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Low environmental impact type ground improvement methods using biomineral precipitation

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Japan

This thesis is presented for the degree of
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R.A.N. Dilrukshi

Declaration

I declare that this thesis, which I submit for the degree of Doctor of Philosophy in Sustainable Resources Engineering, is my own personal effort. Furthermore, I took reasonable care to ensure that the work is original and has not previously been submitted for a degree at any university and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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CHAPTER 1: Introduction

1.1 BACKGROUND

With the rapid growth of population in the world, it has brought many challenges to all facets of society including engineering practices. The range of lands that can be used for human needs is limited with the increase of population as well as with some environmental factors including climate, topography, and soil characteristics. Therefore, the development of infrastructures with lack of useful lands has become a major issue. Engineers are facing new challenges to modify existing ground which are insufficient for the desired land use.

Construction on soft ground area is a great challenge in the field of geotechnical engineering. Many geotechnical engineering problems in the form of slope instability, bearing capacity failure or excessive settlement could occur either during or after the construction phase due to low shear strength and high compressibility of this soil. Dynamic soil instability causes to destroy large number of structures by causing severe loss of human lives and various properties. Particularly in geotechnical engineering, dynamic instability of saturated sandy soils known as liquefaction is one of the most drastic and catastrophic phenomenon induced by earthquake. According to the past experiences, when liquefaction of the ground take place, damage cost has been increased about ten times larger than that without liquefaction occurrence (Sesov and Talaganov, 2001). Settlement, lateral spreading and lateral displacement could be observed due to liquefaction and therefore, severe damages could be occurred in structures. Loose sand can be subject to large settlements, and these settlements tend to occur quickly due to the free-draining granular nature of sands. This can pose foundation challenges and loose sand requires densification in order to provide sufficient settlement control and bearing for new structures. The destructive effects due to liquefaction can be remembered from the devastation brought by Good Friday earthquake

(Moment Magnitude Scale, $M_w = 9.2$) in Alaska and Niigata earthquake (Surface-wave magnitude, $M_s = 7.5$) in Japan both in the year 1964. Beside, recent earthquakes such as Kobe (1995), Kocaeli (1999), Chi-chi (1999) and Bhuj (2001), the liquefaction were a major cause of damage to civil engineering structures. Therefore, a proper study of liquefaction potential of a place has become very important to minimize such disastrous after-shock effect.

Currently, various ground improvement techniques such as cement and chemical grouting, micro piles, jacked piers, driven piers, ground anchors, shoring, soil nailing, vibro compaction, concrete columns, piers, etc. are experienced for the weak soils in order to meet the engineering requirements of soil (Karol, 2003). However, these techniques are related with major environmental issues such as inevitable CO_2 emissions during cement production, and high energy cost for cement production and re- excavation of cement improved ground. In addition to that, all chemical grouts are toxic and/or hazardous up to some extent (Karol, 2003) and therefore, groundwater pollution may occur. The most common chemical grouts are sodium silicate, acrylate, lignin, urethane, and resin grouts (US Army Corps of Engineers, 1995). In 1974, acrylamide grout was associated with five cases of water poisoning in Japan, resulting in the ban of nearly all chemical grouts (Karol, 2003). Therefore, a successful planning is necessary to improve the soil characteristics by different ground improvement techniques in order to assure the minimum damage to the environment.

In order to address the disadvantages of current ground improvement techniques one could look at sustainable or “green” materials and processes using biomimetic in which lessons learned from nature form the basis for evolution of novel technological materials for the development of sustainable materials in the construction industry. When it comes to the field of geotechnical engineering, the most common “green” practices are trying to be applied for the soil improvement. This thesis discusses about such sustainable ground improvement techniques those are managed by science of biology, chemistry and physics together and the

potential of applying this knowledge to multifunctional geotechnical engineering applications under two main topics named as soil improvement with calcium phosphate compounds (hereafter, CPCs) and soil improvement using plant-derived urease induced calcium carbonate (hereafter, CC) precipitation (hereafter, PDUICCP).

1.2 LITERATURE REVIEW

In nature, various minerals, such as calcium carbonate, calcium sulfate, calcium phosphate, calcium oxalate, silicate, and iron oxide are precipitated by living organisms. These biominerals are promising as engineering materials because they have considerable strength and low environmental impact due to less release of greenhouse gases and less energy to produce (Khanafari et al., 2011).

Different mineral formation mechanisms using biological actions have been introduced and these formed materials are called “biogrouts” (Van Paassen et al., 2009). Some of these mechanisms are listed as carbonate precipitation using urea and ureolytic bacteria (Harkes et al., 2010) or urea and plant urease enzyme (Park et al., 2014; Nam et al., 2014; Neupane et al., 2013) or using glucose and yeast (Kawasaki et al., 2006), iron-oxidizing bacteria based iron/manganese compound precipitation (Weaver et al., 2011), siloxane bond formation using glucose and yeast (Terajima et al., 2009), CPC based chemical grouts (CPC-Chem) formation by its self-setting mechanism (Akiyama and Kawasaki, 2012a) and CPC biogROUT (CPC-Bio) formation by the addition of ureolytic microorganisms and an ammonia source to CPC-Chem (Akiyama and Kawasaki, 2012b). Although any biological action is not involved for the formation of CPC-Chem, final product can be considered as a type of biomineral, because CPCs exist as phosphate rocks (e.g., fluorapatite) in the natural environment and also as an important inorganic substance (e.g., hydroxyapatite, HA) in living organisms (Dorozhkin, and Epple, 2002). Therefore, CPCs are also considered as an

environment friendly cementing material similar to carbonates. However, it is very important to study these mechanisms widely in order to bring these novel methods environmentally safe, cost effective and to develop this technology from lab to field scales.

1.2.1 Soil improvement using CPCs precipitation

CPCs are very important inorganic materials in different fields such as geology, medicine and dentistry. CPCs exist as phosphate rocks (mainly fluoroapatite) in the natural environment and as an important inorganic substance (mainly hydroxyapatite, HA) in living organisms (Dorozhkin and Epple, 2002). These biominerals are promising as engineering materials because they have adequate strength and low environmental impact. There are eleven types of CPCs can be identified according to calcium- to- phosphate (Ca/P) molar ratios varying from 0.5 to 2.0 in the ternary system $\text{Ca}(\text{OH})_2\text{-H}_3\text{PO}_4\text{-H}_2\text{O}$. Table 1.1 shows the abbreviations, chemical formula of different CPCs.

As technology advances, the opportunities for biominerals are becoming greater and greater, and have even given rise to new fields of study such as engineering, which primarily focuses on the artificial production of biominerals to use in the field of engineering. Research and development of materials comprised of CPCs are currently in progress, especially in the fields of medicine and dentistry (Martin and Brown, 1995; Bohner et al., 2006; Ginebra et al., 2006). However, medical CPC paste is extremely expensive and has high viscosity, which makes it unfeasible for engineering applications. Therefore, past researchers have been considered the use of CPCs from an engineering viewpoint and aimed to develop a grout material using CPCs that is precipitated under normal temperature and pressure.

Before developing such a grout material, it is very important to be familiar with the characteristic of CPCs. Solubility is very important property of CPCs. The term solubility is a thermodynamic property indicating the equilibrium between the solid and the dissolve

Table 1.1: Biologically relevant calcium orthophosphates (Dorozhkin and Epple, 2002).

Ca/P ratio	Compound	Abbreviation
0.5	Monocalcium phosphate monohydrate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$)	MCPM
0.5	Monocalcium phosphate anhydrate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$)	MCPA
1	Dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$)	DCPD
1	Dicalcium phosphate anhydrate (CaHPO_4)	DCPA
1.33	Octacalcium phosphate ($\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$)	OCP
1.5	A-tricalcium phosphate ($\alpha\text{-Ca}_3(\text{PO}_4)_2$)	α -TCP
1.5	B-tricalcium phosphate ($\beta\text{-Ca}_3(\text{PO}_4)_2$)	β -TCP
1.2-2.2	Amorphous calcium phosphate ($\text{Ca}_x(\text{PO}_4)_y \cdot n\text{H}_2\text{O}$)	ACP
1.5-1.67	Calcium-deficient hydroxyapatite ($\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$) ($0 < x < 1$)	CDHA
1.67	Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$)	HA
2	Tetracalcium phosphate ($\text{Ca}_4(\text{PO}_4)_2\text{O}$)	TTCP

components in a solution. The solubility of various CPCs with pH of the surrounding environment is shown in Fig. 1.1. The other property is the stability. Gel-like or amorphous CPCs change into HA over time (Fig. 1.2) (Tung, 1998 and Chow, 1991). The change rate is dependent on both pH and the concentrations of calcium and phosphate ions. HA forms directly in solutions with low super saturation levels (indicated by arrow 1 in Fig. 1.2), as the concentration of phosphate ions increase, less stable phases of CPCs (e.g. ACP and OCP) form initially and transform into HA over time (indicated by arrows 2-4 in Fig. 1.2) . Therefore, CPCs hardens after injection into soil and rock because of the self-setting mechanism.

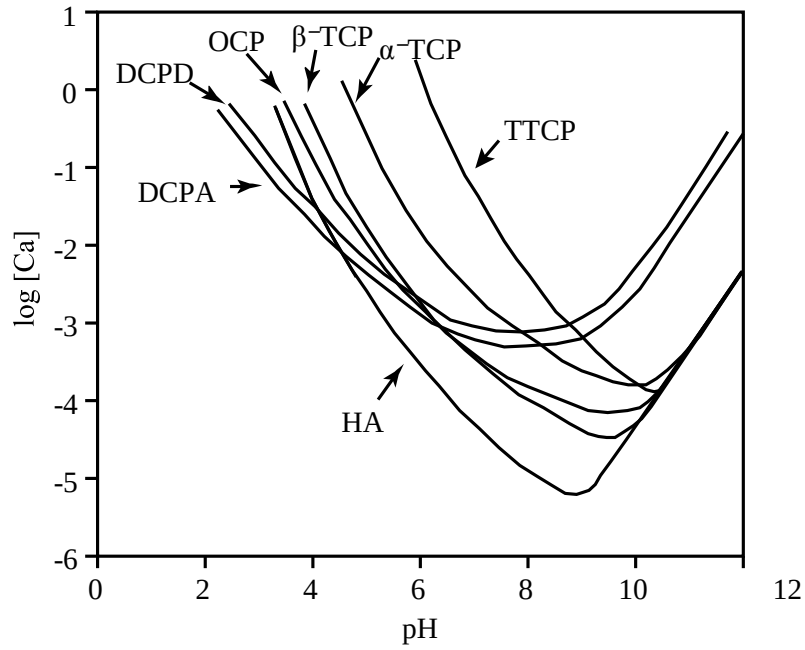


Fig. 1.1: Solubility phase diagrams for the ternary system, $\text{Ca}(\text{OH})_2\text{-H}_3\text{PO}_4\text{-H}_2\text{O}$, at 25°C , showing the solubility isotherms of CaHPO_4 (DCPA), $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (DCPD), $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ (OCP), $\alpha\text{-Ca}_3(\text{PO}_4)_2$ ($\alpha\text{-TCP}$), $\beta\text{-Ca}_3(\text{PO}_4)_2$ ($\beta\text{-TCP}$), $\text{Ca}_4(\text{PO}_4)_2\text{O}$ (TTCP), and $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (HA) (Tung, 1998).

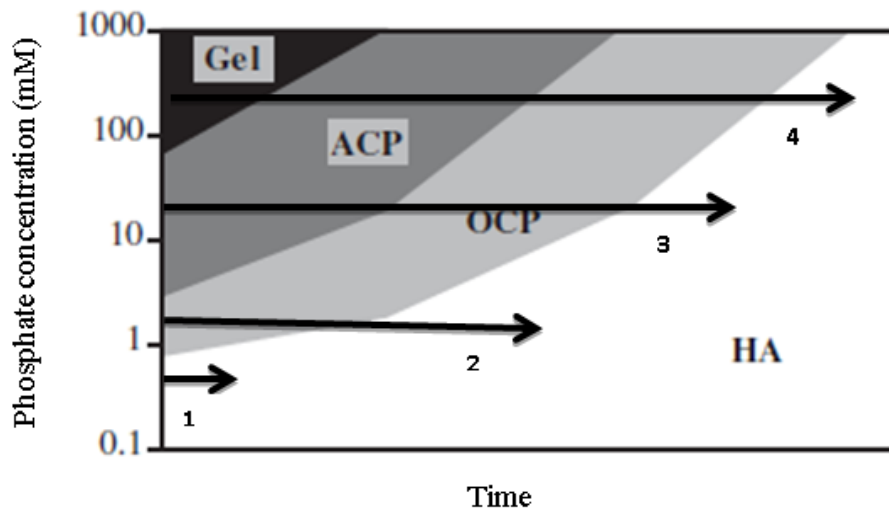


Fig. 1.2: Formation, stability, and hydrolysis of calcium phosphates as a function of phosphate concentration ($\log (\text{P})$) in solutions of amorphous calcium phosphate (ACP) at neutral pH. OCP: Octacalcium Phosphate; HA: Hydroxyapatite (Tung, 1998).

In addition to that, the distribution of orthophosphate species in a solution as a function of pH is shown in Fig.1.3. Between pH values of 4.0 and 6.0, most of the P in the solution is presented as the H_2PO_4^- ion and this form is soluble in water. Between pH 6.5 and 7.5, P in the solution is present partly as the H_2PO_4^- and partly as the HPO_4^{2-} ion. HPO_4^{2-} is also soluble in water but not as readily as H_2PO_4^- ion. Between pH 8.0 and 10.0 the HPO_4^{2-} ion is dominant. Beyond pH 10.0 the dominant ionic form of P is PO_4^{3-} .

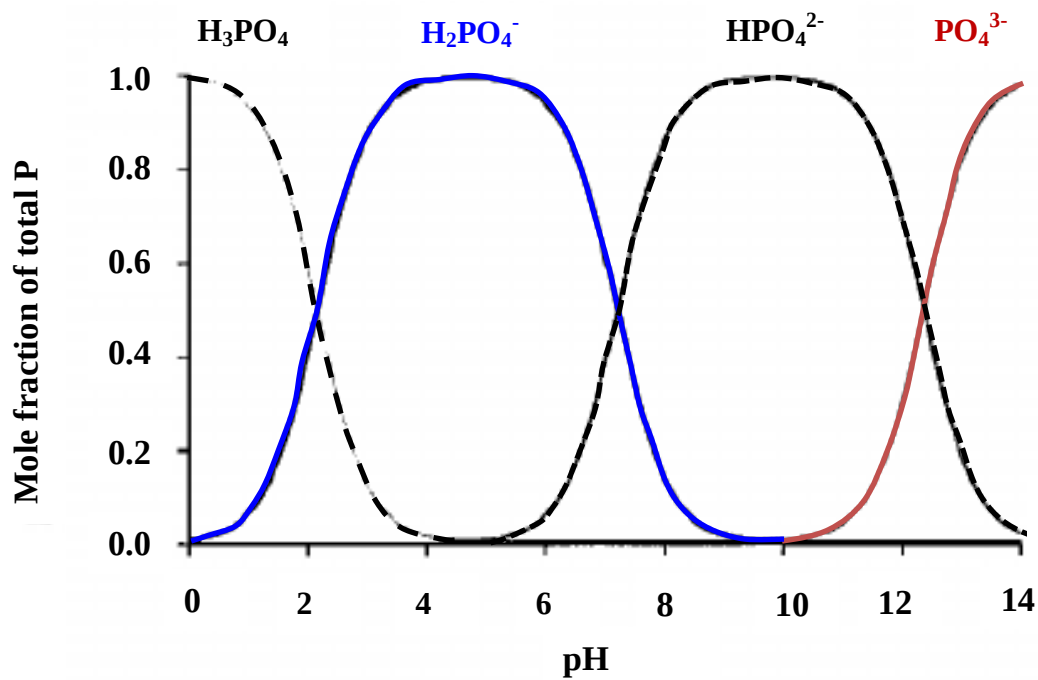


Fig. 1.3: Distribution of orthophosphate species in a solution as a function of pH (Hinsinger P, 2001).

With the purpose of the use of CPCs for engineering applications especially in geotechnical engineering applications, CPC precipitation has been determined in an in vitro examination by using phosphate and calcium stock solutions (Akiyama and Kawasaki, 2012a). To evaluate the potential utility of a new CPC-based biogrout (CPC biogrout) instead of CPC-based chemical grout (CPC-Chem) (Akiyama and Kawasaki, 2012a), CPC biogrout has been produced using soil microorganisms and by the addition of an ammonia

source. Such a CPC biogrout would enable the control of solidification speed. Furthermore, the strength of the ground can be increased by using a combination of CPC-Chem, for rapid solidification, and CPC biogrout, for long-term solidification (Akiyama and Kawasaki, 2012b). The maximum unconfined compressive strength (hereafter, UCS) of sand test pieces cemented with CPCs was found to be 63.5 kPa after 14 days of curing time (Akiyama and Kawasaki, 2012a) using calcium acetate (CA) and diammonium phosphate (DAP) as Ca^{2+} and PO_4^{3-} sources respectively. Furthermore, they have evaluated the UCS under different Ca^{2+} and PO_4^{3-} sources and the UCS values have been changed with Ca^{2+} and PO_4^{3-} sources.

According to another research finding on CPC paste, it has been reported that the UCS of CPC exceeded 10 MPa under normal temperature and pressure conditions (Fernandez et al., 1998). In addition, it has been shown that the UCS of DCP and α -TCP mixed paste has been increased from 35 MPa to a maximum of 56 MPa by using CC as the seed crystal (Fernandez et al., 1998). The addition of TCP or CC powder into CPCs has also been caused to achieve the UCS of 261.4 kPa and 209.7 kPa respectively after 28 days of curing (Kawasaki and Akiyama, 2013). These observations indicate that the existence of seed crystals can reinforce the strength of CPCs.

1.2.2 Soil improvement using plant-derived urease induced CC precipitation

(a) Urease enzyme induced carbonate precipitation mechanism

Urease (EC 3.5.1.5) is a nickel dependent metalloenzyme which catalyzes the hydrolysis of urea. The three dimensional structure of the urease enzyme is shown in Fig. 1.4. The primary common feature of the urease enzymes is the presence of metal centers in their active sites, whose task is to activate the substrate and water for the reaction. Functionally, ureases belong to the superfamily of amidohydrolases and phosphotriestereases (Holm and Sander, 1997). The members of the amidohydrolase superfamily catalyze a variety of

hydrolysis reactions utilizing amides, urease, phosphotriesters and substituted triazines. Among other dinuclear metallohydrolases in the superfamily, ureases are unique in that they possess Ni (II) ions in the active site.

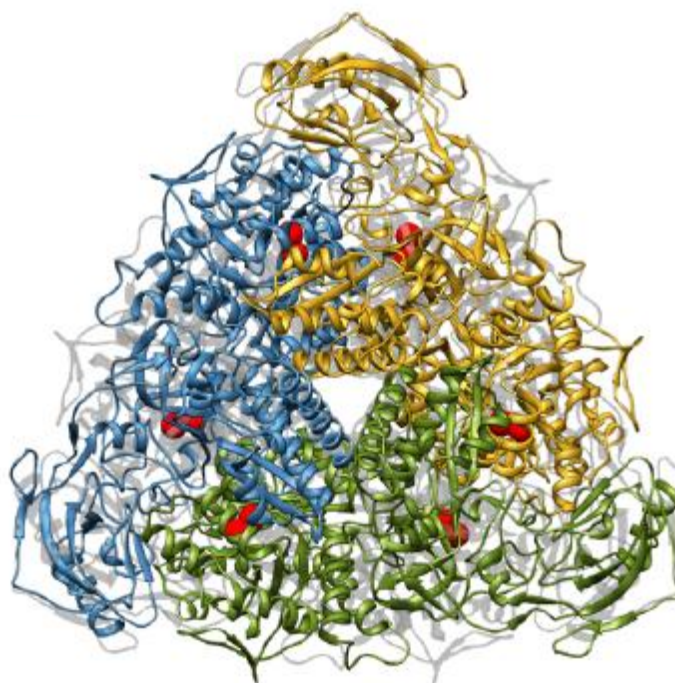


Fig. 1.4: Three dimensional structure of urease enzyme from Jack bean-*Canavalia ensiformis*) (Maroney and Ciurli, 2014).

