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Minireview

Mechanisms of Actions of Sodium Hypochlorite

in Cleaning and Disinfection Processes

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Sodium hypochlorite (NaOCI) is the most widely used disinfectant in the food industry de-spite the increasing availability of other disinfectants. Sodium hypochlorite fulfills many re-quirements as the ideal disinfectant and furthermore it has an excellent cleaning action. The effectiveness of sodium hypochlorite in the cleaning and disinfection processes depends on the concentration of available chlorine and the pH of the solution. Hypochlorous acid (HOCI) is a weak acid and dissociates to the hypochlorite ion (-OCI) and proton (H+) depending on the solution pH. It is generally believed that HOCI is the active species in the germicidal ac-tion, whereas the concentration of -OCI is a key factor determining the cleaning efficiency. This implies that the optimal pH region of the germicidal activity of sodium hypochlorite dif-fers from that of its cleaning activity. This paper describes the theory and practice of the cleaning and disinfecting operations based on the use of sodium hypochlorite solution.

Key words:Sodium hypochiorite/Cleaning action/Germicidal activity/Strong electrophile/
Electrolyzed water.

INTRODUCTION

Nowadays, cleaning and disinfecting operations
have been reconfirmed as the fundamental proce-
dures to sustain hygienic conditions in the food indus-
try setting. The main objects to be cleaned and disinfected are the processing equipment, products or food ingredients, packaging materials, and worker's hands and fingers. The cleanliness of all the product-contact surfaces is one of the most important factors to prevent secondary contamination and to produce safe and wholesome products. Cleaning and disinfection are related complementarily to each other, and are performed as a separate or combined operation. The process of cleaning should precede that of disinfection because it is far easier to disinfect

a surface unsoiled with organic matter than a soiled surface. In a word, the role of cleaning as a process

preceding disinfection is to reduce the load of the disinfection process by removing as much soil as possible and by decreasing the number of microbes before they propagate.
At present, sodium hypochiorite (NaOCI) is the most widely used disinfectant in the food industry. Although alcohol, quaternary ammonium compounds, ozone, peracetic acid, hydrogen peroxide, and or-ganic acids are also utilized to reduce microbial populations, the effectiveness of those chemicals has been always examined in comparison with that of so-dium hypochiorite. Some advantages and disadvan-tages of sodium hypochiorite are summarized in Table 1. The advantages of sodium hypochlorite are that it fulfills many requirements of the ideal disinfec-tant (Rutala and Weber, 1997) and has an excellent cleaning action; furthermore, it is easily combined with some other cleaning elements and detergents. Actually in many food-manufacturing factories, the re-moval of the organic soils (cleaning) and control of microbes (disinfection) are performed with sodium hypochlorite in a single process. However, the

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148S.FUKUZAKl

TABLE 1. Characteristics of sodium hypochlorite solution as the ideal disinfectant.

mechanisms of sodium hypochlorite actions in clean-ing and disinfection processes are originally different from each other. lt is dangerous to misunderstand the action of sodium hypochiorite or to use it inappropri-ately because the effectiveness of its performance can be overestimated. To perform cleaning and disin-fecting operations effectively, it is necessary to un-derstand both the chemical properties of sodium hypochlorite and the mechanisms by which soils are removed and microbes are killed.
l n this article, l intend to review the mechanisms of the actions of hypochlorous acid, the hypochlorite ion, and hydroxide ion, which are the principal ingre-dients of a sodium hypochiorite solution, in cleaning and disinfecting operations. ln addition, the basic properties of electrolyzed water and weak acidic hypochlorite solution (diluted solution compounded with sodium hypochlorite and acid solution) and their applications in the disinfection process are also de-scribed.

BASlC PROPERTlES

l n industrial plants, sodium hypochlorite is manu-factured by the absorption of gaseous chlorine in so-dium hydroxide solution (eq. 1):

(1)

The principal ingredients of aconcentrated sodium hypochlorite solution are hypochlorite and sodium

hydroxide. Most common commercial products of so-
dium hypochiorite are strong base solutions (pH:
12.5 to 13.5) containing 5 to 12% of available chlo-
rine (AC). NaCl formed in eq. 1 is eliminated ade-quately from the NaOCl product. lt is desirable to store sodium hypochiorite solution under dark and cool conditions because it is unstable when exposed to ultraviolet irradiation.

