/s.m^2) has been reported to increase permeability of ions across the membrane of *Chara corallina* (Doughty and Hope 1973), attributed to the alteration of membrane properties such as the depolarization of the membrane potential and decreased membrane resistance. The effect of the UV radiation was highly reversible and membrane properties resumed their original state within 40 min after cessation of the UV radiation. In another study, Shimizu and Sekiguchi (1979) reported that UVC radiation (214 cm distance from the bulbs; 0.06 J/s.m^2) effectively permeabilized the membrane of *E. coli* N212 (4×10^7 cells/ml in culture broth), thus allowing the transport of T4 endonuclease V (molecular weight=18,000 g/mol) across the membrane while retaining its colony-forming ability.

In addition to membrane permeabilization of cells, which allows efficient mass transfer, UV radiation also plays an important role in promoting enzymic activity and production of bioactive compounds by living cells. Hung and Liao (1996) investigated the effect of UV radiation on organophosphate hydrolase activity of *E. coli* JCL 1194, JCL 1095, andf JCL 1096 (10^8 cells/mL) in potassium phosphate buffer and found that UV radiation (UVC; 121 mN/mm^2 for 220 s) increased the specific activity of these strains by at least two-fold compared to the untreated control. Such enzymic activity is beneficial for the degradation or detoxification of organophosphate, a compound that causes acute neurotoxicity. The authors suggested that the increased specific enzyme activity was most likely due to the increased membrane permeability that enhances transport of substrate into the cells.

In another study, Petrea (2008) demonstrated that UV radiation (254 nm for 20–50 s) improved the ethanol production of *S. cerevisiae* (1.8×10^2 cells/mL) in yeast-peptone-glucose medium by a rate of 1.15 % compared to the untreated control. Similarly, Zarif et al. (2011) also reported that treatment of *S. cerevisiae* (1.3×10^8 cells/ml plated on yeast extract peptone dextrose agar) with UV radiation (280 nm; 290 s; 20 cm from the 30-W UV bulb) increased the bioethanol production by 36.7 % compared to the native strains. In the study, UV radiation was applied to induce mutation and produce superior strains with enhanced bioethanol yield. These indicate the possibility of using such technology for enhanced production of biofuel and high-alcohol beverages.

In addition, we have also demonstrated that UV radiation (UVA, UVB, and UVC; 15 cm from the bulb; 30–90 J/m^2) significantly enhanced the intracellular and extracellular β-glucosidase activity of lactobacilli and bifidobacteria. The increased enzymic activity subsequently increased the bioconversion of isoflavone glucosides to bioactive aglycones in prebiotic soymilk (Table 1). Thus, this suggests that UV radiation could be used for the production of functional beverages with enhanced bioactivity.

Mutation

It has been suggested that the altered bioactive properties of UV-radiated microorganisms could be due to mutation or DNA

Table 2 Application of ultrasound, electroporation, and ultraviolet radiation in bioprocessing

Parameter	Ultrasound	References	Electroporation	References	Ultraviolet	References
Main application	Recovery of intracellular proteins and ompounds	Chisti and Moo-Young 1986	Gene transfection and extraction of DNA plasmid	Calvin and Hanawalt 1988	Biotransformation and production of bioactive compounds	Petrea 2008; Zarif et al. 2011
	Fermentation Industry	Wu et al. 2000	Biotransformation and production of bioactive compounds	Loghavi et al. 2007	Yield of enzymes	Hung and Liao 1996
	Increased lactose hydrolysis	Wang et al. 1995	Yield of enzymes	Shiina et al. 2004; Ohshima et al. 1995		
	Increased biotransformation of yield of bioactive compound and/or enzymes	Chuanyun et al. 2003; Yang et al. 2010				
Effective range[a]	<2 W/cm^2	Herran et al. 2008	2–6 kV/cm; 1–3 ms	Tryfona and Bustard 2008	UVC 4 J/m^2	Villarino et al. 2003
					UVB 50–100 J/m^2	Kang et al. 2007
					UVA <10×10^4 J/m^2	Jagger 1981
Factors Affecting Effectiveness of the Treatments	Type of bacteria: Gram-positive bacteria are more resistant to the treatment compared to Gram-negative bacteria	Monsen et al. 2009	Presence of resting gap between each pulses of treatment	Tryfona and Bustard 2008	Presence of visible/ blue light for photoreactivation	Takahashi et al. 2005; Armstrong and Kunz 1992; You et al. 2001
			Type of bacteria: Gram-positive bacteria are more resistant to the treatment compared to Gram-negative bacteria	Garcia et al. 2007		
			Culture Age: mid, exponential or stationary phases are less susceptible to inactivation	Noci et al. 2009; Tryfona and Bustard 2008		