During the hydrolysis of urea, one mole of urea is hydrolyzed intracellularly to one mole of ammonia and one mole of carbamate (eq. (1.1)), which is spontaneously hydrolyzed to one mole of ammonia and one mole of carbonic acid (eq. (1.2)). Ammonia and carbamate subsequently equilibrate in water to form bicarbonate and 2 moles of ammonium and hydroxide ions as described in eqs. (1.3) and (1.4) (De Muynck et al., 2010).



Total reaction:



The presence of calcium ion in the system will lead to the precipitation of CC once a certain level of super saturation is reached. Hausinger (1993) has shown that urease accelerates the hydrolysis of urea by a factor of 10^{14} in comparison with the spontaneous reaction. This fifth reaction raises the pH of the solution. This raise in pH creates an optimum condition for carbonate precipitation. The NH_4^+ and CO_3^{2-} produced from this reaction actually represent the final products of series of reactions. The NH_4^+ ions actually start out as NH_3 . When the ammonia reacts with water, it creates OH^- ions, which raise the pH of the system. This raise in pH causes the carbonate precipitation.

(b) Different sources of urease enzyme

(i) Plant-derived urease

Several families of common plants are very rich in urease, including some varieties of beans, melons and squash, and the pine family (Das et al., 2002). The main plant urease sources are jack beans (*Canvalia ensiformis*), soybean (*Glycine max*) leaf and seeds, pigweeds (*Chenopodium album*) and mulberry leaf (*Morusalba*), etc. (Sirko and Brodzik, 2000) and they help to catalyze the reaction of urea hydrolysis to form ammonium and carbonate ions. Many researches are investigating new plant species such as leaves rather than the seeds having urease activity. Some plant species having urease activity gathered from the literature are summarized in Table.1.2. The best-studied plant urease is that from jack bean (Andrews et al., 1984) which is identified as the first nickel metalloenzyme (Dixon et al., 1975) and the urease from jack bean is the first enzyme crystallized (Sumner, 1926). In

1926, Sumner shows that urease is a protein. Molecular weight of jack bean urease is 480,000 Da (480 kDa) (Goring et al., 1962). There are forty seven -SH groups in the urease species of molecular weight 480,000 Da (480 kDa) and it has been estimated that 4 to 8 of these groups are essential for the activity (Goring and Chin, 1965).

Table 1.2: Urease activities in some plant species.

Plant Species	Urease Activity	Unit	References
<i>Canavalia ensiformis</i> (Jack bean)	2700-3500	$\mu\text{mol urea/min.mg}$	Krajewska, 2009
<i>Glycine max</i> (Soy bean)	650-800		
<i>Cajanus cajan</i> (pigeon pea)	3120		
<i>Gossypium hirsutum</i> (Cotton seeds)	14.5		
<i>Rumex japonicus</i> Houtt.	42.2	$\mu\text{g NH}_4\text{-N/hour. g}$	Watanabe, et al., 1983
<i>Mirabilis jalapa</i> L.	83.8		
<i>Phytolacca americana</i> L.	26.1		
<i>Portulaca oleracea</i> L.	26.4		
<i>Portulaca pilosa</i> L.	35.4		
<i>Canavalia gladiata</i> DC.	251.2		
<i>Hydrocotyle yabei</i> Mak.	33.9		
<i>Plantago lanceolata</i> L.	56.4		
<i>Trichosanthes cucumeroides</i> Maxim.	86.3		
<i>Luffa cylindrica</i> Roem.	47.9		
<i>Cucumis sativus</i> L.	57.1		
<i>Cucurbita moschata</i> Duch.	105.0		
<i>Erigeron philadelphicus</i> L.	56.0		
<i>Helianthus tuberosus</i> L.	48.1		
<i>Eupatorium japonicum</i> Thunb.	38.3		
<i>Siegesbeckia pubescens</i> Makino	60.3		
<i>Artemisia vulgaris</i> L.	52.8		
<i>Setaria viridis</i> Beauv.	54.8		
<i>Eleusine indica</i> Gaertner	45.1		
<i>Pennisetum japonicum</i> Trinius.	62.1		
<i>Eragrostis ferruginea</i> P. Beauvois	44.7		
<i>Miscanthus sinensis</i> Anderss.	39.1		
<i>Iris nertschinskia</i> Lodd.	53.1		

Maximum catalytic activity of jack bean urease occurs at 65°C (Sumner, 1951) and it is inactive at temperatures above 70°C (Frankenberger and Tabatabai, 1982). The optimum pH for jack bean urease usually lies in between 6.0-7.0 (Lai and Tabatabai, 1992; Boyd and Mortland, 1985). The urease isolated from chickpea seeds (*Cicer arietinum* L.) yields maximum activity at pH 7.2 (Pervin et al., 2013). This result is similar to those reported for urease from jack bean (Sung et al., 1989) and pigeon pea (Das et al., 2002) but different from that isolated from mulberry leaves (Hirayama et al., 2000). Maximum urease activity of chickpea seeds has been obtained at temperature of 40°C and beyond that, the enzyme has been denatured rapidly and thus, lost its activity (El-Hefnawy et al., 2014). This result is closely related to those reported by Das et al. (2002) and Srivastava et al. (2001), but differs from that stated by El-Shora (2001). Urease activity increases with substrate concentration. After reaching an optimum value, the urease activity decreases with rising urea concentration (Pervin et al., 2013). The rate of hydrolysis of urea is increased with an increase in urea concentrations until a maximum is reached, beyond which hydrolysis is decreased. The results can be explained by substrate inhibition at higher urea concentrations (Singh and Nye, 1984; Fidaleo and Lavecchia, 2003).

Pervin et al. (2013) has also studied the effect of various metal ions and chemicals on the activity of chickpea urease. The urease activity has increased in the presence of calcium ion at low concentrations like 3 mM or less and decreased at higher calcium ion concentrations. Divalent cations like Ba^{2+} and Mg^{2+} slightly stimulate the enzyme at a concentration of 1-3 mM and Na^+ and K^+ produce little or no effect on the activity (El-Shora, 2001). Heavy metals, such as Cu^{2+} , Zn^{2+} , Pb^{2+} and Hg^{2+} almost completely inhibit the enzyme activity. This has also been proved for pigeon pea urease (Srivastava et al., 2002) and jack bean urease (Krajewska et al., 2004; Zaborska et al., 2004). The urease enzyme activity is completely inactivated when the concentration of Hg^{2+} is high enough (Du et al., 2012).

Muskmelon (*Cucumis melo*) is another plant source of urease enzyme (Wright et al., 2007). Kharbooza and Banggi are common names for muskmelon. Recently, urease from mulberry leaves (*Morus alba*) has also purified and characterized (Hirayama et al., 2000).

(ii) Microbial urease

Microbial (bacteria, fungi and algae) urease is an enzyme produced by ureolytic microorganisms and hydrolysis of urea is catalyzed into NH_4^+ and CO_3^{2-} by this enzyme. The metabolic activities of these microbial sources promote the precipitation of CC using produced carbonate, and Ca^{2+} ions in the system. These crystals form bonds between sand grains and increase the strength and stiffness of the sand (Van Paassen et al., 2010; Martinez and DeJong, 2009). Microorganisms with ureolytic activity are found in soil and water as well as in human and animal bodies (Mobley and Hausinger, 1989). There are several bacterial sources for this enzyme such as *Bacillus sphaericus*, *Sporosarcina pasteurii*, *Sporosarcina psychrophila*, *Proteus vulgaris*, *Myxococcus xanthus*, *Sporosarcina ureae*, *Pararhodobacter* sp., *Beijerinckia indica*, *Phanerochaete chrysosporium*, *Bacillus subtilis*, etc. Wijngaarden et al. (2012) explain that the majority of studies on microbially induced carbonate precipitation (hereafter, MICP) have been used the micro-organism named *Sporosarcina pasteurii* (previously *Bacillus pasteurii*) having urease enzyme for stabilizing soils.

Potential geotechnical and geoenvironmental applications of MICP are reducing the liquefaction potential of soil (Chu et al., 2009; Montoya et al., 2013), improvement of the strength and stiffness of granular soils (Van Paassen et al., 2010; DeJong et al., 2006; DeJong et al., 2010; Whiffin et al., 2007), increasing the bearing capacity of foundations (Shahin et al., 2017), sealing subsurface fractures (Phillips et al., 2016), preventing piping of earth dam (Jiang et al., 2016), reduction of the permeability of fractured rock (Cuthbert et al., 2013),

plugging (Ferris and Stehmeier, 1992), remediation of cracks in concrete (Ramachandran et al., 2001), coral sand solidification to form artificial beach rock (Danjo and Kawasaki, 2016), sand immobilization and suppression of dust (Bang et al., 2011; Stabnikov et al., 2013), removal of heavy metals from contaminated environment (Li et al., 2013), waste water treatment (Hammes et al., 2003), surface treatment for limestone conservation (De Muynck et al., 2013; Le Metayer-Levrel et al., 1999), etc.

(iii) Soil urease

Soil ureases are partly extracellular being liberated during microbial and plant root metabolism and death. They are also intracellular as part of the soil biomass (Dharmakeerthi and Thenabadu, 1996). Soil urease activity is related to the vegetation of the soil (Palma and Conti, 1990). Higher urease activity in soils under vegetation compared to vegetation free soils has been observed by Reddy et al. (1987) and they attributed it to the higher microbial proliferation and microbial activity at the rhizosphere. The major factor that influence for the level and distribution of urease activity through the soil profile is the content of soil organic carbon (Myers and McGarity, 1968; Tabatabai, 1977). However, this relationship is obviously modified within particular horizons by other soil properties such as reaction, gleying and soil texture (Myers and McGarity, 1968). According to the Myers and McGarity (1968), urease activity in calcareous soils is lower than in non-calcareous soil. They have reported that in saline soils, urease activity is low. Skujins (1967) has reported that in alkaline soils, the activity is much less, and it is also much less in carbonate rich soils, apparently because of detrimental effect of Ca^{2+} on the urease producing organisms. Numerous studies show that the urease activity in soils increases with the increase in temperature from 10°C to 40°C. In some soils, urease activity increases very markedly with the increase in temperature from 40°C to 70°C (Lai and Tabatabai, 1992; Kumar and Wagenet, 1984; Zantua and

Bremner, 1977) but decreases rapidly above this temperature range (Zantua and Bremner, 1977; Sahrawat, 1984). The optimum pH of soil urease activity reported in past is 6.5 to 7.0 (Paulsan and Kurtz, 1970) and it is similar to that of jack bean urease (Lai and Tabatabai, 1992; Bremner and Mulvaney, 1978; Reithal, 1971).

Equilibrium between ammonium ion and ammonia gas occurs in aqueous solutions of ammonium salts (Fenn and Kissel, 1973). When, enzyme activity is high in soil, the rate of hydrolysis of urea is increased and high soil pH and greater losses of gaseous ammonia can be observed. Although high ammonium ion concentration in soil is non-toxic, ammonia gas is extremely toxic to the plant (Gould et al., 1986). Therefore, the loss of volatile ammonia and ammonia toxicity to germinate seedlings are major problems in urea fertilization.

(c) Methods for evaluating urease activity

There are several methods to determine urease activity such as manometric, titrimetric, colorimetric, potentiometric method as well as spectrophotometric method. Some of those methods are summarized in Table A.1 in **APPENDIX A**. In those methods, urease can be examined by measuring substrate/products of the ureolytic reaction or byproducts of this reaction, such as ammonia, an increase in pH or conductivity as well as generated heat. Numbers of excellent assays are available for quantifying urease activity and analyzing its kinetic behavior (Mobley et al., 1995). More popularly, ammonia release during the ureolytic reaction can be detected with phenol-hypochlorite (Witte and Medina-Escobar 2001), Nessler's reagent (Sumner 1926), diacetyl monoxime method or Ehrlich's reagent method to allow colorimetric determination of activity and can be detected by ion-selective electrodes. It is possible to include pH indicators/pH sensitive dyes in the assays or simply observe changes with pH electrodes since release of ammonia results in an increase in pH. The amount of ammonia can also be monitored spectrophotometrically using a couple system with NADH-dependent glutamate dehydrogenase (ammonia is a substrate for this enzyme).

The other product of the reaction, CO₂, can be trapped and monitored by radiological methods with ¹⁴C-labeled urea as a substrate (Kaltwasser and Schlegel, 1966).

All of the methods described above are distinct in their sensitivities, ease of use, and susceptibility to interference. Two commonly employed assays mentioned above paragraph namely phenol-hypochlorite and Nessler's method are time consuming, incompatible with common buffers, require high temperature, non-homogenous true color solution and have the disadvantage of using harmful chemicals.

Croston et al. (1955) explain some advantages, disadvantages and the cares that should be followed at titrimetric, pH change and conductimetric methods. The end point of the titrimetric method is not sharp and good results require extreme care in titration. The titration method requires appreciably more time than the others but gives direct measure of urease activity. The pH change method is simple in operation, however, the recorded differences between samples at room temperature may be very small and frequent checking of the meter is required to minimize errors. The sensitivity of the titrimetric and pH-change methods can be improved by using 50°C or 60°C reaction temperatures. However, from laboratory experiences of Croston et al. (1955), their opinion is that maximum precision is obtained by the conductimetric method.

It is easy to identify urease activity. However, quantifying the urease activity is time consuming. Although there are some analytical techniques to determine urease activity, the use of spectro-photometric method is still well popular. The urease activity can also be obtained quantitatively from conductivity method proposed by Whiffin (2004).

(d) Use of plant-derived urease in the field of geotechnical engineering

Nowadays, the urease enzyme is widely used in different fields of industries such as medicinal, construction, agricultural, food, etc. Different kinds of ureolytic bacteria and micro algae, soil urease and plant urease have applied for the above fields. Currently, the potential

for using the PDUICCP through urea hydrolysis in several environmental and geotechnical engineering applications has been demonstrated.

Attempts to use PDUICCP have made during past years to diminish the hydraulic conductivity of soils, to reinforce the loose soils and to prevent soil erosion. However, all of these applications are limited to laboratory scale and any kind of field application has not reported so far. Calcite precipitation using jack bean urease has been investigated to improve the mechanical properties of sand (Park et al., 2014). As a result of that, the UCS of 317 kPa has been achieved, compared to that of sand without jack bean urease. The highest strength of the specimen has been obtained using calcium chloride stock solution rather than the other stock solutions of calcium sources (calcium hydroxide and calcium nitrate). Nemati and Voordouw (2003) have investigated plant urease induced calcite formation, plugging studies in an unconsolidated porous media system and plugging studies of Berea sandstone in a core-flooding system. Jack bean urease having urease activity of 26,100 Units/g solid has been used for the study. According to the results of the study on plugging of unconsolidated porous media by enzymatically formed CC, a significant decrease in permeability of porous media has been observed. At low concentration of enzyme (0.03 g/L), the extent of plugging enhance with the increase in temperature from 22°C to 30°C. Although the temperature is a factor that influence for carbonate precipitation, its influence is less with high concentrations of enzyme used in this study. However, Nemati and Voordouw, explain that proportional increase of reactants and enzyme concentrations above a certain level inhibit the urease activity and decrease the quantity of produced CC and plugging of porous media.

Another attempt to find a method to create building materials using precipitated CC has been done by Bull (2014) with the aid of jack bean (*Canavalia ensiformis*) urease from Sigma-Aldrich Co. LLC. Specific activity of the jack bean urease used in the study is 15,000-50,000 Units/g solid. They have obtained uniform cemented specimens by dissolving urea

and CaCl_2 in one solution and urease in another solution. After curing, and air drying for 2 weeks, maximum UCS of 319 kPa and an elastic modulus of approximately 10 MPa have been obtained. Bull has also observed that CC cementation is not successful when solutions with high concentrations of CaCl_2 and urea. Hence, it is beneficial to study tolerable limits of concentrations of each solution for a better output. Neupane et al. (2013) have considered plant urease (jack bean) induced CC precipitation technique for both small scale laboratory samples and samples in drum cans with a diameter of 56 cm and a height of 85 cm to evaluate the applicability of this technique for larger-scale applications. They have reported that the amount of precipitated calcite helps to modify the mechanical properties of sandy soil. However, mechanical tests using the same grouting condition have not conducted. Hamdan et al. (2013) have conducted laboratory column tests (Acrylic tube tests and Triaxial tests) for cemented specimens made using silica sand, urea, CaCl_2 and plant-derived urease, and obtained significant strength increase over non-cemented specimens at the same relative density. X-Ray Diffraction (hereafter, XRD) analysis and Scanning Electron Microscopy (hereafter, SEM) observations confirm that CC (specifically calcite) is the cementing agent, and acid digestion performs to determine the amount of CC.

In addition to the urease from jack bean, some investigations have been done by using other types of seeds urease. Sword beans (*Canavalia gladiata*) urease is an example. The permeability as well as strength characteristics have been examined using sword beans urease (Yasuhara et al., 2012). The permeability of the improved samples has been reduced by more than one order of magnitude. Maximum UCS obtained is 1620 kPa. An increase in compressive strength up to 1.6 MPa has also been obtained for oven dried specimens made using commercially available urease, with the activity of 2970 Units/g (Neupane et al., 2014). The strength enhancement and permeability reduction of subsurface soils are essential features for geotechnical engineering applications. It is necessary to achieve UCS value of

100 kPa to avoid ground liquefaction during earthquakes (Yamazaki et al., 1998). This target value is successfully achieved by the past research studies based on PDUICCP. Hence, this technique can be applied for ground reinforcement and hence to mitigate ground liquefaction. However, it is necessary to investigate the initial ground condition for soil type, soil pH, contaminants in the soil, permeability characteristics, etc. before applying this technique in-situ in large scale. A homogenous, well-controlled distribution of CC should also be achieved prior to the real field applications. Therefore, future investigations should focus on these areas to get maximum output from this technique.

1.3 SCOPE

The scope of this thesis is the investigation of laboratory scale novel ground improvement techniques using artificial biomineral precipitation. The thesis is consisted with two major sections with different sub sections as follows.

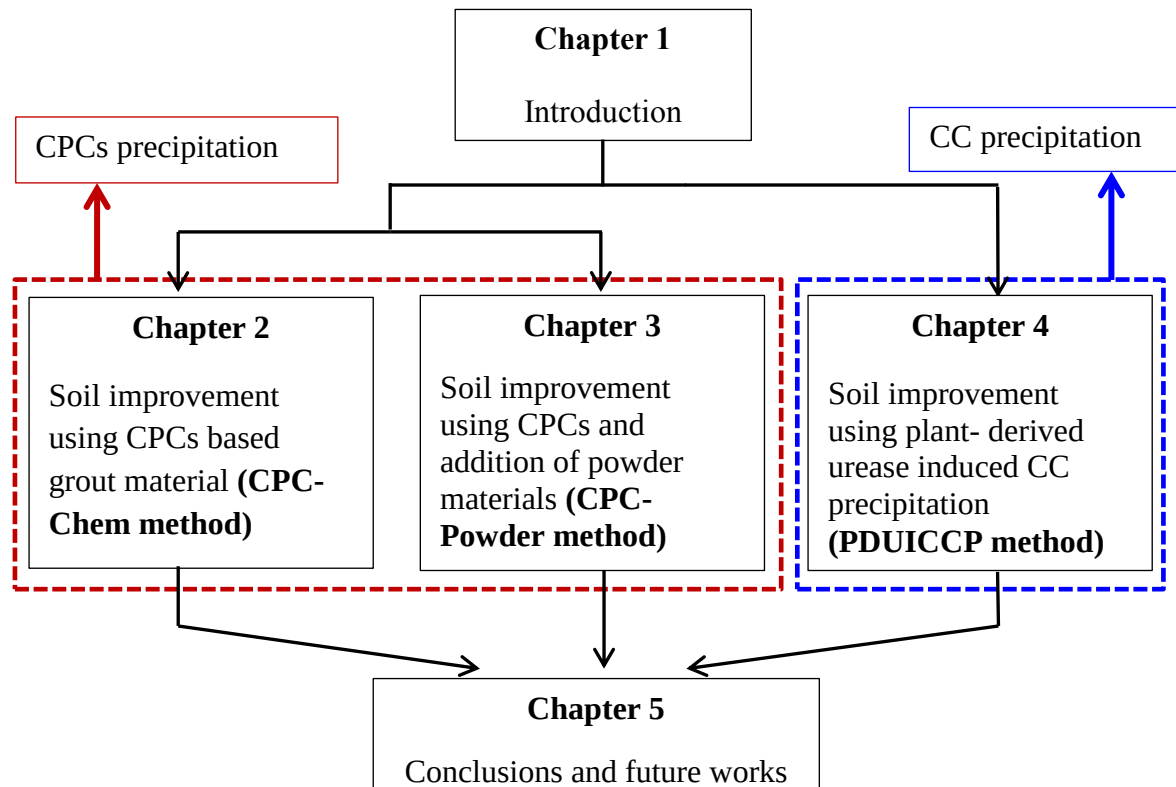


Fig. 1.5: Scope of the thesis.