Oxidizing reaction
Both hypochiorous acid (HOCl) and the hypochlorite ion (-OCl) are strong oxidizing agents. HOCl and -OCl have been reported to react with a wide variety of biological molecules such as proteins (Hawkins and Davies, 1998 and 1999; Hazell et al., 1993 and 1994), amino acids (Nightingale et al., 2000), peptides (Heinecke et al., 1993), lipids (Spickett et al., 2000), and DNA (Prutz, 1998) at

physiological pH conditions. The CI atom in HOCl and -OCl behaves as Cl+, a strong electrophile, and com-
bines with a pair of electrons in parts of the substrate that have high electron densities (Wojtowicz, 1979). Among biological molecules, the C=C double bond, peptide bond (amide bond) , amino groups, and thiol groups are susceptible to the electrophilic attack of Cl+ (Nightingale et al., 2000; Pereira et al., 1973; Winterbourn and Brennan, 1997). The CI atom is re-duced to the chloride ion (Cl-) as it accepts two elec-trons. The standard electrode potentials for the reduction of HOCI and -OCl are given below (Weast, 1988):

(2)

(3)

Dissociation and equilibrium in aqueous solution
Hypochlorous acid (HOCl) is a weak acid (pKa

=7.5, 25•Ž) in aqueous solution (Morris, 1966a) and

it dissociates readily to -OCl and H+ depending on
pH (eq. 4). Figure 1 shows the variation in the per-
centage of HOCl as a function of pH. In general, the
pH of sodium hypochlorite solutions diluted to com-
monly used concentrations at 50 to 200 mg AC/L
ranged between 8.5 to10. ln this pH region, most of
the chlorine exists as -OCl. As the pH of sodium
hypochlorite solution decreases to the weak acidic
pH region between 4 and 6, HOCI becomes the pre-
dominant species. When the pH is further lowered to
less than 4, HOCl is gradually converted to Cl2 (eq.
5). The shift in the equilibrium of eq. 5 to the right is
affected not only by H+ concentration but also by Cl-
concentration. The pHs at which Cl2 begins to

CLEANlNG AND DlSlNFECTlON USlNG SODlUM HYPOCLORlTE 149

FIG. 1. Distribution of HOCl in aqueous solution as a function of pH. Curve for HOCI--OCl equilibrium was derived from the calculation by using pKa of 7.5. Curves
A and B are the hypothetical curves in the absence and presence of additional 100 mM NaCl, respectively.

generate are approximately 2.8 and 4.5 in the ab-sence and presence of additional 100 mM NaCl, re-spectively (lwasawa et al., 2004), as depicted by curves A and B in Fig. 1.

hypochiorite solution depends on the concentration of HOCI but not on the total AC concentration (Brazis et al., 1958, Charlton and Levine, 1935; Dantec, et al., 2002; Sagripanti and Bonifacino, 1996). This is attrib-uted to the penetration of HOCl into the microbial cell across the cell wall and membrane. It is believed that

the mechanism of the germicidal activity of HOCl or -OCl is due to the inhibition of enzyme activity essen-
tial for the growth, damage to the membrane and DNA, and perhaps an injury to membrane transport capacity although it has not been fully elucidated. HOCl stress is also suggested to generate common deleterious oxidative species which can damage cel-lular components (Dunkan and Touati, 1996; Dukan et al., 1999). On the other hand, the germicidal activ-
ity of a concentrated sodium hypochiorite solution is
based on its high pH (-OH action) and -OCl oxida-
tion (Estrela et al., 2002).

Membrane permeability and germicidal activity

A model for the germicidal actions of HOCl and -OCl based on their ability to penetrate into the mi-

(4)

(5)

Thus, in aqueous solution chlorine exists in three different forms, i.e., C12, HOCI, and -0C1, which are called free available chlorine or free residual chlorine.

The solubility of Cl2 (aq) in water is very low and therefore Cl2 (aq) readily escapes from the solution. Also, in the neutral pH region (6.5 to 7.5), undissociated HOCl tends to be decomposed at a relatively high rate (Adam et al., 1992) and its con-centration decreases gradually during the storage pe-riod. On the other hand, dissociated -OCl is more stable in alkaline water, especially at pHs above 10, and AC concentration is almost constant even after a

6-month storage (Siqueira, 2000).
Once free chlorine species reacts with ammonia or N-organo compounds to form monochloramine, dichioramine, and trichioramine or a variety of organo-N-chloro compounds, the term used is either combined chlorine, combined residual chlorine, or combined available chlorine. The free and combined

available chlorine in water are collectively described as total residual (available) chlorine.