[a] Effective range represents the processing conditions that do not cause significant inactivation of cells

alteration induced by UV. UV radiation, especially with UVB and UVC, generated DNA photoproducts such as cyclobutane pyrimidine dimers and 6–4 pyrimidine-pyrimidone that play primary roles in the genotoxic effect of UV. Takahashi et al. (2005) previously demonstrated that the number of cyclobutane pyrimidine dimers in genomic DNA of *Paramecium tetraurelia* significantly increased upon exposure to UVB and UVC. Similarly, UV radiation (UVC; 200 J/m^2) on *E. coli* also significantly induced the formation of cyclobutane pyrimidine dimers and 6–4 pyrimidine-pyrimidone photoproducts in lacI and lacZ genes of the cells (58). This UV-induced premutagenic lesion of DNA could possibly lead to lethality and mutations after replication, if left unrepaired (Friedberg et al. 2006).

The premutagenic lesion of DNA or production of DNA photoproducts can occur at both high/lethal and low/sublethal doses of UV radiation (Witkin 1969). The DNA damage induced by high doses of UV radiation is often irreversible and lethal as the irradiated cells are rapidly overwhelmed by photons generated by the UV radiation (Beggs 2002). On the other hand, DNA damage induced by sublethal UV radiation is reversible and can be efficiently repaired via a pathway known as photoreactivation. Photoreactivation is a direct reversal mechanism for UV-induced DNA-damage, catalyzed by photolyase, which uses visible light as its sole energy source. The DNA photolyases are widely found in plants, animalss and microorganisms including bacteria, Archaea, and yeast (Lin 2002). Experimental evidence has strongly proved that photoreactivation has been observed in both eukaryotic and prokaryotic organisms, and efficiently prevented UV mutagenesis in a broad range of species (Lucas-Lledo and Lynch 2009). Past studies have reported that photoreactivation using visible lamps reduced DNA photoproducts of *Paramecium tetraurelia* to undetectable limits despite irradiation with UVC (150 J/m^2) for 6 h (Takahashi et al. 2005). In another study, exposure of *S. cerevisiae* to photoreactivating light for 30 min was reported to reduce the frequencies of both UVB (9,765 J/m^2) and UVC (60 J/m^2) mutagenesis by approximately 75 % compared to cells without photoreactivation (Armstrong and Kunz 1992). In addition to microbial cells, photoreactivation has also been found effective in mammalian cells. You et al. (2001) demonstrated that photoreactivation of photolyase-expressing mouse embryonic fibroblast cells resulted in almost complete repair of cyclobutane pyrimidine dimers and 6–4 pyrimidine-pyrimidone within 3 h after UVB irradiation at 500 J/m^2. In addition, such a process also promoted the survival and propagation of UV-treated cells. Wade and Trosko (1983) demonstrated that photoreactivation with 40-W fluorescent lights enhanced the colony-forming ability of exponentially growing UVC-irradiated (15.6 J/m^2) rat kangaroo cells. Thus, although UV radiation could exert genotoxicity and lethality at higher doses, damage occurring from sublethal doses can be prevented and/or repaired via intrinsic cellular DNA repair systems.

Conclusions

Physical treatments including ultrasound, electroporation, and UV radiation have shown promising effects in promoting the viability of living cells, mainly due to the reversible impact of sublethal physical treatments on the cellular membrane. Considering that mechanisms of action of the physical treatments mainly involve changes in the cellular membrane, future studies should concentrate on the effect of such treatments on the composition of membrane fatty acids.

The alteration of membrane permeabilization by physical treatment is indeed beneficial for potential bioprocess applications. However, the lack of information in the public domain about selectivity and specificity of the effect of physical treatments is of concern, and further research in this area is warranted. Despite their beneficial applications, potential side effects of physical treatments such as mutation and DNA damage should not be underestimated. Mutation and DNA damage could possibly occur when excessively high treatment doses are applied on the cells. Thus, these treatments should be applied under appropriate process controls to attain the optimum beneficial effects. In conclusion, sublethal physical treatments could serve as a new technology for the advancement of bioprocessing, while preserving cellular survivability and DNA integrity when treatments are conducted under appropriate parameters and conditions (Table 2).

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