In chapter 1, the background for the current research, literature review related to the current research, objectives and originality of the research are described. One of the mixing method for ground improvement named as CPC-Chem method in which sand is cemented by mixing different concentrations of Ca^{2+} and PO_4^{3-} solutions is described in Chapter 2. Precipitated CPCs in between sand particles help to create bridges between sand particles and hence, to strengthen the sandy soil. As an improvement for the CPC-Chem method, CPC-Powder method is introduced and described in Chapter 3. The purpose of the use of CPC-Powder method is to improve the UCS more than CPC-Chem method. As mentioned above, Chapter 2 and Chapter 3 describe sand cementation using artificially formed PO_4^{3-} biominerals. Furthermore, another method for sand cementation using artificially formed carbonate biomineral is described in Chapter 4. Finally, Chapter 5 provides some concluding remarks based on the results obtained from Chapters 2, 3 and 4 as a guide for future studies.

1.4 ORIGINALITY AND USEFULNESS OF THE STUDY

Attempt to use of CPCs for sand cementation is a novel experience for ground improvement. Therefore, literature findings related to sand cementation using CPCs are very few to make it suitable for effective ground improvement method. According to the results of UCS values obtained from previous researches related to CPC-Chem method, the UCS values have been varied according to the Ca^{2+} and PO_4^{3-} concentrations as well as the sources of Ca^{2+} and PO_4^{3-} . At the same time, the rate of self-setting of CPCs has been changed with Ca^{2+} and PO_4^{3-} concentrations and therefore, temporal variation of UCS has been changed accordingly. However, the number of testing cases used in those researches is insufficient to make a conclusion. In order to understand these matters more clearly, the Chapter 1 of this thesis is described strength characteristics of the CPC precipitated sandy materials by changing the concentrations of Ca^{2+} and PO_4^{3-} solutions in a wide range and find out a range

of concentrations of Ca^{2+} and PO_4^{3-} that gives UCS of more than 100 kPa. At the same time, different sources of Ca^{2+} and PO_4^{3-} other than the previous studies were selected. Mineral identification of the different types of precipitated CPCs and find out the relationship between mineral type and UCS was another novelty of this study. Apart from that, the effect of particle size of the sand material on UCS was newly investigated during this study. Widening of the investigations of the CPC-Chem method by changing the concentrations of Ca^{2+} and PO_4^{3-} , the sources Ca^{2+} and PO_4^{3-} and the sand material that subjects to the cementation is important to understand and get an idea about the practical use of this method in future.

The CPC-Powder method described in Chapter 2 is differing from previous studies as follows.

Kawasaki and Akiyama, 2013	➡	Ca^{2+} source = CA PO_4^{3-} source= DAP Added Powders= TCP, MgP, CC, MgC
Amarakoon et al., 2014	➡	Ca^{2+} source = CA PO_4^{3-} source= DAP Added Powders= CC from Scallop shell powder
This study	➡	Ca^{2+} source = CA PO_4^{3-} source= DPP Added Powders= TCP, MgP, CC, MgC

To bring the CPC-Powder method to the practical stage at actual ground, it is very important to increase the number of sources of powder materials and Ca^{2+} and PO_4^{3-} sources available for the precipitation of cement materials. Then there is a possibility to use this method easily according to the availability of sources. Furthermore, the usefulness of this study may further improved by adding alternative powder materials from the viewpoint of waste utilization and cost effectiveness. The use of egg shells, oyster shells or scallops shells

as alternative to the commercially available CC is one such example.

The Chapter 4 in this thesis is described about sand cementation using PDUICCP. All the previous researches related to PDUICCP are based on commercially available purified plant urease. However, the price of commercially available urease (urease from jack bean) is expensive (according to the prices of Wako Pure Chemical Industries Ltd, 1 g of urease is about 14,500 JPY) to use it for large scale practical applications. In this study, crude extract of watermelon seeds that are discarded as kitchen garbage were used as an alternative source to the commercially available purified urease. The usefulness of this study from the view point of cost effectiveness may be one hint to promote this method as a large scale practical application in future. Furthermore, previous studies related to the PDUICCP have been focused to obtain several kPa values of UCS. However, this study focused to obtain several kPa to MPa values of UCS for identify the range of applicability of this method such as to mitigate the liquefaction and to create artificial soft rocks.

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CHAPTER 2: Soil improvement using CPCs based grout material (CPC-Chem method)

2.1 INTRODUCTION

Some of the major challenges in the current construction industry are the incompetence of the ground to provide proper supporting capability for overlying structures, vulnerability of slopes and ground to catastrophic natural or anthropogenic events (geo-disasters) such as earthquakes, floods, landslides, and embankment and dam failures. Although, the cement-based stabilizers are widely used for ground improvement and therefore, act as countermeasures against geo-disasters, it involves major environmental issues such as large CO₂ emissions during cement production, high energy cost for cement production, and re- excavation of cement improved ground. Therefore, innovative and sustainable ground improvement practices are necessary to withstand the stresses resulting from geo-disasters and hence to prevent or mitigate them. As a grout material, CPCs have unique physical and chemical properties which are able to overcome some of the above issues arising from cement based hardeners.

Regarding CPCs, the solubility of CPCs depends on the pH of the surrounding environment (Fig. 1.1) (Tung, 1998). Gel-like or amorphous CPCs change into HA over time (Fig. 1.2) (Tung, 1998). Therefore, CPC hardens after injection into soil and rock because of the self-setting mechanism. Phosphate and calcium stock solutions can be made from fertilizers, and calcium and phosphate can also be extracted from the bones of livestock and the shells of aquatic animals, respectively. Furthermore, precipitated CPCs are non-toxic. Unlike concrete, re-excavated muck that consists of soil, rock, and CPC grout is recyclable as agricultural fertilizer. These advantages make it suitable for geotechnical engineering applications (Akiyama and Kawasaki, 2012a).

With the purpose of the use of CPCs for engineering applications especially in geotechnical engineering applications, CPC precipitation has been determined in an in vitro examination by using phosphate and calcium stock solutions (Akiyama and Kawasaki, 2012a). The maximum UCS of sand test pieces cemented with CPCs was found to be 63.5 kPa after 14 days of curing time by using CA and DAP as Ca^{2+} and PO_4^{3-} sources respectively (Akiyama and Kawasaki, 2012a). According to the results of other UCS values obtained by them, they have been varied according to the Ca^{2+} and PO_4^{3-} concentrations, Ca/P ratio and the sources of Ca^{2+} and PO_4^{3-} . At the same time, the rate of self-setting of CPCs has been changed, and temporal variation of UCS is changed accordingly. In order to understand these matters more clearly, detail investigations should be carried out.

2.2 OBJECTIVES

The aim of the Chapter 2 was to examine the variation of strength of sandy materials by changing the concentrations of Ca^{2+} and PO_4^{3-} solutions and find out a range of concentrations of Ca^{2+} and PO_4^{3-} that gives UCS of more than 100 kPa, which is the required strength for mitigating ground liquefaction (Yamazaki et al., 1998), and hence, to apply this method as a countermeasure for liquefaction.

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2.3 MATERIALS AND EQUIPMENT

2.3.1 Materials

(a) Commercially available sand

Two types of clean sands with different particle sizes named as Toyoura and Mikawa that can be obtained commercially in Japan were used after drying in an oven up to 110°C to simulate a granular ground material (Table 2.1).

Table 2.1: Physical properties of Toyoura and Mikawa sand.

Physical property	Toyoura sand	Mikawa sand
Maximum Density (cm ³)	1.645	1.476
Minimum Density (cm ³)	1.335	1.256
Particle Density (g/cm ³)	2.64	2.66
Mean Diameter (μm)	200	870

(b) Reagents

Calcium acetate- $\text{Ca}(\text{CH}_3\text{COO})_2$ (CA) and dipotassium phosphate- K_2HPO_4 (DPP) with relatively high solubility were chosen for convenient handling during practical application.

2.3.2 Equipment

(a) Cylindrical plastic molds and accessories

Cylindrical plastic molds were used for preparing sand specimens for UCS test. The diameter of the mold is 50 mm and the height is 100 mm. A collar was placed on top of the mold while preparing top layer of sand specimen. A mold remover was used to separate the plastic mold and test specimen.

(b) Hand rammer

A Hand rammer (Weight = 758.91 g, Length= 250 mm, Diameter of the cross section = 30 mm) was used to compact the sand into the mold.

(c) Magnetic stirrer

A Magnetic stirrer was used to dissolve chemical reagents in water.

(d) Uniaxial compression test apparatus

A uniaxial compression test apparatus was used for measuring UCS of solidified test specimens. It consists with three devices: uniaxial compression testing machine (Makoto Research sha Co., Ltd., a desktop -type high capacity compression testing machine, T266-31100), logger (Hamada Denki, HMD multi logger Jr) and PC software (Hamada Denki, HM1616Sx / Tx series).

(e) pH meter (Eutech Instruments Pte., Ltd., Singapore)

(f) Low vacuum SEM machine

A SEM machine (TM 3000 Miniscope, HITACHI) was used to observe the microscopic images of cemented sand test pieces.

(g) Rigaku multiflex Powder X-ray Diffractometer for XRD analysis

2.4 METHODOLOGY

2.4.1 Preparation of sand test pieces for UCS test and conducting UCS test

In this Chapter 2, CA and DPP were chosen for convenient handling during practical application. Required concentrations of each chemical reagent were prepared, and hereafter, concentrations are presented as M (mol/L).

Initially, standard sand test pieces were made from 320.09 g of Toyoura sand and 73.3 mL of CPC-Chem only (36.65 mL from each of CA and DPP solution) following a previous report (Akiyama and Kawasaki, 2012a). Weight of sand and volume of solution required to fill voids are calculated as follows. The weight of sand required (g) and the volume of solution required (cm³) for Mikawa sand can also be calculated as below. The obtained values for Mikawa sand are 287.21 g and 86.6 mL respectively.

$$\text{Mold height, } h \text{ (mm)} = 100$$

$$\text{Mold diameter, } d \text{ (mm)} = 50$$

$$\text{Thickness of overhead projector sheet (mm)} = 0.1$$

Therefore,

$$\text{Actual mold height, } h_1 \text{ (mm)} = 100 - 0.1 = 99.9$$

$$\text{Actual mold diameter, } d_1 \text{ (mm)} = 50 - 0.1 - 0.1 = 49.8$$

$$\text{Total volume, } V_t \text{ (cm}^3\text{)} = \pi d_1^2 \times h_1 = 194.6$$

$$\text{Particle density, } \rho_s \text{ (Toyoura sand) (g/cm}^3\text{)} = 2.64$$

$$\text{Maximum density, } \rho_{\max} \text{ (Toyoura sand) (g/cm}^3\text{)} = 1.645$$

$$\text{Weight of sand required, } m_g \text{ (g)} = \rho_{\max} \times V_t = 1.645 \times 194.6 = \underline{320.09}$$

$$\text{Volume of solution required (cm}^3\text{)} = V_t - m_g / \rho_s = 194.6 - 320.09 / 2.64 = \underline{73.3}$$

CPC-Chem was added to 320.09g of Toyoura sand and the mixture was uniformly mixed in a stainless steel ball for 2 min. and then divided in to quarters. Each quarter was then placed in to the plastic mold. Before placing the mixture of sample into the mold, the inner wall of each mold was covered with a 0.1 mm thick overhead projector sheet to avoid disturbances to the test pieces during their removal from the mold. The mixture was tamped down 30 times by a hand rammer at each filling of quarter to the mold. Finally, the upper edges of the test pieces were slightly trimmed to be flat and covered with Parafilm M (Structure Probe, Inc., West Chester, PA) to avoid desiccation. The molded test pieces were subsequently cured in an airtight container at high humidity for 1, 7, 14 and 28 days as required. The test pieces were removed carefully from the mold after curing and the UCS was measured using uniaxial compression at an axial strain rate of 1% /min. All test pieces were prepared, cured and tested at 25 °C. In order to investigate the repeatability of measurements of UCS test, two test pieces were made for each test case under identical conditions. However, only one test piece was made for few cases. The pH of the test pieces was calculated as an average of three measurements (top, bottom, and middle of each test piece) using a pH Spear.

2.4.2 SEM observation

Segments of the UCS test pieces were observed using SEM machine (TM 3000 Miniscope, HITACHI) just after the UCS tests.

2.4.3 XRD Analysis

XRD analysis was conducted for the powdered CPCs. Different concentrations of Ca^{2+} and PO_4^{3-} sources were mixed to identify the mineral change with concentrations of Ca^{2+} and PO_4^{3-} sources. The prepared CPC homogel mixtures were kept at 25°C until required curing period (for this study, 28 days curing time was selected for XRD analysis). After required curing period, the mixtures were dried at 110°C about 1 day, and ground using a mortar and pestle to get a powder form of CPC.

2.5 RESULTS

2.5.1 Relationship between curing time (days) and UCS of Toyoura sand test pieces

Temporal variation of UCS under different concentrations of CA and DPP and two different Ca/P ratios are represented in Fig. 2.1. According to the results, when the concentrations of CA and DPP increase, UCS values increase in both Ca/P ratios (Ca/P=1 and Ca/P=0.5). Furthermore, the initial rate of increment of UCS increased with the increase of both concentrations of Ca^{2+} and PO_4^{3-} sources.

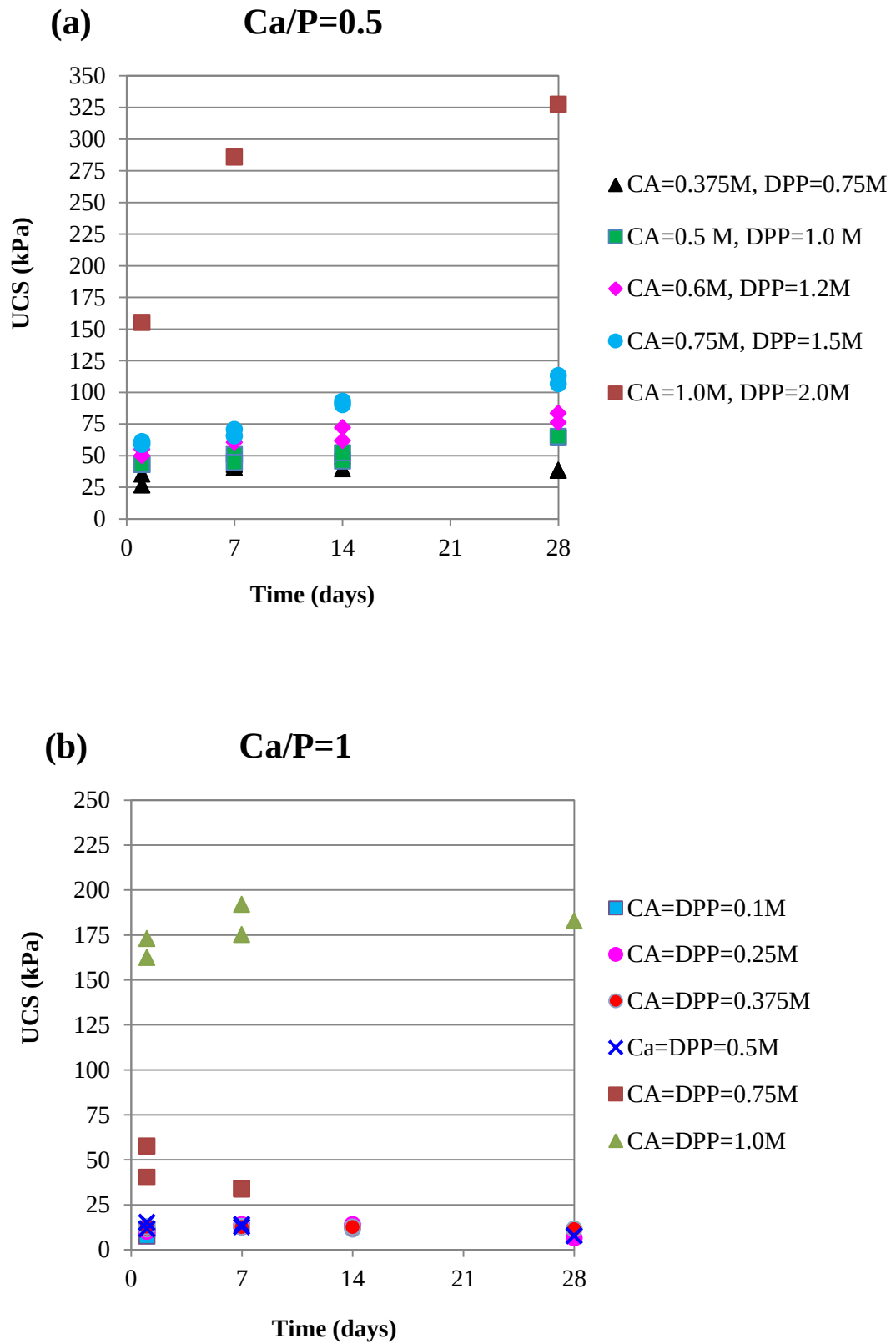


Fig. 2.1: Relationship between curing time (days) and UCS of Toyoura sand test pieces (a) Ca/P = 0.5 and (b) Ca/P = 1.0.

2.5.2 Relationship between pH and UCS of Toyoura sand test pieces

According to the Fig. 2.2, pH was not a governing factor to increase UCS under same Ca/P ratio, because pH was not varied under same Ca/P ratio (Ca/P=0.5 and Ca/P=1 in this study). When Ca/P=0.5, pH is neutral or weakly alkaline and when Ca/P=1, pH is weakly acidic. Furthermore, we observed a decrement of pH with the increment of Ca/P ratio in detail as shown in Fig. 2.3.