MECHANlSM OF THE GERMlCIDAL ACTlON

Sodium hypochlorite has a broad antimicrobial spectrum. The germicidal activity of a diluted sodium

FlG. 2. A model illustrating the mechanisms of the ger-micidal actions of HOCl and -OCl based on their ability to penetrate the membrane into the microbial cell. l onized -OCl has a poor germicidal activity because of its inability to diffuse through microbial l plasma mem-brane, and it exerts an oxidizing action only from outside of the cell (circle A). HOCl can penetrate the lipid bilayer in the plasma membrane by passive diffusion due to its electrical neutrality. HOCl can attack the microbial cell both from the outside (circles A') and inside the cell (circles B and C), which is responsible for the potent germicidal activity of HOCl.

150S.FUKUZAKl

crobial cell is shown in Fig. 2. This model explains that the germicidal activity is governed by the abilities of HOCl and -OCl to diffuse through the microbial cell membrane. Basically, ionized -OCl is not able to penetrate the microbial cell membrane because of the existence of the lipid bilayer, a hydrophobic layer, of the plasma membrane. Occasionally, some structures

of the microbial cell wall also protect the cell from -OCl
penetration. Mycobacteria and corynebacteria possess a peculiar cell wall structure in which the peptidoglycan is covalently linked to mycolic acids, consisting of long fatty acids up to 90 carbon atoms. The mycolic acids represent a hydrophobic barrier to

-OCl entry. Therefore, -OCl exerts an oxidizing ac-
tion only form outside the cell (circle A). ln the firt step of disinfection by -OCl, the rupture or disintegra-tion of microbial cell wall and membrane appears to occur, and then -OCl would inactivate functional pro-teins localized in the plasma membrane. This is re-sponsible for the poor germicidal activity of -OCl. On the other hand, HOCl can penetrate the lipid bilayer of the plasma membrane by passive diffusion. Apparently, the germicidal activity of sodium hypochlorite is a direct function of the HOCl concen-tration. The penetration of HOCl is due to its electrical neutrality and to its modest molecular size being comparable to that of water. This is true of neutral and small molecules such as H2O2. As a result, HOCl can attack the microbial cell not only from outside the cell (circle A') but also from inside the cell (circles B and C), thereby accelerating the inactivation rate and enhancing the germicidal activity.
Studies on the inactivation of microorganisms by disinfectants indicate that the disinfectant concentra-

tion and contact time are the two key variables deter-mining the efficiency of disinfection. The most commonly used model has been derived from the modified Chick-Watson law (Chick, 1908; Watson, 1908):

(6)

where No is the initial number of microorganisms, N is
the number of surviving microorganisms at the con-
tact time T, C is the disinfectant concentration, and k
is the lethality coefficient (inactivation rate constant)
of the microorganism. lt has been generally observed
that the inactivation of microorganisms follows first-
order kinetics with regard to the C•~T value.
Therefore, the C•~T value is commonly used as a unit
of germicidal activity that will achieve, for example,
99 to 99.99% inactivation of the microorganisms. lt
has been reported that based on a C•~T concept,
HOCl is 80 times more effective a disinfectant to
Escherichla coil (Morris, 1966b) and 40 times more

effective a disinfectant to Pseudomonas spp.-
dominating heterotrophic bacteria than -OCl (Le-
Chevallier et al., 1988). ln general, vegetative
bacteria are easily inactivated by disinfectants includ-
ing chlorine, but bacterial spores are highly resistant
to chemical disinfectants (Russell, 1990). lt is likely
that the penetration of disinfectant into the spores
may be a major rare-limiting factor, causing a large
difference between the disinfection efficiencies of
HOCl and -OCl. lt has been reported that HOCl is ap-
proximately 100 times (Brazis, 1958) and 1,000
times (Nebel, 1981) more germicidal to Bacillus spp.
spores than -OCl. Penetration of HOCl/-OCl is also
attained in the presence of methanol and other
alcohols, resulting in powerful sporicidal activity
(Coates and Death, 1978; Death and Coates, 1979).
l n addition, chlorine disinfection is more efficient at
higher temperatures. Studies on the effect of tem-
perature on the inactivation by sodium hypochlorite
have shown that the rate constant for the inactivation
of Bacillus subtilis spores has an Arrhenius-type tem-
perature dependence (Dantec, et al., 2002; Page,
2003; Sagripanti and Bonifacino, 1996).
The behavior of HOCl, a weak acid, in membrane
penetration is also the same as those of
undissociated organic acids used as antimicrobial
acidulous agents to preserve foods. The antimicrobial
action of organic acids is due not only to pH reduc-
tion in the environment but also to the entry of organic
acids in undissociated forms into the cell, causing a
drop of intracellular pH by the dissociation of protons
from the acid (Fukuzaki et al., 1990). The excess
protons have to be extruded out from the cell to main-
tain a functional proton gradient (•¢pH). The extru-
sion of protons by the ATPase would require the
hydrolysis of ATP, meaning a decrease in ATP avail-
ability for the growth and metabolism of the organ-
isms. Consequently, organic acids might act as
uncouplers of the membrane proton gradient and
hence of oxidative phosphorylation (Herrero et al.,
1985). ln the case of HOCl, however, the principale
germicidal activity is attributed to the oxidizing action
of HOCl rather than to the reduction of the •¢pH or
ATP availability of the cell.

HOCl-induced damage
Molecules that have highly nucleophilic sites are
supposed to react rapidly with HOCl (or -OCl).
Among the cellular components, these include
porphyrins and hemes, ferredoxin-like iron-sulfur cen-
ters, purine and pyrimidine bases, conjugated
polyenes, amines, amino acids, and sulfhydryl groups
(Albrich et al., 1981). The oxidation of these compo-
nents by HOCl results in the loss of physiological

CLEANlNG AND DlSINFECTlON USlNG SODIUM HYPOCLORlTE 151

functions. During HOCl stress for E coli, loss of cata-lytic function of sulfhydryl enzymes and decrease in antioxidants such as glutathione have been sug-gested to be the bactericidal events (Albrich et al., 1981; Dukan et al., 1999; Thomas, 1979). HOCl has also been found to disrupt oxidative phosphorylation (Barrette et al., 1989), metabolic pathways involved in ATP utilization or generation (Barrette et al., 1987), and other membrane-associated activities (Champer and McFeters, 1979). Furthermore, HOCl can cause DNA damage resulting from the formation of chlorinated derivatives of nucleotide bases (Dunkan and Touati, 1996; McKenna and Davies, 1988). At present, it can be summarized that the pri-mary effect of HOCl is either or both (i) the oxidation of sulfhydryl (SH) groups of essential enzymes and antioxidants and (ii) deleterious effects on DNA syn-thesis.

l nvolvement of reactive oxygen species
Under the stress of HOCl, the possible involvement of reactive oxygen species (ROS) generated in mi-crobial cells has been suggested (Dunkan and Touati, 1996; Dukan et al., 1999). Aerobic organisms must deal with ROS such as superoxide anions (O-2), hydrogen peroxide (H2O2), and hydroxyl radicals (OH) that are generated from sequential univalent reductions of oxygen during oxidative phosphoryla-tion:

(7)

Oxidative phosphorylation is localized in the mitochondrial inner membrane of eukaryotic cells (circle C in Fig. 2) or in the plasma membrane of pro-karyotic cells (circle B in Fig. 2). Cells are equipped with several defenses including antioxidants such as glutathione, and enzymes including catalases, peroxidases, and superoxide dismutases that are in-volved in eq. 7. lf antioxidants are exhausted or the activities of these enzymes are once inactivated by HOCl, O2- and H2O2 would accumulate. H2O2 can readily diffuse into the cytoplasm through the mitochondrial inner membrane or plasma membrane. On the other hand, free iron is found to be released from microbial iron centers, i.e., heme and non-heme iron proteins, during exposure to HOCl (Rusen and Klebanoff, 1982). This can lead to the production of highly reactive OH via the Fenton reaction:

(8)

The formed Fe3+ is reduced to Fe2+ by O2- (Harber-Weiss reaction). Besides the Fenton reaction, it has been reported that the reaction between HOCl and iron is also a potential source of OH in activated

neutrophils (Folkes et al., 1995):

(9)

Thus, it is likely that the endogenously formed OH is
also responsible for the potent germicidal activity of
HOCl.

A concentrated NaOCl solution
A concentrated sodium hypochlorite solution is a
strong base solution due to the presence of NaOH,
i.e., -OH. High concentrations of -OH , i.e., pH > 12.5,
can alter the integrity of the cell wall and plasma
membrane through degradation of constituents such
as mucopolysaccharides, proteins, phospholipids or
unsaturated fatty acids of the plasma membrane
(Estrela et al., 2002). ln this pH region, -OH may act

primarily as an active antimicrobial agent. ln addition, -OCl i
nactivates the essential enzymes via the oxida-
tion of SH groups and the chloramination of amino
groups, resulting in the dysfunction of membrane ac
tivity. Mixtures of sodium hypochlorite at 200 mg
AC/L with 1.5 to 4•“ NaOH are much more rapidly
sporicidal than either NaOH or sodium hypochlorite
used alone (Cousins and Allan, 1967). This is due to
the fact that high concentrations of -OH can dissolve
the spore coat that consists mainly of proteins,
thereby increasing -OCl penetration or inducing
spore lysis.