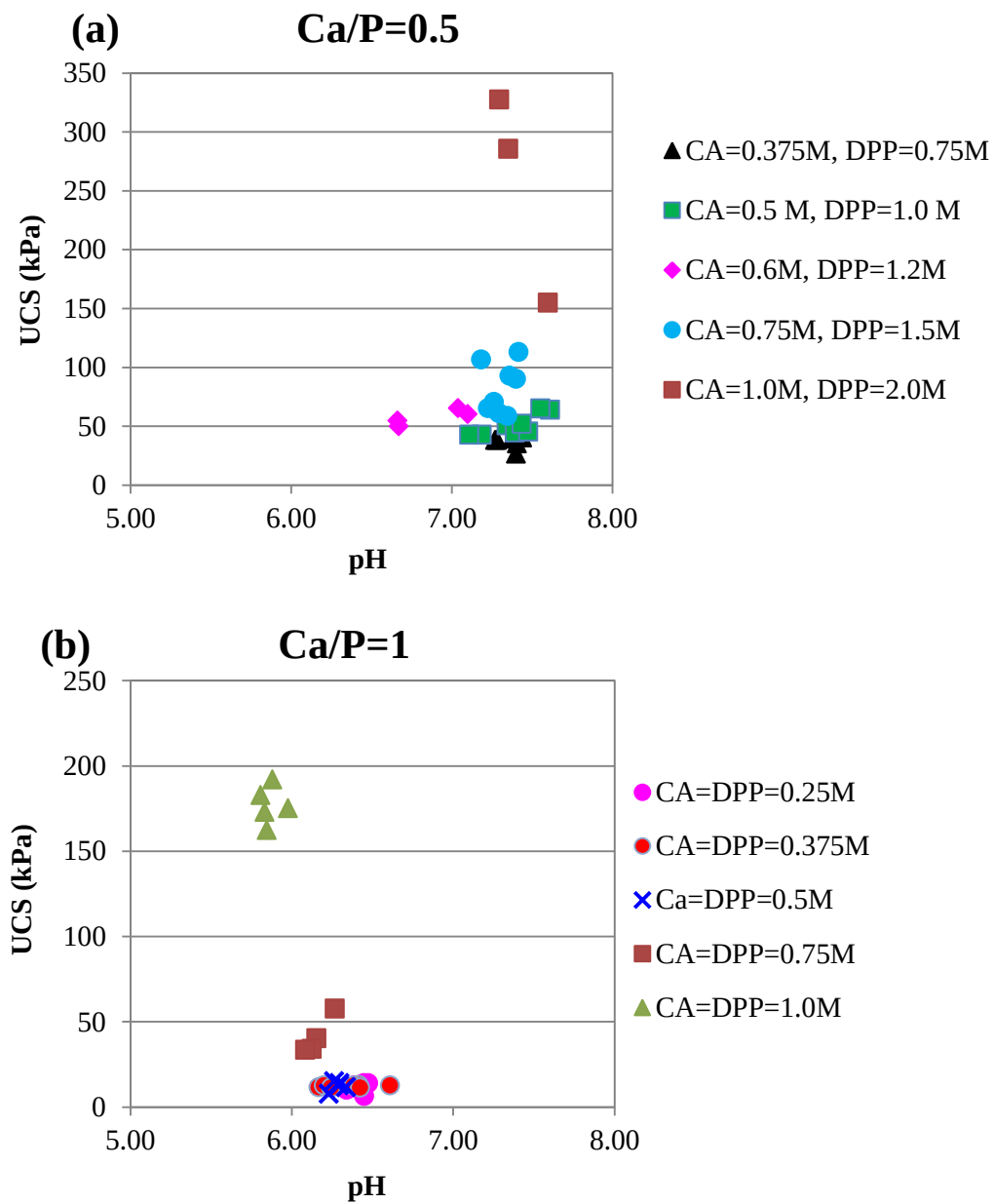


Fig. 2.2: Relationship between pH and UCS of Toyoura sand test pieces (a) Ca/P = 0.5 and (b) Ca/P = 1.0.

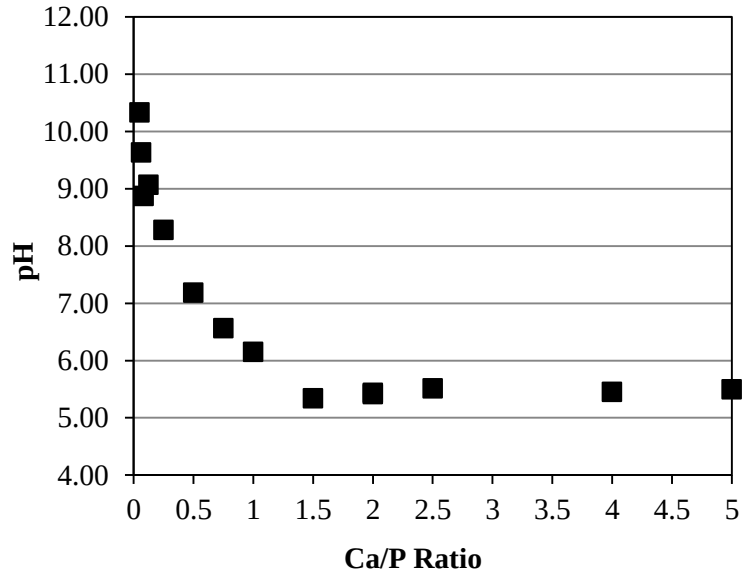


Fig. 2.3: pH variation with Ca/P ratio.

2.5.3 Relationship between wet density and UCS of Toyoura sand test pieces

In both Ca/P ratios, UCS increased with the increase of wet density of test specimen just before UCS test (Figs .2.4 and 2.5).

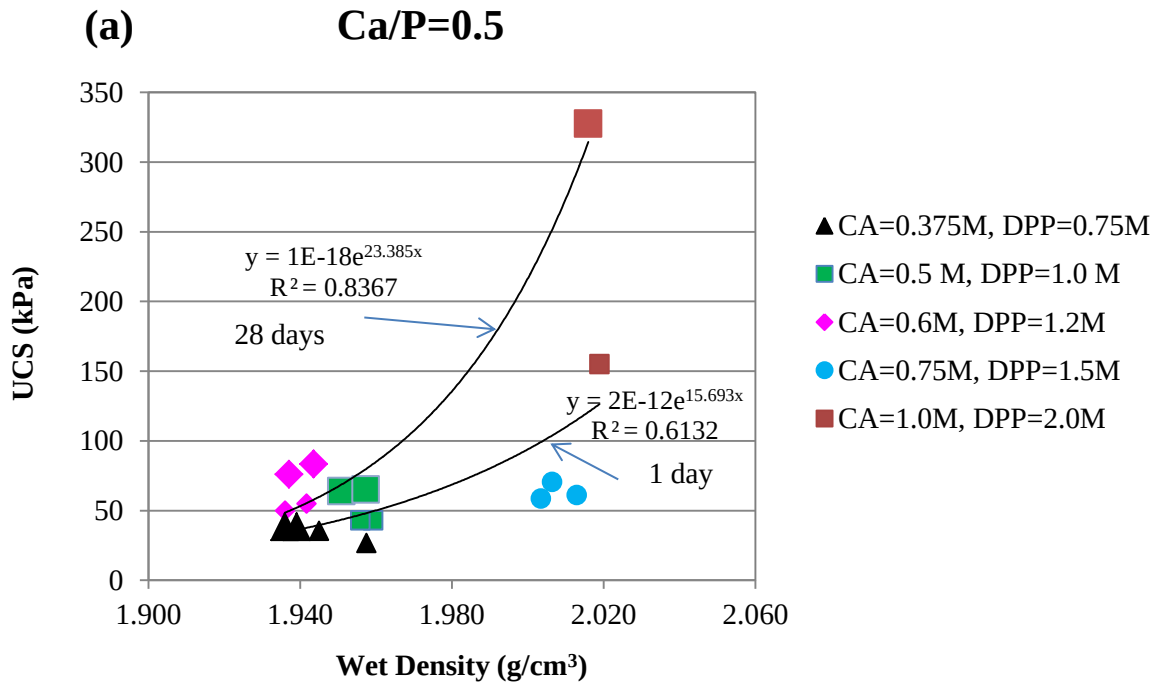


Fig. 2.4: Relationship between wet density and UCS of Toyoura sand test pieces with Ca/P = 0.5.

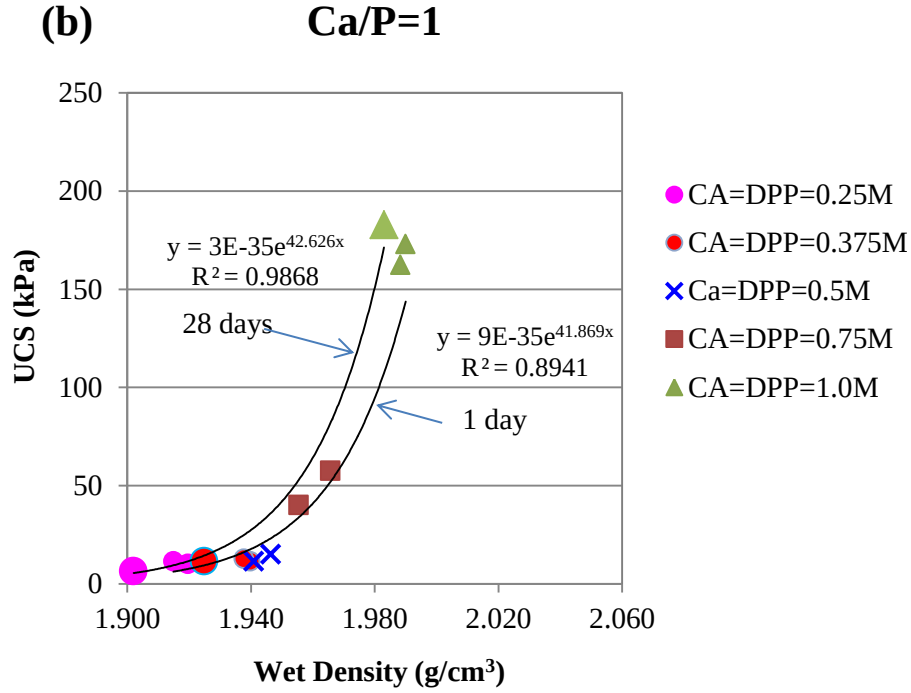


Fig. 2.5: Relationship between wet density and UCS of Toyoura sand test pieces with Ca/P = 1.

2.5.4 SEM observation

SEM images clearly show the binding of sand particles with precipitated CPCs. The precipitated amount of CPCs increased with the increase of the concentrations of CA and DPP (Figs. 2.6 and 2.7) for same Ca/P ratio. When Ca/P ratio is 1, plate-like crystals could be observed (Fig. 2.7).

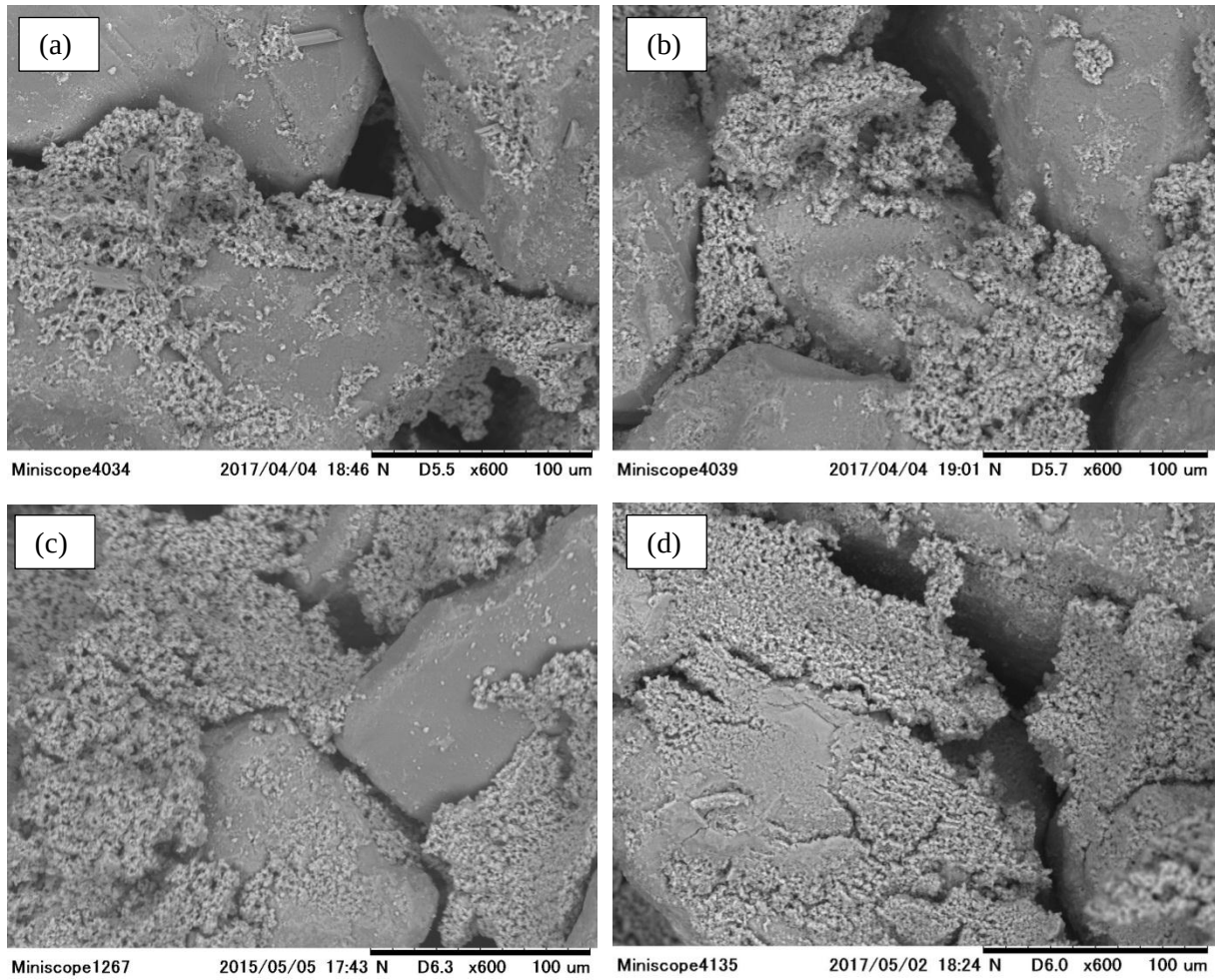


Fig. 2.6: SEM images of Toyoura sand test pieces (28 days of curing period) cemented by CA and DPP with $\text{Ca/P} = 0.5$ (x 600) (a) $\text{CA}=0.375\text{M}$, $\text{DPP}=0.75\text{M}$ (b) $\text{CA}=0.5\text{M}$, $\text{DPP}=1.0\text{M}$, (c) $\text{CA}=0.75\text{M}$, $\text{DPP}=1.5\text{M}$ (d) $\text{CA}=1.0\text{M}$, $\text{DPP}=2.0\text{M}$.

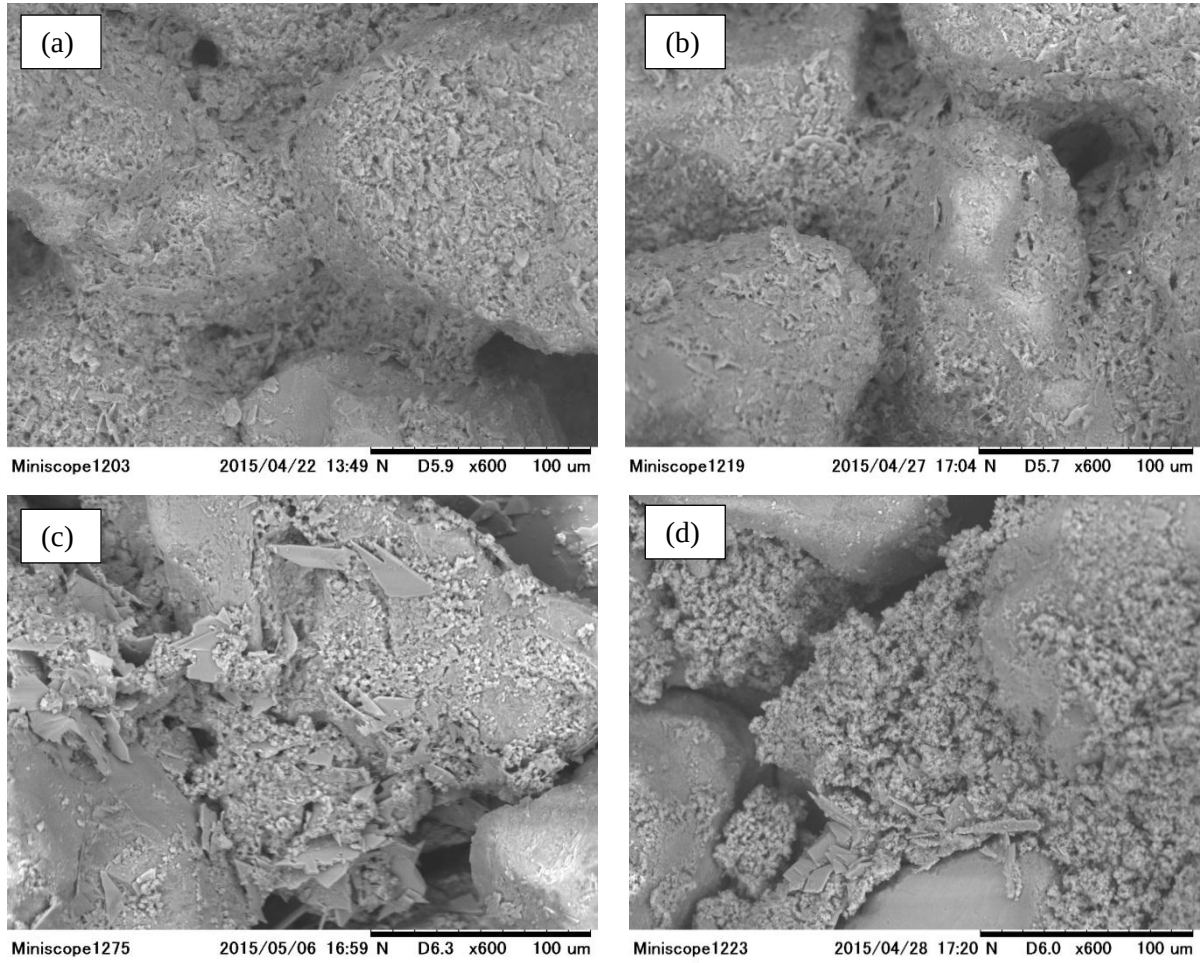


Fig. 2.7: SEM images of Toyoura sand test piece (28 days of curing period) cemented by CA and DPP with $\text{Ca/P} = 1.0$ (x 600) (a) $\text{CA}=0.375\text{M}$, $\text{DPP}=0.375\text{M}$ (b) $\text{CA}=0.5\text{M}$, $\text{DPP}=0.5\text{M}$, (c) $\text{CA}=0.75\text{M}$, $\text{DPP}=0.75\text{M}$ (d) $\text{CA}=1.0\text{M}$, $\text{DPP}=1.0\text{M}$.

2.5.5 UCS of sand test specimens made by different types of sands

In the Section 2.3.1, we introduced two different silica sands for this study and those sands were used to prepare sand test specimens for measuring the UCS and finally, to compare the UCS values of test pieces made using two types of sand. The results are shown in Figs. 2.8, 2.9 and 2.10. According to Fig.2.8, at low concentrations, test pieces made using Toyoura sand showed higher UCS than the test pieces made using Mikawa sand. At

intermediate concentrations, UCS is almost same for both sands, and at higher concentrations, the test pieces made using Mikawa sand showed UCS higher than Toyoura sand.

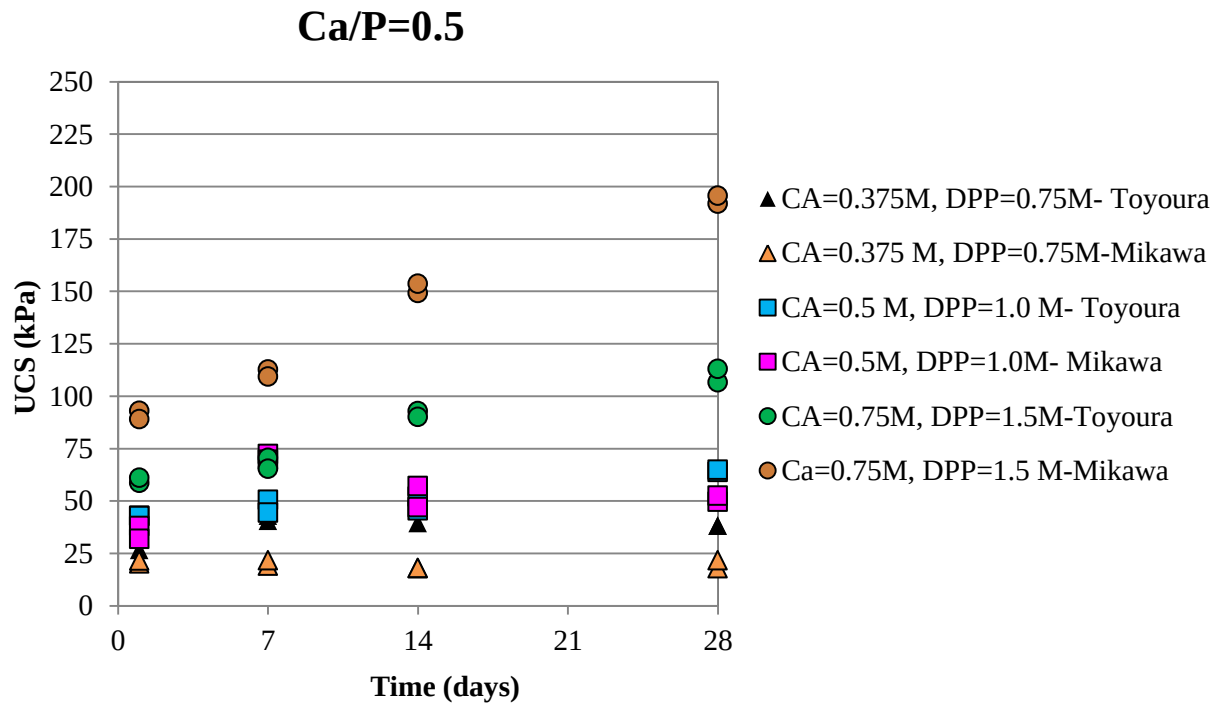


Fig. 2.8: Temporal variation of UCS (Ca/P=0.5).

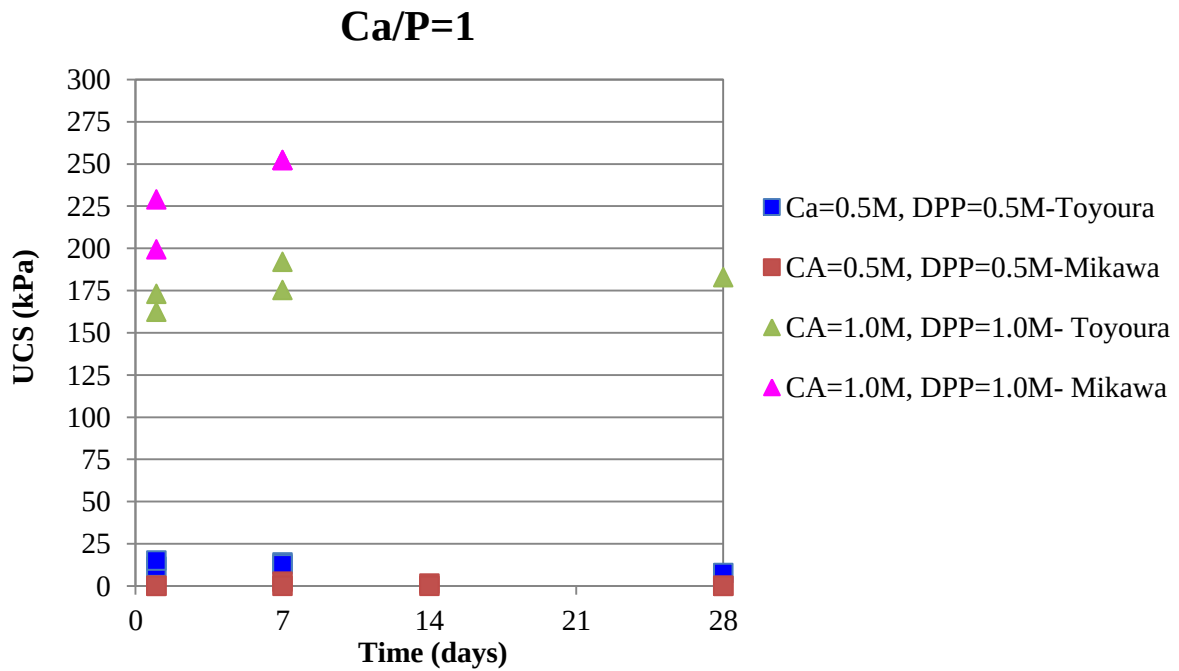


Fig. 2.9: Temporal variation of UCS (Ca/P=1).

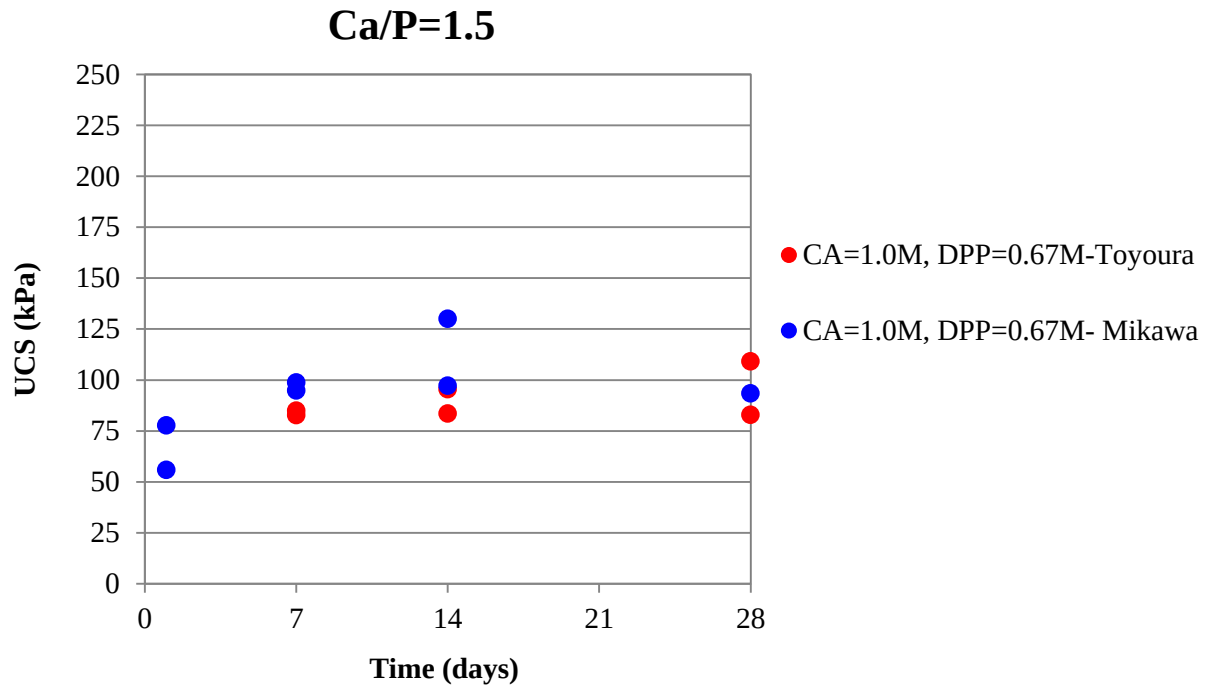


Fig. 2.10: Temporal variation of UCS (Ca/P=1.5).

2.5.6 Behavior of stress (σ) - strain (ϵ) curves obtained at the UCS measurement

As discussed in the section 2.4.1, for most of the test cases, two identical test pieces were prepared and tested for measuring UCS. According to the compressive stress (σ)–strain (ϵ) curves of the test pieces obtained while measuring the UCS, the two stress-strain curves obtained for two identical test pieces made for each test case showed almost the same behavior in terms of shape (Fig. 2.11). Moreover, the compressive strain decreased and shifted leftward and Young's modulus increased with the increment of UCS without in the case CA=0.75M, DPP=1.5M in Fig. 2.11(a).

According to the compressive stress (σ)–strain (ϵ) curves of the test pieces made using Toyoura and Mikawa sands, specimens made using coarse sand (Mikawa sand in this study) showed lower compressive strain than fine sand (Toyouura sand in this study) (Fig. 2.12).

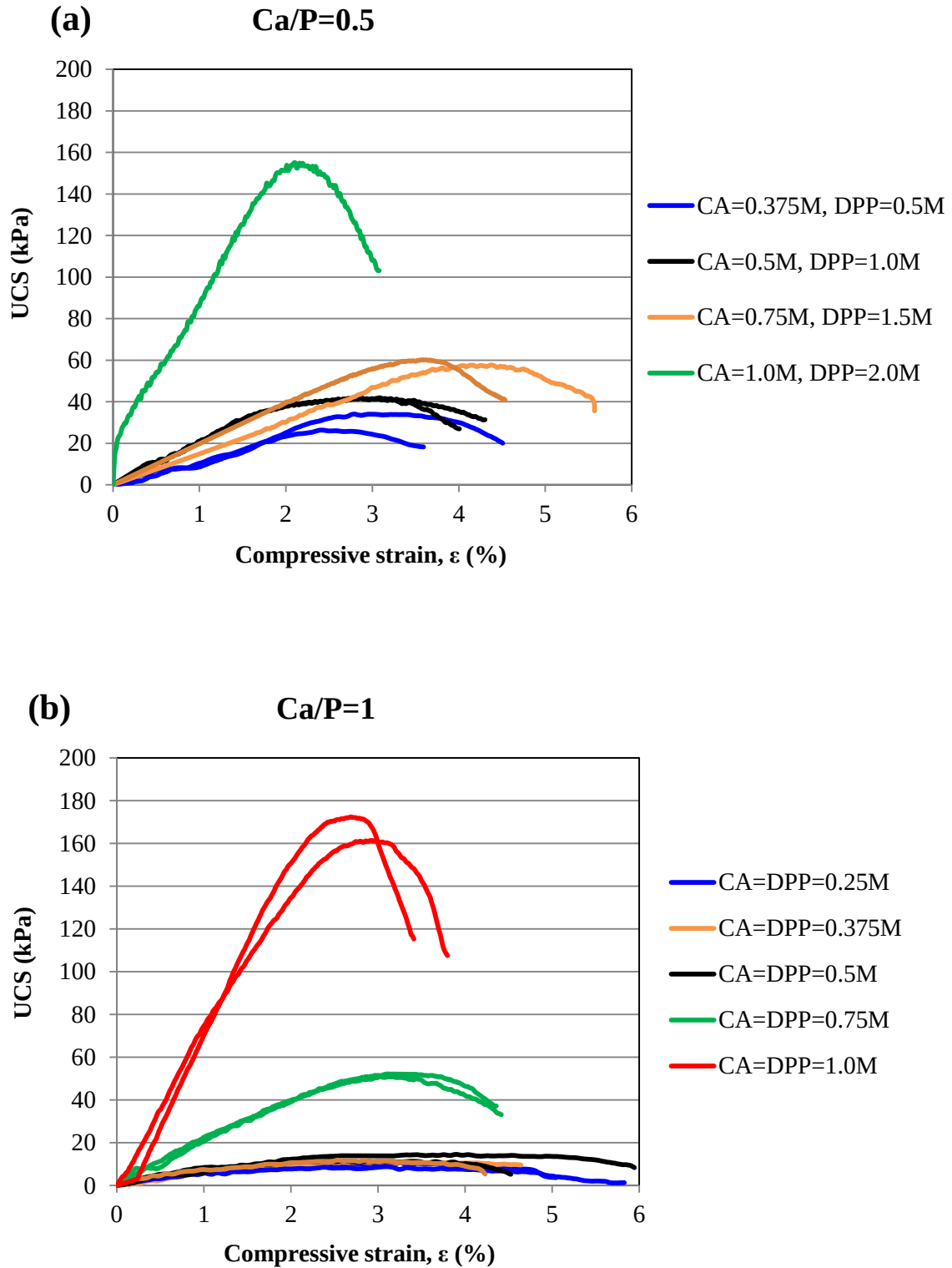


Fig. 2.11: Stress-strain curves of the test pieces made using Toyoura sand (a) Ca/P=0.5 and (b) Ca/P=1.

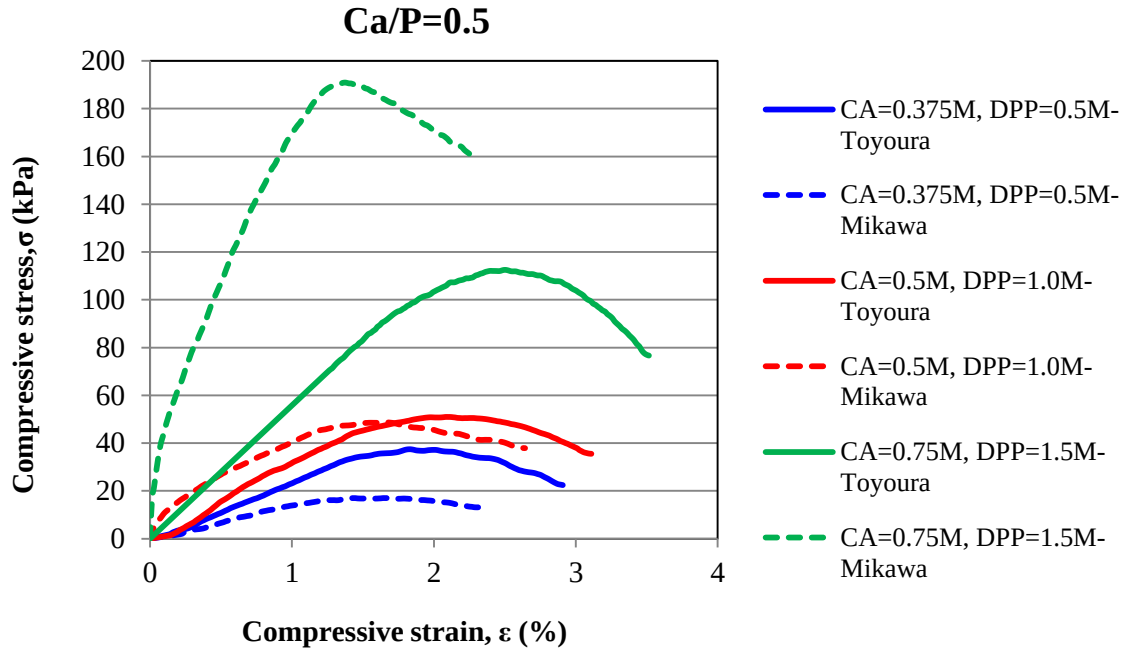


Fig. 2.12: Stress-strain curves of the test pieces made using Toyoura and Mikawa sand.

2.5.7 Summary of UCS with different concentrations of Ca^{2+} (CA) and PO_4^{3-} (DPP)

The summary of UCS values obtained by changing Ca^{2+} and PO_4^{3-} concentrations is shown in Fig. 2.13. According the values of UCS, we could identify three different areas A, B and C as in Fig. 2.13. As an average, the 1 day UCS values in Area A were less than 20 kPa. Even after 28 days of curing, any improvement could not be seen in the test cases relevant to Area A. It means the self-setting of CPCs was very low or almost zero in the area A during 28 days of curing period. The UCS in Area B was around in between 20 kPa and 100 kPa after 1 day and the UCS increased with time due to self-setting mechanism and in some cases, the UCS exceeded the value of 100 kPa after 28 days. The UCS in Area C was more than 100 kPa even after 1 day curing period. The self-setting of CPCs was happen in Area B and Area C and therefore, strength improvement could be seen within 28 days of curing period. Because of the self-setting of CPCs was very low or almost zero in the Area A, the UCS increment was not observed in Area A within 28 days of curing period. (All the

UCS results obtained in this study are shown in Tables 2.2 and 2.3 with their wet densities and pH values).

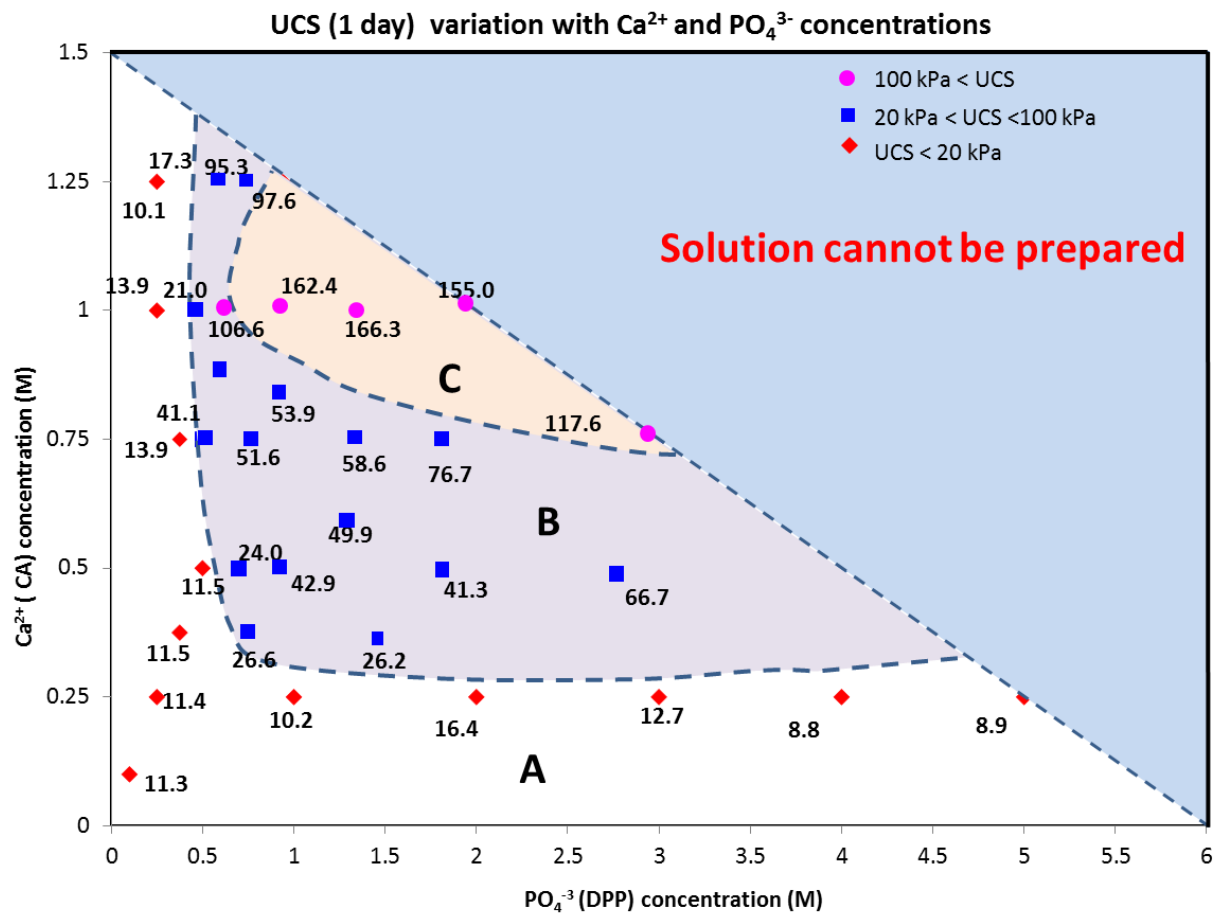


Fig. 2.13: Variation of UCS (1 day) with Ca^{2+} and PO_4^{3-} concentrations.