Effect of organic load
l t is known that the germicidal efficacy of sodium
hypochlorite solution is lowered in the presence of or-
ganic materials because of a decrease in the AC con-
centration. Bloomfield et al. (1991) demonstrated
that adding 0.5 and 1.0% (w/v) albumin to a chlorine
solution containing 250 mg AC/L reduced the killing
of several bacteria from >6 log units to 0.3-1.9 log
units and to 0.5-1.4 log units, respectively. Takehara
and Fukuzaki (2006) studied the decrease in the AC
concentration (100 mg/L) in the presence of 0.3%
(w/v) bovine serum albumin (BSA) over the pH
range of 2 to 12 and found that the maximum de-
crease in AC concentration occurred at pH 9.5,
whereas the minimum was observed at pH 4.5. This
finding implies that HOCl which has the highest ger-
micidal activity is less affected by the coexisting pro-
tein. It is of importance to note that pH values at
which decomposition of AC is promoted in the pres-
ence of protein are in agreement with the pH region
(8.5 to10) of sodium hypochlorite solutions at com-
monly used AC concentrations.

152S.FUKUZAKl

MECHANlSM OF THE CLEANING ACTlON

l t has been pointed out that the primary function of
a detergent is to minimize the magnitude of attractive
forces between soil and the solid surface by adsorp-
tion of detergent components both on soil and on the
solid surface (Jennings, 1965). ln addition, the de-
composition of organic soil into low-molecular-weight
fragments by detergents can accelerate the progress
of cleaning. The cleaning action of sodium
hypochlorite is based on the synergism of the oxidiz-
ing power of -OCl and the ability of -OH to dissolve
organic soils.

Role of -OH
Hydroxide ions in a concentrated sodium
hypochlorite can dissolve a wide range of organic
materials, i.e., proteins, polysaccharides, and fats.
Sodium hydroxide is often added to sodium
hypochlorite solution not only to strengthen the clean-
ing power, but also to stabilize free available chlorine
in the form of -OCl, and to reduce the corrosive effect
of -OCl on metal. Hydroxide ions can be adsorbed
both on most hydrophilic solid surfaces and on bio-
polyelectrolytes such as proteins (=desorption of
H +)
, making their surfaces more negatively charged.
As a result, -OH affects the interfacial properties of
solid surfaces and soils in an aqueous solution, and
their action is largely due to "surface-activity"
(Koopal, 1985). ln the case of reaction of NaOH and
fat soils (i.e., saponification reaction), -OH degrades
fats, transforming them into glycerol and sodium salts
of carboxylic acids containing long chains of carbon
atoms (soap), which in turn reduce the interfacial
tension of the remaining fat-solution interfaces.
Figure 3 shows the effect of the pH of the cleaning
solution on the removal efficiency of different kinds of
proteins from stainless steel surfaces during batch
cleaning (Takahashi et al., 2003b). Although BSA,
ƒÀ -lactoglobulin
, casein, and gelatin have different
properties, e.g., thermal stability, solubility, and mo-
lecular structure, there are no significant differences
in the relationship between the removal efficiency and
the pH among the four proteins. At pHs above 11, the
removal efficiency increased markedly with increas-
ing pH. ln the pH range of 4.0 to 9.0, only small or no
removal of proteins was observed. Although the
amount of removed proteins increased slightly at a
pH below 3.0, low-pH solutions often induce the ag-
gregation of proteins, resulting in persistent residual
soils. Thus, most naturally occurring proteins are sus-
ceptible to caustic alkali cleaning. lt has also been re-
ported that the rate of the removal of milk soils from
the stainless steel surface is first-order with respect

FlG. 3. Effect of the pH of the cleaning solution on the
removal of BSA (A) ,ƒÀ-lactoglobulin (B) , casein (C),
and gelatin (D) from stainless steel surfaces during
batch cleaning at 40•Ž. The pH values of cleaning solu-
tions were adjusted to pH 1.0 to 13.5 with HNO3 or
NaOH solution.

to the OH concentration in the range of 0.01 to
0.15M (Jennings, 1959). At high pH values,
intramolecular electrostatic repulsion and the swelling
of the proteins can be enhanced, and the adsorbed
proteins become more soluble and dispersable in the
NaOH solution. ln addition, the repulsive force be-
tween proteins and stainless steel surfaces becomes
greater, and attractive forces to hold protein molecule
on stainless steel surfaces will diminish, thereby ac-
celerating the removal of the adsorbed proteins. lt is
thought that preferential displacement by -OH ions is
one of the principal cleaning mechanisms on hydro-
philic solid surfaces (Fukuzaki et al., 2002; Takahashi
and Fukuzaki, 2006).
l n general, the rate of proteineous soil removal by
NaOH solution is also enhanced with the increase in
temperature. The combination of sodium hydroxide
and heat stimulates the swelling of the soil layer and
brings about the hydrolysis of proteins, thereby short-
ening lag time at the beginning of cleaning and accel-
erating the removal of proteins (GraƒÀhoff, 1983,
1988; Jennings, 1959 and 1963; Takahashi et al.,
2003a). Kinetic studies on the effect of temperature
on the rate of removal of proteineous soils from stain-
less steel in NaOH cleaning showed that the first-