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand) (To be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.375	0.75	0.5	1	26.6	7.40	1.957
CA	DPP	0.375	0.75	0.5	1	35.3	7.41	1.945
CA	DPP	0.375	0.75	0.5	7	42.9	7.43	1.951
CA	DPP	0.375	0.75	0.5	7	40.5	7.44	1.947
CA	DPP	0.375	0.75	0.5	14	49.5	7.48	1.961
CA	DPP	0.375	0.75	0.5	14	39.4	7.38	1.956
CA	DPP	0.375	0.75	0.5	28	38.2	7.27	1.939
CA	DPP	0.375	0.75	0.5	28	38.2	7.28	1.936
CA	DPP	0.5	1.0	0.5	1	42.9	7.19	1.959
CA	DPP	0.5	1.0	0.5	1	42.8	7.11	1.956
CA	DPP	0.5	1.0	0.5	7	50.6	7.34	1.961
CA	DPP	0.5	1.0	0.5	7	44.5	7.39	1.969
CA	DPP	0.5	1.0	0.5	14	45.5	7.48	1.963
CA	DPP	0.5	1.0	0.5	14	52.1	7.44	1.967
CA	DPP	0.5	1.0	0.5	28	63.9	7.61	1.951
CA	DPP	0.5	1.0	0.5	28	65.0	7.55	1.957
CA	DPP	0.6	1.2	0.5	1	49.9	6.67	1.936
CA	DPP	0.6	1.2	0.5	1	54.8	6.66	1.942
CA	DPP	0.6	1.2	0.5	7	60.3	7.10	1.941
CA	DPP	0.6	1.2	0.5	7	65.4	7.04	1.942

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand) (To be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.6	1.2	0.5	14	61.7	7.06	1.927
CA	DPP	0.6	1.2	0.5	14	72	7.03	1.942
CA	DPP	0.6	1.2	0.5	28	75.9	7.01	1.937
CA	DPP	0.6	1.2	0.5	28	83.3	7.12	1.944
CA	DPP	0.75	1.5	0.5	1	58.6	7.35	2.003
CA	DPP	0.75	1.5	0.5	1	61.1	7.29	2.013
CA	DPP	0.75	1.5	0.5	7	70.5	7.26	2.006
CA	DPP	0.75	1.5	0.5	7	65.4	7.23	-
CA	DPP	0.75	1.5	0.5	14	92.8	7.36	-
CA	DPP	0.75	1.5	0.5	14	90.1	7.40	-
CA	DPP	0.75	1.5	0.5	28	106.6	7.18	-
CA	DPP	0.75	1.5	0.5	28	113.1	7.42	-
CA	DPP	1.0	2.0	0.5	1	155	7.60	2.019
CA	DPP	1.0	2.0	0.5	7	285.6	7.35	2.018
CA	DPP	1.0	2.0	0.5	28	327.4	7.30	2.016
CA	DPP	0.25	0.25	1.0	1	11.4	6.35	1.915
CA	DPP	0.25	0.25	1.0	1	10.1	6.34	1.920
CA	DPP	0.25	0.25	1.0	7	12.6	6.39	1.912
CA	DPP	0.25	0.25	1.0	7	13.9	6.47	1.914
CA	DPP	0.25	0.25	1.0	14	12.6	6.43	1.913

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand) (To be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.25	0.25	1	14	13.9	6.45	1.910
CA	DPP	0.25	0.25	1	28	6.4	6.45	1.886
CA	DPP	0.375	0.375	1	1	11.5	6.17	1.940
CA	DPP	0.375	0.375	1	1	12.7	6.20	1.938
CA	DPP	0.375	0.375	1	7	12.8	6.42	1.932
CA	DPP	0.375	0.375	1	7	12.5	6.38	1.930
CA	DPP	0.375	0.375	1	14	11.4	6.42	1.918
CA	DPP	0.375	0.375	1	14	12.6	6.61	1.923
CA	DPP	0.375	0.375	1	28	11.4	6.24	1.925
CA	DPP	0.5	0.5	1	1	11.5	6.34	1.941
CA	DPP	0.5	0.5	1	1	15.1	6.26	1.946
CA	DPP	0.5	0.5	1	7	13.9	6.30	1.941
CA	DPP	0.5	0.5	1	7	12.7	6.29	1.931
CA	DPP	0.5	0.5	1	28	7.7	6.23	1.929
CA	DPP	0.75	0.75	1	1	40.2	6.15	1.955
CA	DPP	0.75	0.75	1	1	57.6	6.27	1.966
CA	DPP	0.75	0.75	1	7	34.0	6.12	1.971
CA	DPP	0.75	0.75	1	7	33.6	6.08	1.954
CA	DPP	1.0	1.0	1	1	173	5.83	1.990
CA	DPP	1.0	1.0	1	1	162.4	5.85	1.988

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand) (To be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	1.0	1.0	1	7	192	5.88	1.990
CA	DPP	1.0	1.0	1	7	175.2	5.98	1.987
CA	DPP	1.0	1.0	1	28	182.8	5.81	1.968
CA	DPP	1	0.75	1.33	1	106.6	5.63	1.931
CA	DPP	1	0.75	1.33	1	112.8	5.60	1.924
CA	DPP	1.25	0.94	1.33	1	97.6	5.74	1.972
CA	DPP	1.25	0.94	1.33	7	135.2	5.25	1.972
CA	DPP	1.25	0.94	1.33	28	154.5	5.38	1.983
CA	DPP	1	0.67	1.5	7	85	5.48	1.908
CA	DPP	1	0.67	1.5	7	82.8	5.31	1.903
CA	DPP	1	0.67	1.5	14	83.6	5.35	1.903
CA	DPP	1	0.67	1.5	14	95.5	5.22	1.916
CA	DPP	1	0.67	1.5	28	82.9	6.11	1.910
CA	DPP	1	0.67	1.5	28	109.1	5.95	1.921
CA	DPP	0.75	0.5	1.5	1	41.1	5.34	1.897
CA	DPP	0.75	0.5	1.5	1	45.2	5.41	1.892
CA	DPP	0.75	0.5	1.5	7	46.4	5.54	-
CA	DPP	0.75	0.5	1.5	7	53.0	5.54	-
CA	DPP	0.75	0.375	2	1	13.9	5.27	-
CA	DPP	0.75	0.375	2	1	15.2	5.21	-

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand) (To be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	1	0.5	2	1	21.0	5.44	1.874
CA	DPP	1	0.5	2	1	38.4	5.41	1.880
CA	DPP	1.25	0.625	2	1	95.3	5.36	1.941
CA	DPP	1.25	0.625	2	7	93.1	5.19	1.957
CA	DPP	1.25	0.625	2	28	55.6	5.33	1.941
CA	DPP	1.25	0.5	2.5	1	17.3	5.51	-
CA	DPP	1.25	0.5	2.5	1	35.0	5.61	-
CA	DPP	1.25	0.5	2.5	7	45.7	5.30	1.891
CA	DPP	1.25	0.5	2.5	28	28.1	5.52	1.894
CA	DPP	1	0.25	4	1	13.9	5.45	1.892
CA	DPP	1	0.25	4	7	11.4	5.18	1.868
CA	DPP	1	0.25	4	28	12.7	5.40	1.879
CA	DPP	1.25	0.25	5	1	10.1	5.50	1.858
CA	DPP	0.25	5	0.05	1	8.9	10.33	2.038
CA	DPP	0.25	5	0.05	7	10.3	9.61	2.060
CA	DPP	0.25	5	0.05	28	10.0	9.63	2.056
CA	DPP	0.25	4	0.06	1	8.8	9.63	2.014
CA	DPP	0.25	3	0.08	1	12.7	8.87	2.002
CA	DPP	0.25	3	0.08	7	15.2	8.96	2.007
CA	DPP	0.25	3	0.08	28	13.9	8.75	1.986

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand) (To be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.25	2	0.13	1	16.4	8.92	1.974
CA	DPP	0.5	4	0.13	1	12.7	9.07	2.054
CA	DPP	0.5	4	0.13	7	12.6	8.76	2.047
CA	DPP	0.5	4	0.13	28	12.7	8.64	2.048
CA	DPP	0.5	3	0.17	1	66.7	8.30	2.039
CA	DPP	0.5	3	0.17	7	54.5	8.37	2.038
CA	DPP	0.5	3	0.17	28	47.1	8.09	2.034
CA	DPP	0.25	1	0.25	1	10.2	8.02	1.937
CA	DPP	0.25	1	0.25	7	17.7	7.96	1.931
CA	DPP	0.25	1	0.25	28	20.2	7.83	1.938
CA	DPP	0.375	1.5	0.25	1	26.2	-	-
CA	DPP	0.375	1.5	0.25	7	40.2	-	-
CA	DPP	0.375	1.5	0.25	28	40.9	-	-
CA	DPP	0.5	2	0.25	1	41.3	8.28	1.951
CA	DPP	0.5	2	0.25	1	48.3	8.11	1.960
CA	DPP	0.75	3	0.25	1	117.6	8.00	2.040
CA	DPP	0.75	3	0.25	7	117.6	7.86	2.048
CA	DPP	0.75	3	0.25	28	85.3	7.91	2.062
CA	DPP	0.75	2	0.38	1	76.7	8.26	2.003

Table 2.2: Summary of UCS results with their wet density and pH values (Toyoura sand).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.75	2	0.38	7	100.0	7.84	2.022
CA	DPP	0.75	2	0.38	28	158.0	8.23	2.003
CA	DPP	1	1.5	0.67	1	166.3	6.92	1.951
CA	DPP	0.56	0.75	0.75	1	24.0	6.95	-
CA	DPP	0.56	0.75	0.75	7	28.8	7.05	-
CA	DPP	0.56	0.75	0.75	28	31.3	6.77	-

Table 2.3: Summary of UCS results with their wet density and pH values (Mikawa sand) (to be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.375	0.75	0.5	1	20.2	6.91	1.934
CA	DPP	0.375	0.75	0.5	1	21.6	7.04	1.943
CA	DPP	0.375	0.75	0.5	7	19.1	7.12	1.942
CA	DPP	0.375	0.75	0.5	7	21.7	7.26	1.948
CA	DPP	0.375	0.75	0.5	14	17.9	7.02	1.941
CA	DPP	0.375	0.75	0.5	14	18.0	7.25	1.939
CA	DPP	0.375	0.75	0.5	28	17.9	7.42	1.935
CA	DPP	0.375	0.75	0.5	28	21.7	7.62	1.938
CA	DPP	0.5	1	0.5	1	38	7.38	1.951
CA	DPP	0.5	1	0.5	1	31.9	7.19	1.947
CA	DPP	0.5	1	0.5	7	72.4	7.46	1.954
CA	DPP	0.5	1	0.5	7	69.7	7.16	1.951
CA	DPP	0.5	1	0.5	14	47	7.47	2.283
CA	DPP	0.5	1	0.5	14	57.2	7.48	2.282
CA	DPP	0.5	1	0.5	28	49.7	7.37	1.989
CA	DPP	0.5	1	0.5	28	52.5	7.35	1.992
CA	DPP	0.75	1.5	0.5	1	93.0	7.17	1.985
CA	DPP	0.75	1.5	0.5	1	89.1	7.20	1.990
CA	DPP	0.75	1.5	0.5	7	112.8	7.25	1.959
CA	DPP	0.75	1.5	0.5	7	109.3	7.23	1.980

Table 2.3: Summary of UCS results with their wet density and pH values (Mikawa sand) (to be continued...).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	0.75	1.5	0.5	14	149.2	7.26	1.942
CA	DPP	0.75	1.5	0.5	14	153.6	7.29	1.978
CA	DPP	0.75	1.5	0.5	28	191.9	7.48	1.991
CA	DPP	0.75	1.5	0.5	28	195.7	7.48	2.003
CA	DPP	0.5	0.5	1	1	0	6.66	1.923
CA	DPP	0.5	0.5	1	1	0	6.67	1.908
CA	DPP	0.5	0.5	1	7	2.6	6.31	1.910
CA	DPP	0.5	0.5	1	7	0	6.49	1.915
CA	DPP	1.0	1.0	1	1	199.4	6.03	1.864
CA	DPP	1.0	1.0	1	1	229.0	6.13	1.927
CA	DPP	1.0	1.0	1	7	252.2	5.86	1.953
CA	DPP	1.0	1.0	1	7	252.3	5.93	1.914
CA	DPP	1	0.75	1.33	1	120.5	5.57	1.969
CA	DPP	1	0.75	1.33	1	143	5.64	1.977
CA	DPP	1	0.75	1.33	7	173.5	4.84	1.971
CA	DPP	1	0.75	1.33	7	214.0	4.94	1.970
CA	DPP	1	0.67	1.5	1	77.7	5.67	1.973
CA	DPP	1	0.67	1.5	1	55.9	5.69	1.978
CA	DPP	1	0.67	1.5	7	94.9	5.56	1.965
CA	DPP	1	0.67	1.5	7	98.7	5.51	1.968

Table 2.3: Summary of UCS results with their wet density and pH values (Mikawa sand).

Calcium source	Phosphate source	Ca ²⁺ (M)	PO ₄ ³⁻ (M)	Ca/P	Days	UCS (kPa)	Average pH	Wet Density (g/cm ³)
CA	DPP	1	0.67	1.5	14	97.2	5.55	1.969
CA	DPP	1	0.67	1.5	14	130.1	5.63	1.974
CA	DPP	1	0.67	1.5	28	93.4	5.30	1.979
CA	DPP	1	0.5	2	1	9.0	5.57	1.932
CA	DPP	1	0.5	2	1	13.9	5.62	1.942

2.5.8 XRD analysis

XRD analyses done for different testing cases with different concentrations of Ca^{2+} and PO_4^{3-} sources are shown in Figs. 2.14, 2.15, 2.16, 2.17, 2.18, 2.19, 2.20, 2.21, 2.22 and 2.23. Out of them, Figs. 2.14 and 2.15 belong to Area A. Figs. 2.16, 2.17, 2.18, 2.19 and 2.20 belong to Area B and Figs. 2.21, 2.22 and 2.23 belong to Area C.

Tripotassium calcium hydrogen phosphate and hydroxylapatite were the main CPC compounds found in Area B and Area C. However, the CPC compounds found in Area A was not pure Tripotassium calcium hydrogen phosphate and/or hydroxylapatite. They were associated with other compounds. Especially in Figs. 2.14 and 2.16, we could observe some part of the added PO_4^{3-} source (dipotassium hydrogen phosphate (DPP)) was remained without involving for making CPCs. The variation of UCS in Area B and Area C may be due to the formation of Tripotassium calcium hydrogen phosphate and/or hydroxylapatite rather than other compounds found in Area A.

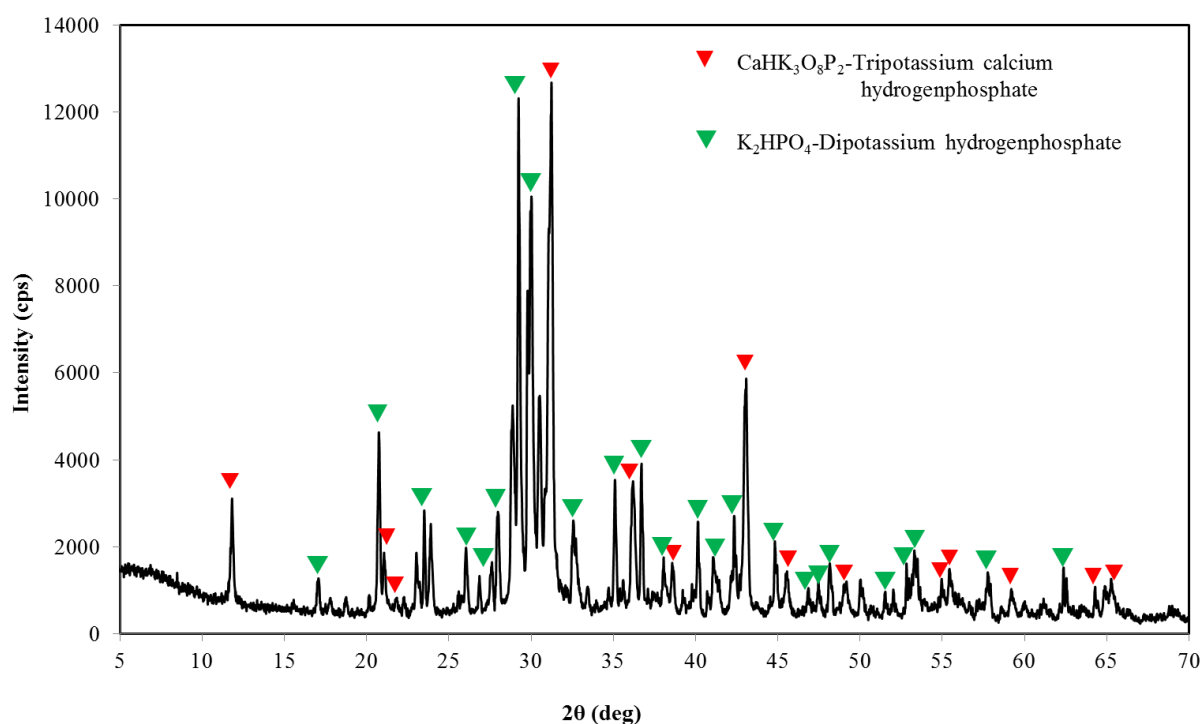


Fig. 2.14: XRD analysis of CPC with 0.25 M CA and 0.5 M DPP (after 28 days of curing).

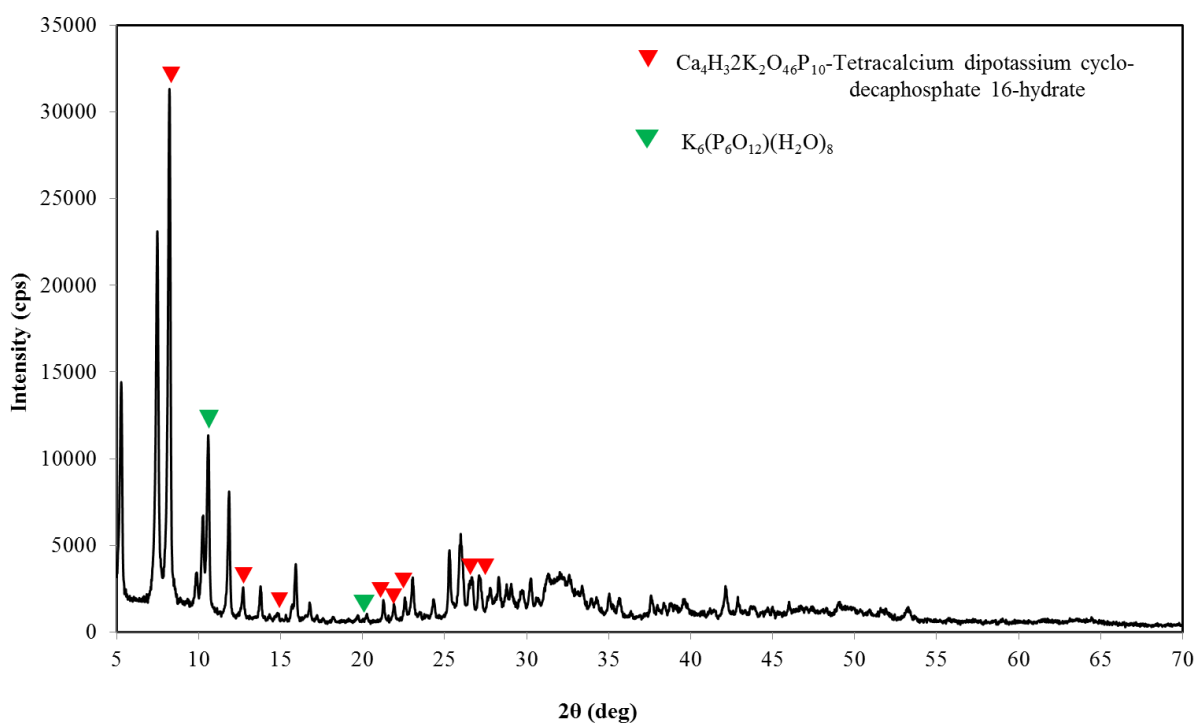


Fig. 2.15: XRD analysis of CPC with 1.0 M CA and 0.25 M DPP (after 28 days of curing).

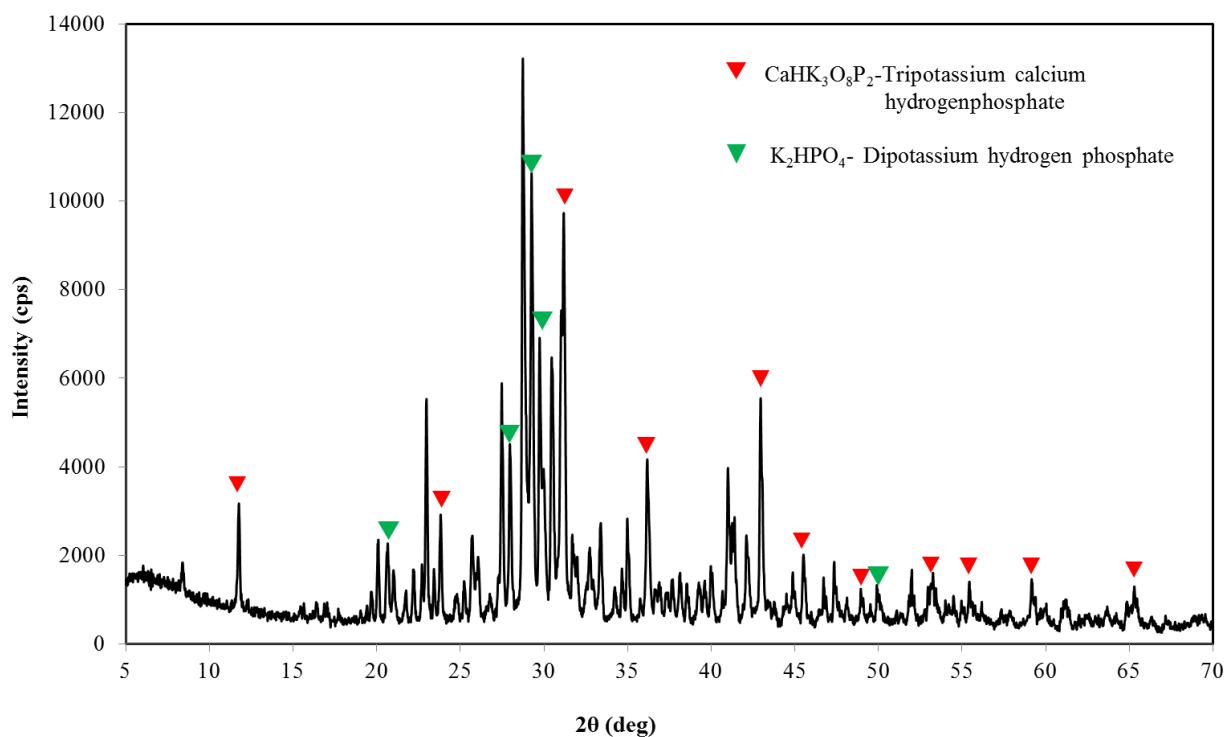


Fig. 2.16: XRD analysis of CPC with 0.5 M CA and 3 M DPP (after 28 days of curing).