CLEANING AND DlSlNFECTlON USlNG SODlUM HYPOCLORlTE 153

order rate constant had an Arrhenius-type tempera-
ture dependence and increased by 1.4 to 1.6 times
for every 10•Ž rise in temperature (Jennings, 1959;
Takahashi and Fukuzaki, 2003).

Role of -OCl
Which has the stronger cleaning power in a dilute
sodium hypochlorite solution, HOCl or -OCl? Recent
investigations at the author's laboratory reveal that
the -OCl concentration is a major factor determining
the actual AC concentration required for the removal
of protein (BSA), acidic polysaccharide (pectin), and
bacterial cells (Pseudomonas fluorescens) from hard
solid surfaces (Al2O3 surfaces) (Urano and Fukuzaki,
2005; Fukuzaki et al., 2006a). ln the absence of
NaOCl, no significant desorption (< 10%) of BSA,
pectin, and P fluorescens cells from Al2O3 surfaces
occur over the pH range of 2 to 10. ln this pH region,
their removal from Al2O3 surfaces occurs by the addi-
tion of NaOCl, depending on the -OCl concentration.
Figure 4 shows the efficiency of the removal of BSA,
pectin, and P. fluorescens cells from Al2O3 surfaces,
obtained at various AC concentrations (100 to 2,000
mg/L) in the pH range of 2 to 10, as a function of the
OCl concentration. These graphs clearly indicate
that the removal of BSA, pectin, and P.fluorescens
cells occurs in an -OCl -dependent manner. At mod-
erate -OH concentrations (pH > 10), -OH leads to
the hydration and swelling of the adsorbed molecules
of protein and pectin. This can facilitate the mass
transfer of -OCl into the adsorbed layer toward the
contact surfaces between the protein and solid sur-

faces. lt is also found that the first-order rate con-

stants for BSA and pectin removal increase exponen-tially with the - OCl concentration (Urano and Fukuzaki, 2005). ln these studies, it is indicated that no significant desorption, especially of BSA, takes place in the pH range of 1.5 to 6.5 even in the pres-ence of the sufficient AC concentration of 1,100 mg/L, indicating that undissociated HOCl exerted lit-tle action in the removal of these organic soils.

As to the removal of BSA, the primary function of -OCl is to decompose the BSA molecules into low-
molecular-weight fragments by its oxidative action (Urano and Fukuzaki, 2005). The chlorination reac-tions of proteins with -OCl occur mainly on the free amino groups of amino acid residues to give chloramines under physiological conditions (Clark et al., 1986; Domigan et al., 1995). Hawkins and Davies (1998 and 1999) have reported that free amino groups of lysine residues, which are present in many proteins at a much higher level than other reactive residues, were converted into unstable chloramines via the reaction with HOCl/-OCl, which were in turn broken down into nitrogen-centered radicals via the homolysis of N-Cl bonds. They concluded that the chloramine formation and nitrogen-centered radicals are key species involved in the HOCl/-OCl-induced backbone fragmentation of proteins. On the other
hand, despite little occurrence of the decomposition
of pectin and P. fluorescens cells, their removal can
be enhanced in the presence of -OCl. Conceivably,
OCl might also attack the pectin-Al2O3 and cell-
Al2O3 interfaces and partially displace or cleave ad-

FlG. 4. Removal of BSA (A), pectin (B), and P. fluorescens cells (C) from Al2O3 surfaces as a function of the-OCl
concentration (batch cleaning at 40•Ž). Cleaning experiments were conducted at total AC concentrations of 120 to
2200 mg/L at various pH values. The data obtained in the pH range of 2 to 10, where no significant desorptionoc-
curred by the action of -OH alone, were plotted against the -OCl concentration. Symbols (total AC concentration
used in experiment, mg/L): •¢, 120 mg/L; • , 200 mg/L; •ž, 250 mg/L; •Ÿ 500 mg/L; •Z, 1000 or 1100 mg/L;
•¡, 2200 mg/L.