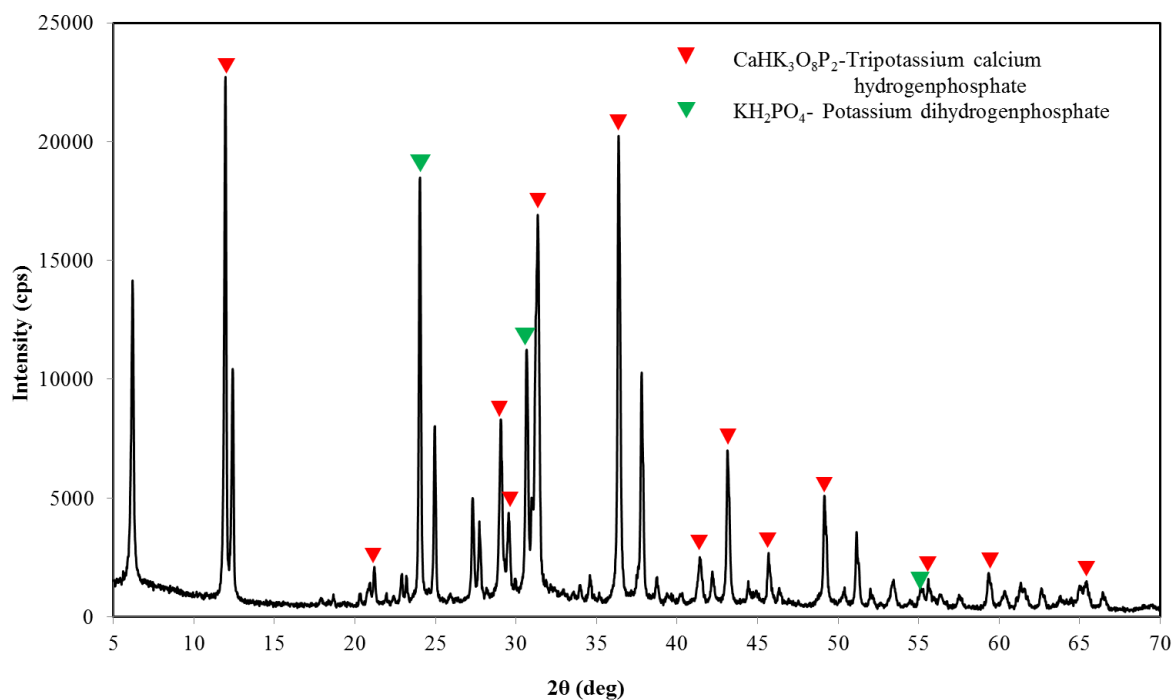


Fig. 2.17: XRD analysis of CPC with 0.75 M CA and 1.5 M DPP (after 28 days of curing).

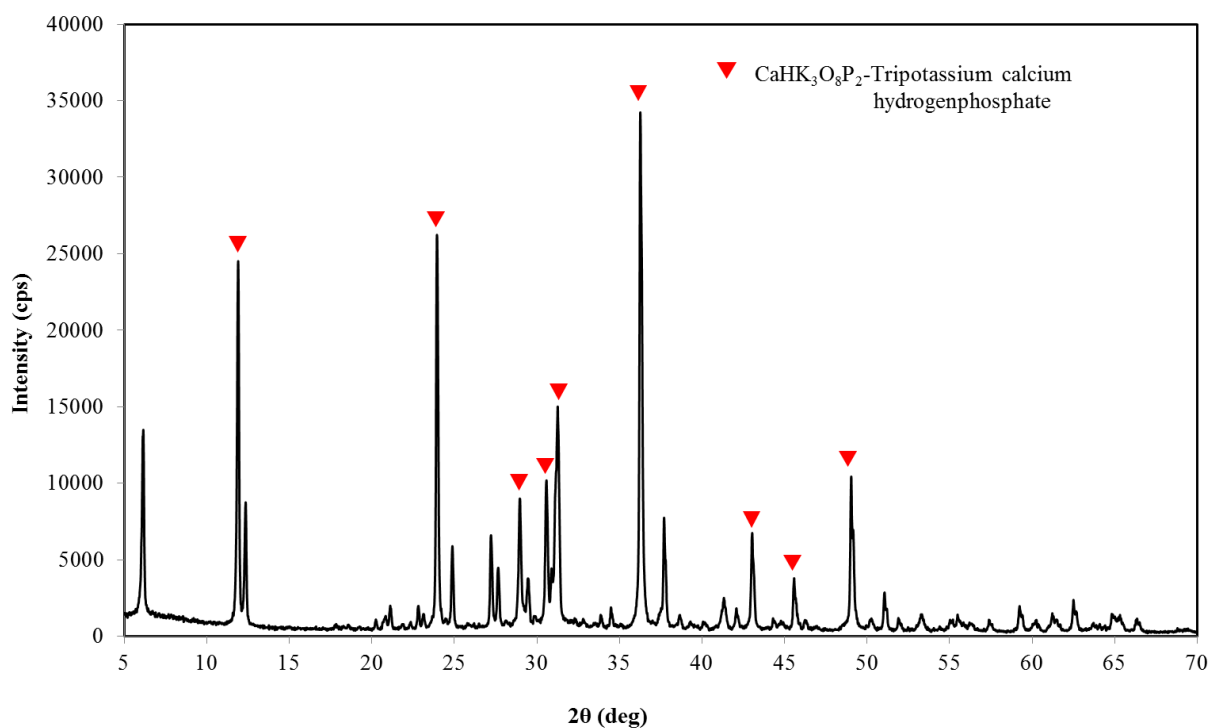


Fig. 2.18. XRD analysis of CPC with 0.75 M CA and 2.0 M DPP (after 28 days of curing).

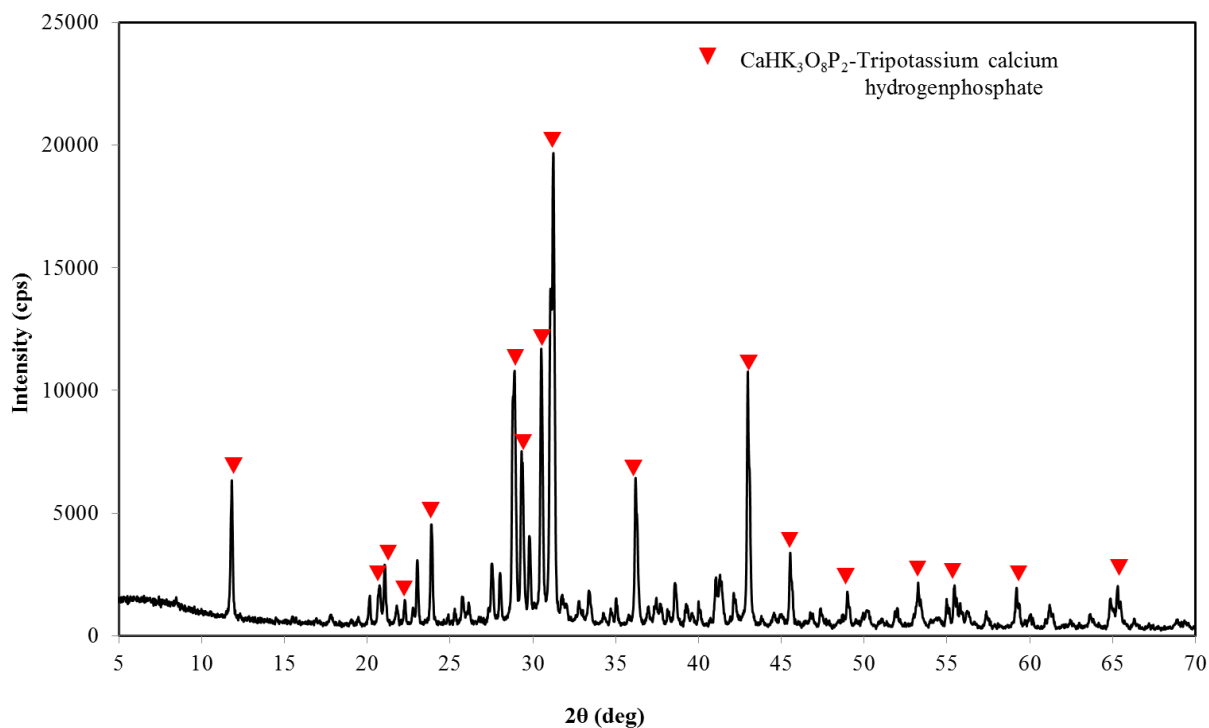


Fig. 2.19: XRD analysis of CPC with 0.75 M CA and 3.0 M DPP (after 28 days of curing).

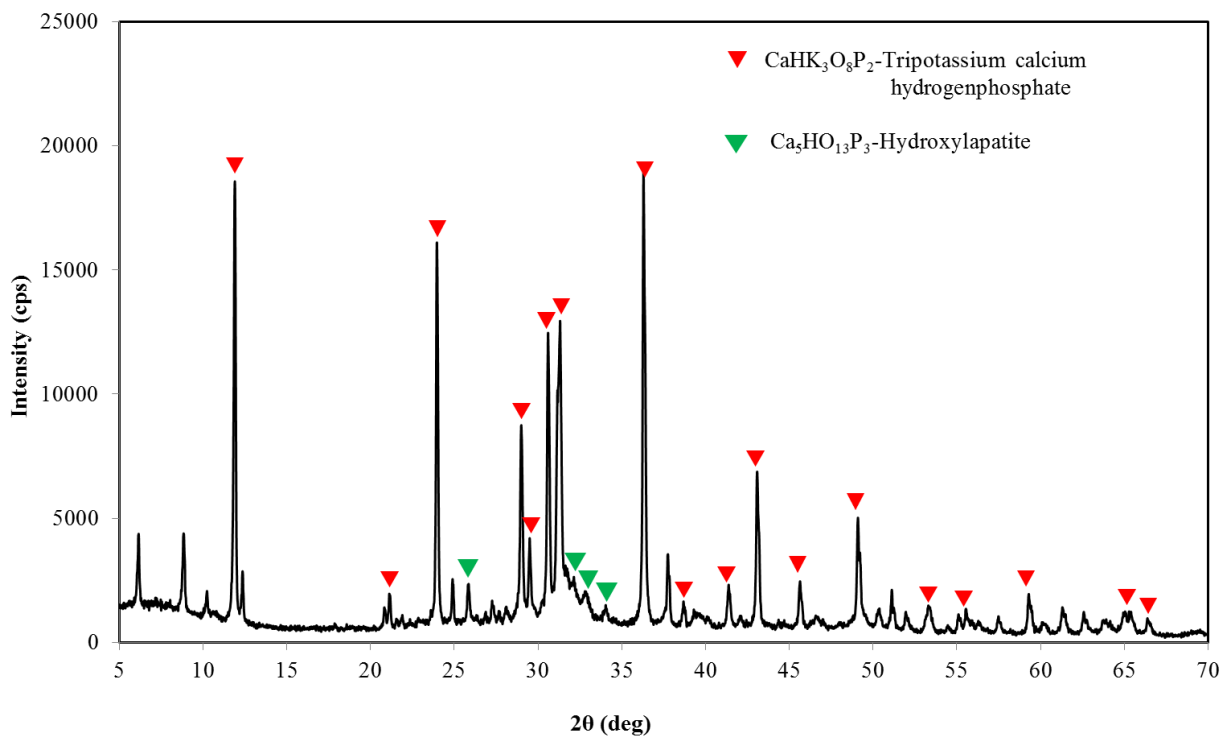


Fig. 2.20: XRD analysis of CPC with 0.56 M CA and 0.75 M DPP (after 28 days of curing).

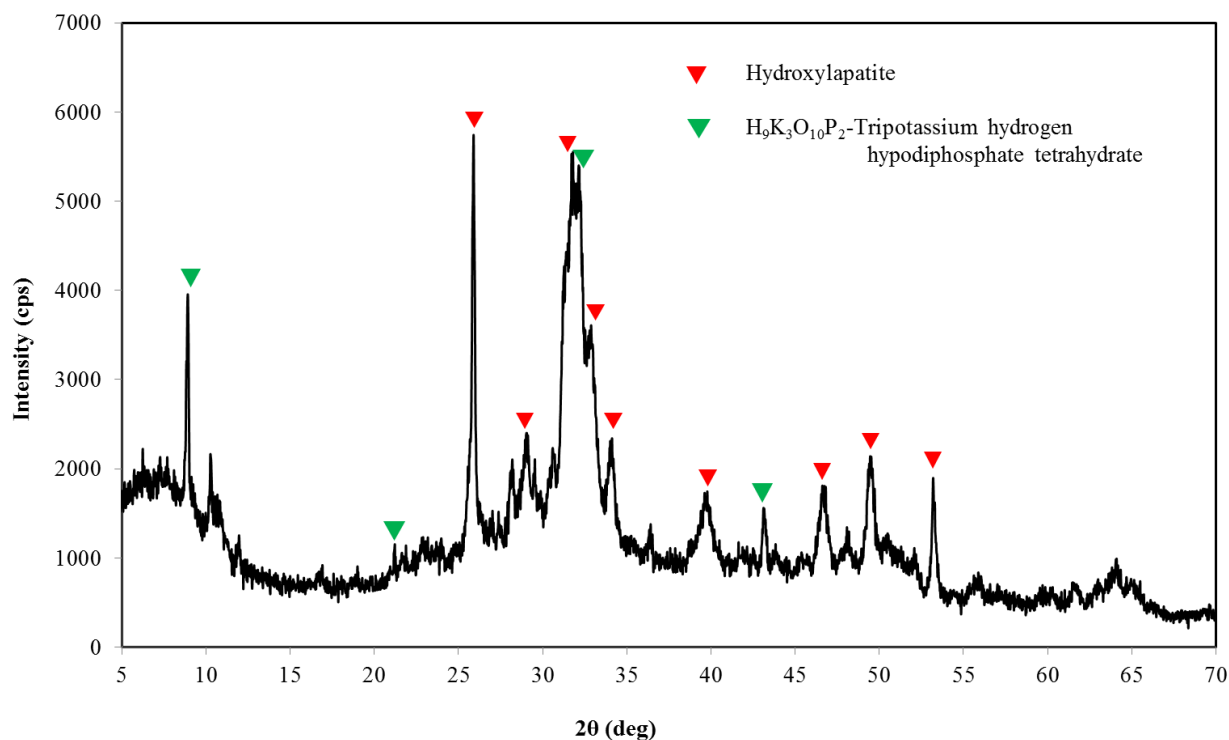


Fig. 2.21: XRD analysis of CPC with 1.25 M CA and 0.94 M DPP (after 28 days of curing).

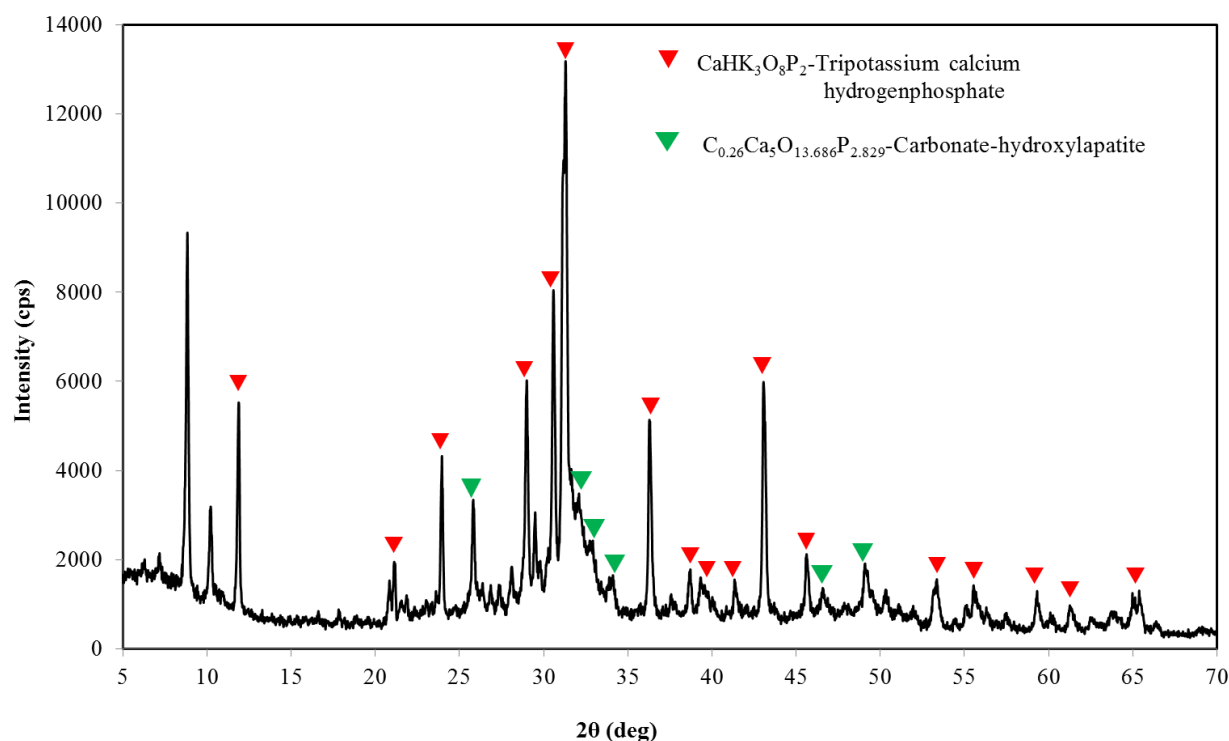


Fig. 2.22: XRD analysis of CPC with 1.0 M CA and 1.0 M DPP (after 28 days of curing).

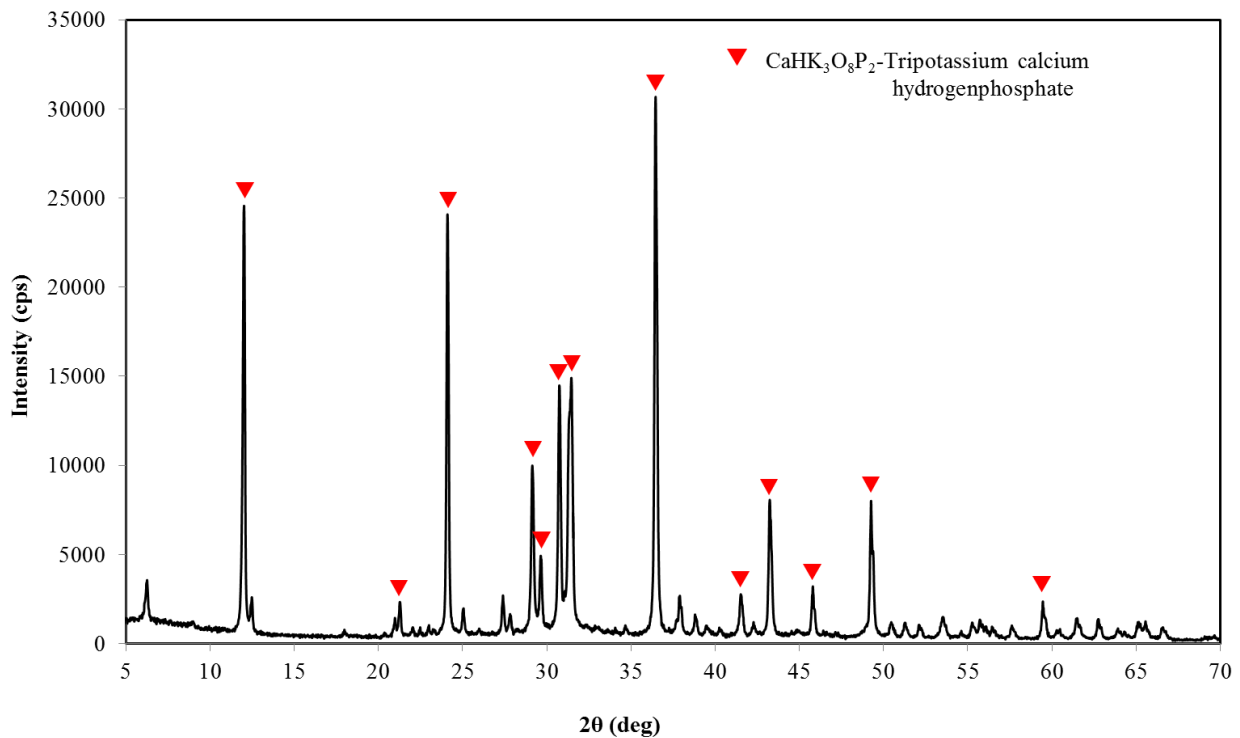


Fig. 2.23: XRD analysis of CPC with 1.0 M CA and 2.0 M DPP (after 28 days of curing).