154 S. FUKUZAKl

sorbing groups on pectin molecules and the bacterial cells. At present, the mode of action of -OCl at solid-liquid interfaces has not been fully elucidated and a study on this mechanism is in progress at the author's laboratory.
The findings in Fig. 3 indicate that -OCl is available for the removal of biofilm. Biofilm consists of both mi-

crobes and the extracellular polymeric substances (EPS), composed of (acidic) polysaccharides or globular glycoproreins (Costerton et al., 1981). ln the early stage of biofilm formation, bacteria along with organic and inorganic molecules, e.g., proteins and polysaccharides, are adsorbed on a solid surface forming a conditioning film. The conditioning film al-ters the physicochemical properties of the solid sur-face which may also induce the subsequent adhesion of microbial cells. The attached microbes grow and produce EPS that contribute to the anchorage of the cells to the surface and serve to protect microbes from hostile environments. Biofilm bacteria grown on various solid surfaces are 150 to 3,000 times more resistant to HOCl (pH 7) than are unattached cells (LeChevallier et al., 1988). To prevent microbes from forming biofilm, it is necessary to eliminate as much as possible both the conditioning film and EPS. ln this regard, the repeated use of alkaline hypochlorite so-lution (-OCl) and weak acidic hypochlorite solution (HOCl) is expected to be effective for the successful control of biofilm.

At the pH region above 12, -OCl and -OH can syn-ergistically enhance the efficiency and the rate of the removal of, especially, protein. Besides the preferen-tial displacement by OH, high concentrations of -OH cause the deprotonation of the N atom involved

in the peptide bond, resulting in the increase in the electron density of the N atom. As a result, the pep-tide bond becomes more susceptible to the electophilic attack of Cl+, thereby accelerating the fragmentation of the protein molecule. ln caustic al-kali cleaning at the -OCl concentration of ca. 1,000 mg/L, the first-order rate constant for BSA desorption increases by approximately 5 times by increasing pH values from 10 to 12.5 (Urano and Fukuzaki, unpub-lished data).

ELECTROLYZED WATER AND WEAK AClDlC HYPOCHLORlTE SOLUTlON

Recently, electrolyzed water has been receiving at-tention as a novel and alternative disinfectant to so-

dium hypochlorite solution. When sodium chloride (NaCl) solution is electrolyzed using an electrolyzer in which the anode and cathode are separated by al membrane, acidic electrolyzed water (pH 2 to 3) is

generated at the anode site, while alkaline electro-lyzed water (pH 11 to 12) is generated at the cath-ode site. ln the case of non-membrane electrolyzer, electrolysis of the NaCl solution provides weak alka-line electrolyzed water containing sodium hypochlorite (pH >7.5), which is chemically identical with a dilute NaOCl solution. Weak acidic electro-

lyzed water (pH 5 to 6.5) is produced by the elec-trolysis of a diluted hydrochloric acid (HCl) in non-membrane electrolyzer, followed by dilution with water. Weak acidic hypochlorite solution is commer-cially and mechanically prepared by addition of HCl to a diluted sodium hypochlorite solution. ln manual preparation, acetic acid is the most suitable and saf-est acid for the preparation of an acidic hypochlorite s olution (Kuroiwa et al., 2003). Aside from the way it is produced, weak acidic hypochlorite solution pre-pared by using HCl is chemically identical with weak acidic electrolyzed water.
Acidic electrolyzed water and weak acidic hypochlorite solution have been demonstrated to have a bactericidal effect on fresh-cut vegetables (lzumi, 1999; Koseki and Itoh, 2001; Koseki et al., 2001; Parish, 2003). The bactericidal activity of elec-trolyzed water can be also explained by the chemical equilibrium of Cl2, HOCl, and - OCl as mentioned above. Len et al. (2000) have studied the inactivation of Bacillus cereus in 30sec exposure to electrolyzed water (10 to 12 mg AC/L) adjusted to a pH in the range of 2 to 9 and reported that more than a 100-fold reduction was observed in the pH range of 2 to 6. They also demonstrated that the maximum bacteri-cidal activity of electrolyzed water occurred at the pH region between 4 and 6 where HOCl concentration is the highest, indicating that HOCl is the primary com-ponent for inactivation. Note that weak acidic electro-lyzed water and hypochlorite solution have a poor ability to remove microbial cells and organic soils from solid surfaces (see Fig. 4).
Weak acidic hypochlorite solution is commonly used in the concentration range of 50 to 200 mg/L with the contact time of 1 to 2 min to disinfect sur-

faces of fresh and fresh-cut produce, and of process-ing equipment (Parish, 2003). Although the germicidal activity of hypochlorite solution becomes the highest at pHs between 4 and 6, pH values be-tween 6 and 7 are typically used to the minimize the corrosion of metals while yielding acceptable chlorine efficacy.
Alkaline electrolyzed water is considered to be a di-lute sodium hydroxide solution and it is useful for the removal of organic soils on solid surfaces (Fukuzaki, et al., 2004; Takehara et al., 2001) and hence also for preliminary cleaning before a disinfecting operation

CLEANlNG AND DlSlNFECTlON USING SODIUM HYPOCLORlTE 155

with acidic electrolyzed water (Koseki et al., 2001;
Yoshida, et al., 2005).

COMBlNED USE OF SODlUM
HYPHOCHLORlTTE AND
A SURFACTANT

The high surface tension (ƒÁ) is one of disadvan-
tages of sodium hypochlorite from the viewpoints of
cleaning and disinfecting operations (Table 1). This
is just a weak point of water in terms of the
wettability. When NaOCl or NaOH is added to water,
the ƒÁ value undergoes a slow but regular increase.
The ƒÁ values of the NaOH solution and NaOCl solu-
tion of pH 12 are 71 to 73 mN/m at 20•Ž. The prop-
erty of high ƒÁ prevents the NaOCl solution from
wetting solid surfaces and soils, and from penetrating
into the deposited soil layer. Surface-active agents
(surfactants) are regarded as substances capable of
reducing surface (interfacial) tension. The addition of
only 0.01 to 0.1% of a surfactant makes the ƒÁ values
of NaOCl and NaOH solution lower to approximately
29 to 35 mN/m. The marked decrease in the ƒÁ of
NaOH and NaOCl solutions in the presence of a sur-
factant results in the improvement of the wetting abil-
ity of the solutions. Outer rinds and waxy cuticle on
the surface of many fruits and vegetables, and poly-
mer surfaces are hydrophobic in character and hence
they show poor wettability to water-based cleaning or
disinfecting solutions. Surfactants can improve the
wettability of hydrophobic surfaces to water, thereby
augmenting the efficiency of cleaning or disinfection.
Ono et al. (2005) demonstrated that 2.5- to 2.8-log-
unit reduction in vegetative bacteria on cucumbers
and long green onions could be achieved reliably by
the combined use of weak acidic hypochlorite solu-
tion (pH 6, 100 mg AC/L) and a nonionic surfactant
(food-emulsifying agent, 0.025% [w/v]) for 1 min, in
comparison to 0.8- to 1.6-log-unit reduction by weak
acidic hypochlorite solution alone. The addition of a
nonionic surfactant (0.02%, w/v) to NaOH solution
(pH 12) could improve the efficiency of the cleaning
of hydrophobic polyethylene terephthalate (PET) sur-
faces fouled with protein and catechin (Fukuzaki et
al., 2006b). This indicates that the principal point of
the working of -OH ions is the soil-PET interfaces
rather than soil-liquid interfaces.
The technique of foam cleaning, combining surfac-
tants, water, and compressed air, has been applying
increasingly in the food industry. The use of alkaline
NaOCl solution as the ingredient of foam cleaning is
more suitable for the removal of organic soils and mi-
crobes even though the AC concentration and pH of
NaOCl solution are relatively low (e.g. 200 mg AC/L

at pH 10). Foam cleaning has many advantages, e.g., of lowering the interfacial tension, prolonging the con-tact time between detergents and soils to loosen all soils from equipment surfaces, low mechanical ac-tion, reducing the amount of detergent, and applica-bility to horizontal, vertical, and inverted surfaces, and to the parts of the machinery not accessible to human hands. Foam can suck up soils released from solid surfaces, which in turn makes rinsing easier.

FlNAL REMARKS

To utilize sodium hypochlorite, electrolyzed water, and weak acidic hypochlorite solution effectively, it is important that we should thoroughly know the basic property of each solution and should utilize each ex-cellent function correctly. lt is especially necessary to make it clear whether the objective is "cleaning" or

"disinfecting". Entirely removing organic soils is an
important pretreatment step to perform disinfection efficiently. lt is indispensable that we use pH prepara-tions and surfactants effectively to complement or to strengthen the cleaning and disinfecting actions of sodium hypochlorite solution. The findings presented here provide useful information concerning the clean-ing and disinfecting operations using NaOCI solution. At present, the research into and development of al-ternative cleaning agents and disinfectants to chlo-rine has continued to progress. However, despite the introduction of new cleaning agents and disinfec-tants, sodium hypochlorite solution is likely to con-tinue to find wide use in food industry settings because of its numerous advantages.

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156 S. FUKUZAKl

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CLEANlNG AND DlSINFECTlON USING SODlUM HYPOCLORlTE 157

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