2.6 DISCUSSION

2.6.1 Relationship between curing time (days) and UCS of Toyoura sand test pieces

The amount of precipitated CPCs increased with the increase of CA and DPP concentrations. That means, the voids between sand particles replaced with precipitated CPCs and this caused to increase UCS. At higher concentrations of CA and DPP, an increment of UCS could be observed with time due to its self-setting property. Furthermore, at low concentrations of CA and DPP, an increment of UCS could not be observed during 28 days of curing in both Ca/P ratios. When the 1 day UCS is less than about 20 kPa, we could not observe an increment of UCS with time. At low concentrations, even after the 28 days of curing, a gel-like formation was observed rather than a crystal formation of CPCs. Therefore, at low concentrations, it was difficult to enhance the UCS with curing time.

2.6.2 Relationship between pH and UCS of Toyoura sand test pieces

According to the Fig. 2.2, the pH was not a governing factor to increase UCS under same Ca/P ratio using same Ca^{2+} and PO_4^{3-} sources because pH was not varied under same Ca/P ratio (Fig. 2.3). To compare the pH effect on UCS, it is suitable to combine different Ca^{2+} and PO_4^{3-} sources (Table 2.4) which are having different initial pH as in Table 2.5.

Table 2.4: Different combinations of Ca^{2+} and PO_4^{3-} sources.

Case No.	Ca^{2+} source	PO_4^{3-} source
Case 1	Basic	Basic
Case 2	Basic	Acidic
Case 3	Acidic	Basic
Case 4	Acidic	Acidic

Table 2.5: Different Ca^{2+} and PO_4^{3-} sources having different initial pH.

Ca^{2+} source		PO_4^{3-} source	
Name	Initial pH	Name	Initial pH
1. $\text{Ca}((\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O})$ - (CA)	6.5-8.5	1. K_2HPO_4 -(DPP)	8.7-9.4
2. $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ - (CN)	4-6	2. $\text{NH}_4\text{H}_2\text{PO}_4$ -(MAP)	4-4.5
3. $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ - (CL)	4.5-8.5		

2.6.3 Relationship between wet density and UCS of Toyoura sand test pieces

The amount of precipitated CPCs increase with the increase of concentrations of CA and DPP, and finally it may cause to increase the wet density. That means, the voids between sand particles is replaced with precipitated CPCs and it caused to increase UCS. A change in

wet density from 1 day to 28 days in same testing case could not be observed (Figs. 2.4 and 2.5). However, the UCS increased from 1 day to 28 days without changing the wet density.

2.6.4 SEM observation

According to the SEM observation, when Ca/P ratio is 1, plate-like crystals could be observed (Fig. 2.7). There are few CPCs, e.g., octacalcium phosphate (OCP) (Wang et. al., 2004) and dicalcium phosphate (DCP) (Toyama et al., 2001), whose precipitation leads to plate-like crystals. As described by Dorozhkin and Epple (2002), when Ca/P= 1, there is a possibility to precipitate DCP (DCPD- Brushite or DCPA- Monetite). Therefore, precipitated plate-like crystals may be DCP. Non-HA CPC ultimately changes into HA (Tung, 1998). Thus, the crystal form in test pieces may change after a long curing period, thereby increasing the strength of the test pieces.

2.6.5 UCS of sand test specimens made by different types of sands

Fine sand, (Toyoura sand in this study) typically has smaller particles and smaller pores than coarse sand (Mikawa sand in this study) consists with large particles and large pores. Therefore, Toyoura sand has a greater ability to hold and retain pore fluid within the inter-particle spaces. In contrast, large pores in Mikawa sand do not have a greater ability to hold the pore fluid.

As explained in the Section 2.6.1, at low concentrations, even after the 28 days of curing, a gel-like formation was observed rather than a crystal formation of CPCs. Therefore, at low concentrations, it was difficult to enhance the UCS with curing time. Furthermore, according to Fig. 2.8, at low concentrations, test pieces made using Toyoura sand showed higher UCS than the test pieces made using Mikawa sand. We can explain it by imitating the formed gel in between sand particle to pore fluid in between the sand particle described

above. When we apply a compression load, Toyoura sand test specimen can bear more load than Mikawa sand test specimen because of the above explanation. At higher concentrations, the test pieces made using Mikawa sand showed higher UCS than Toyoura sand. At higher concentrations, only crystal precipitation of CPCs was observed in the void spaces between sand particles. Therefore, more CPCs precipitated in between Mikawa sand test pieces compare with Toyoura sand test pieces because larger pore spaces are available in Mikawa sand. This may be a possible reason to get higher UCS for the test pieces made using Mikawa sand rather than Toyoura sand at higher concentrations of Ca^{2+} and PO_4^{3-} .

2.7 CONCLUSIONS

In this study, the UCS of sand test pieces made using CA and DPP as Ca^{2+} and PO_4^{3-} sources respectively was analyzed by changing Ca^{2+} and PO_4^{3-} concentrations, Ca/P ratio, curing time and sand type.

According to the results of the UCS values obtained, the UCS values were varied according to the Ca^{2+} and PO_4^{3-} concentrations. When the concentrations of both sources increased, the UCS increased regardless of the Ca/P ratio. The Ca/P ratio changed the final pH in the CPC mixture. When Ca/P=0.5, pH was neutral or weakly alkaline and when Ca/P=1, pH was weakly acidic, and when Ca/P ratio was increased further, pH remained in acidic range. However, by considering previous researches, the behavior of pH with Ca/P ratio is mainly dependent on the Ca^{2+} and PO_4^{3-} sources. The rate of self-setting of CPCs was changed with Ca^{2+} and PO_4^{3-} concentrations. At low concentrations of Ca^{2+} and PO_4^{3-} , the rate of self- setting was low compare with high concentrations.

The objective of current study was to increase the UCS more than 100 kPa which is the required strength for mitigating ground liquefaction. According to the results of UCS test carried out for Toyoura sand test pieces, a UCS more than 100 kPa was obtained after 28

days of curing time from the test case with CA=0.75M and DPP= 1.5M (Ca/P=0.5). Furthermore, the best concentration range to achieve 100 kPa at early stage rather than 28 days is CA=0.75M- 1.0 M and DPP=1.5M- 2.0M. For Ca/P=1, the above range is CA=DPP= 0.75-1.0M. These ranges are also suitable for Mikawa sand. However, it gives little higher values rather than Toyoura sand during selected ranges of concentration.

Specific crystal structure could not be identified from SEM images for the test cases relevant to Ca/P=0.5. When Ca/P ratio is 1, plate-like DCP crystals could be observed. The relationships between the strength and the various CPC precipitation parameters (concentration and pH of the reaction mixture, curing time, etc.) are clear using CA and DPP as Ca^{2+} and PO_4^{3-} source respectively. However, continued research is needed to identify the process or processes that link crystal precipitation to the increase in strength more clearly. Furthermore, shearing and permeability tests using pieces cemented by CPC should be conducted to evaluate the applicability of CPCs for some purposes such as permeability control and reinforcement of soil and rock.

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CHAPTER 3: Soil improvement using CPCs and addition of powder materials (CPC-Powder method)

3.1 INTRODUCTION

Sand cementation using CPCs (CPC-Chem method) was described in Chapter 2. The values of UCS depended on test conditions such as the chemical composition of Ca^{2+} and PO_4^{3-} sources and the curing time of sand test pieces in CPC-Chem method. This chapter presents another method as an attempt to enhance the values of UCS obtained from CPC-Chem method. With the purpose of improving UCS, hereafter, the use of different powder materials as seed crystals with CPCs (CPC-Powder method) is described.

A seed crystal is a small piece of single crystal/polycrystal material from which a large crystal of the same material typically is to be grown. The oversaturated solution and seed crystals generally coexist to yield effective crystal growth (Miyazaki et al., 2008). The CPC powders appear to play two roles during solidification of the paste: as sources of phosphate and calcium for HA and as seed crystals that promote the precipitation and growth of HA (Nancollas and Wefel, 1976; De Rooij et al., 1984; Changsheng and Shen, 1997; Moriyama et al., 2001). The existing biogROUT also uses bacterial cells as a nucleus of CC precipitation (De Muynck et al., 2010). These features indicate that the presence of seed crystals can improve the speed and efficiency of crystal precipitation.

3.2 OBJECTIVES

In the present study, my aim was to improve the strength of CPC-Chem by using two kinds of phosphate powders (tricalcium phosphate, TCP; magnesium phosphate, MgP) and two kinds of carbonate powders (calcium carbonate, CC; magnesium carbonate, MgC) as seed crystals, and exceed the UCS more than 100 kPa, which is the strength required to mitigate ground liquefaction during earthquakes (Yamazaki et al, 1998).

3.3 MATERIALS AND EQUIPMENT

3.3.1 Materials

The key materials used for the CPC-Powder method are the solutions of CA, and DPP with final concentrations of 0.75 M and 1.5 M respectively, and two kinds of phosphate powders (TCP and MgP) and two kinds of carbonate powders (CC and MgC) as seed crystals for CPC precipitation. All the other materials used are similar to the materials used in Chapter 2. Table 3.1 lists the four powders used as seed crystals for CPC precipitation. TCP is a type of CPCs and hence, it has the self-setting property and it can be changed in to hydroxyapatite (HA) over time (Tung, 1998). When compare with other CPCs, TCP releases less phosphate ions at the process of hydrolysis (Chow, 1991) (Table 3.2) and it helps to keep a neutral to weakly alkaline pH range to possess insolubility (Tung, 1998). Furthermore, TCP is an approved food additive in Japan, so it is non-toxic and easy to acquire (Kawasaki and Akiyama, 2013). The second type of phosphate powder we used was MgP. It has been used as a quick hardener

Table 3.1: Properties of powder materials used (Kawasaki and Akiyama, 2013).

Compound	Abbreviation	Established price (JPY/ 500 g)	CAS no.	Mean diameter (μ m)	Density (g/cm ³)
Tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$	TCP	2800	7758- 87-4	12.0	3.14
Magnesium phosphate $\text{Mg}_3(\text{PO}_4)_2$	MgP	3200	13446- 23-6	8.2	2.19
Calcium carbonate CaCO_3	CC	2500	471- 34-1	17.1	2.93
Magnesium carbonate MgCO_3	MgC	2900	56378- 72-4	20.7	2.16

for the emergency repair of roads and airport runways (Seehra et al., 1993; Mestres and Ginebra, 2011). It may produce struvite-K ($\text{KMgPO}_4 \cdot 6\text{H}_2\text{O}$) among the sand particles after reacting with the stock solution of phosphate we used in this study.

Two carbonate powders we selected were CC and MgC. According to a study of Fernandez et al., 1998, the UCS has been increased from 35 MPa to a 56 MPa by using CC crystal. CC is the main component of oyster and scallop shells, which are disposed as marine industrial waste (410,000 t/year in Japan; Ports and Harbors Bureau, 2004) and it is the main component of limestone. It is non-toxic and inexpensive. Mg-containing carbonates such as magnesite (MgCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$) have a lower solubility than CC (Chou et al., 2000). Therefore, strength and chemical stability can be expected with MgC. Hence, MgC was also selected as promising candidate to reinforce the ground along with CPCs. It is also a non-toxic and recyclable material.

Table 3.2: Hydrolysis of calcium phosphate compounds (Kawasaki and Akiyama, 2013).

Compound	Ca/P	Abbreviation	Hydrolysis
Monocalcium phosphate	0.5	MCP	$5\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O} \rightarrow \text{Ca}_5(\text{PO}_4)_3\text{OH} + 7\text{H}_3\text{PO}_4 + 4\text{H}_2\text{O}$
Dicalcium phosphate	1.0	DCP	$5\text{CaHPO}_4 + \text{H}_2\text{O} \rightarrow \text{Ca}_5(\text{PO}_4)_3\text{OH} + 2\text{H}_3\text{PO}_4$
Octacalcium phosphate	1.33	OCP	$5\text{Ca}_8(\text{PO}_4)_6\text{H}_2 \cdot 5\text{H}_2\text{O} \rightarrow$ $8\text{Ca}_5(\text{PO}_4)_3\text{OH} + 6\text{H}_3\text{PO}_4 + 17\text{H}_2\text{O}$
Tricalcium phosphate	1.5	TCP	$5\text{Ca}_3(\text{PO}_4)_2 + 3\text{H}_2\text{O} \rightarrow 3\text{Ca}_5(\text{PO}_4)_3\text{OH} + 2\text{H}_3\text{PO}_4$
Hydroxyapatite	1.67	HA	$\text{Ca}_5(\text{PO}_4)_3\text{OH}$
Tetracalcium phosphate	2.0	TTCP	$3\text{Ca}_4(\text{PO}_4)_2\text{O} + 3\text{H}_2\text{O} \rightarrow 2\text{Ca}_5(\text{PO}_4)_3\text{OH} + 2\text{Ca}(\text{OH})_2$

3.3.2 Equipment

(a) Permeability test apparatus and accessories



Fig. 3.1: Permeability test apparatus and accessories.

3.4 METHODOLOGY

3.4.1 Preparation of sand test pieces for UCS test and conducting UCS test

At the preparation of control samples by using CPC only, a volume of 36.65 mL from CA solution and the same volume from DPP solution were added to 320.09 g of Toyoura sand to fill the total voids between sand particles as calculated by Akiyama and Kawasaki (2012a) as shown in the Section 2.4.1. The powder-added test pieces were made according to the weight combination ratios of sand and powder material. First, 1% (3.2 g), 5% (16.0 g), and 10% (32.0 g) from the total weight of sand were selected as the weight of powder and the weight of sand added were 316.89, 304.09, and 288.09 g, respectively. The sand-powder mixture was uniformly mixed and thereafter, CA and DPP solutions were added to the mixture in stainless steel ball, and mixed for 2 min and then divided into quarters. Each quarter was then placed into the plastic mold described above. Before placing the sample into the mold, 0.1 mm thick overhead projector sheet was used to cover the inner wall of the mold to avoid any disturbance of the test pieces during their removal from the mold. The mixture was tamped down 30 times by a hand rammer when each quarter was filled into the mold. Finally, the top surface of the test pieces were slightly trimmed so that they were flat, and covered with Parafilm M (Structure Probe, Inc., West Chester, PA) to avoid desiccation. The molded test pieces were cured in an airtight container at high humidity. After curing, test pieces were removed carefully from the mold, and the UCS was measured with a UCS apparatus T266-31100 (Seiken-sha Co.Ltd., Japan) at an axial strain rate of 1% /min. A temperature of 25 °C was maintained while preparing, curing and testing of the test pieces. The repeatability of measurements of UCS was studied by testing two test pieces for each test case under same condition. The pH of top, bottom, and middle of each test pieces was measured and calculated as an average of three measurements using a pH Spear (Eutech Instruments Pte., Ltd., Singapore).

3.4.2 SEM observation

Segments of the UCS test pieces were observed by SEM (TM 3000 Miniscope, HITACHI) just after the UCS tests.

3.4.3 Measurement of water impervious property (permeability) of cemented sand test pieces

The soil permeability is a very important factor to study the behavior of soil in its natural condition with respect to water flow. The falling head permeability test is a common laboratory testing method used to determine the permeability of fine grained soils with intermediate and low permeability. Therefore, the falling head method was selected to measure the reduction of permeability of Toyoura sand due to the addition of CPC and powder materials (TCP in this study). Test pieces were made only from CPC with sand and, CPC along with TCP powder and sand. They were prepared into a stainless steel mold with 5 cm inner diameter and 5 cm height. The preparation and curing procedure of test pieces were similar to the procedure used to make and cure the test pieces for UCS test. In addition to that, permeability of Toyoura sand was measured following the same procedure for the purpose of comparison.

The falling head permeability test involves flow of water through a relatively short soil sample connected to a standpipe which provides the water head and also allows measuring the volume of water passing through the sample. Before starting the flow measurement, the soil sample was vacuumed about 10 min using a vacuum pump and a desiccator to remove entrapped air and saturated about 24 hours. The standpipe was filled with distilled water to a given upper level. The test was started by allowing water to flow through the sample until the water in the standpipe reaches a given lower limit. The time required to drop water from the upper level to the lower level in the standpipe was recorded.

Often, the standpipe was refilled and the test was repeated for 3 times. On the basis of the test results, the permeability (m/s) of the sample was calculated as in eq. (3.1). All the dimensions are clearly shown in Fig. 3.2.

$$K = -2.303 \times \frac{aL}{A\Delta t} \times \log \frac{h_1}{h_2} \quad \text{eq. (3.1)}$$

Where: a = Cross section of tube (cm^2)

A = Cross section of sample (cm^2)

L = Height of sample (m)

Δt = Time required to drop 10 cm water height (s)

h_1 = Height of water table after a time Δt (cm)

h_2 =Initial height of water table (h_1+10) (cm)

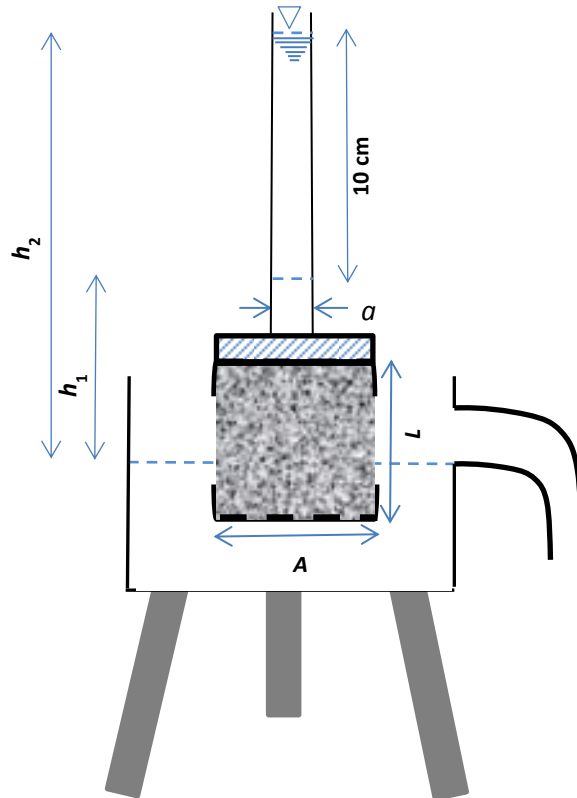


Fig. 3.2: Schematic diagram of the arrangement of permeability test.

3.5 RESULTS

3.5.1 Variation of UCS of sand test pieces with powder type and powder percentage

The UCS values of all test pieces were graphically summarized in Figs.3.3 and 3.4 with their pH values for the ease of comparison. The pH of the TCP- and MgP-added test pieces ranged from weakly acidic to weakly alkaline (6.0-8.0) pH. The pH values of the CC- and MgC-added test pieces remained between 8.0-9.5 pH. The addition of TCP and CC caused to increase the UCS with respect to the percentage of powder added. However, we could not observe clear increment of UCS with the addition of MgP and MgC into CPCs. Moreover, the result shows that the addition of magnesium compounds is not effective for increasing UCS using CPCs.

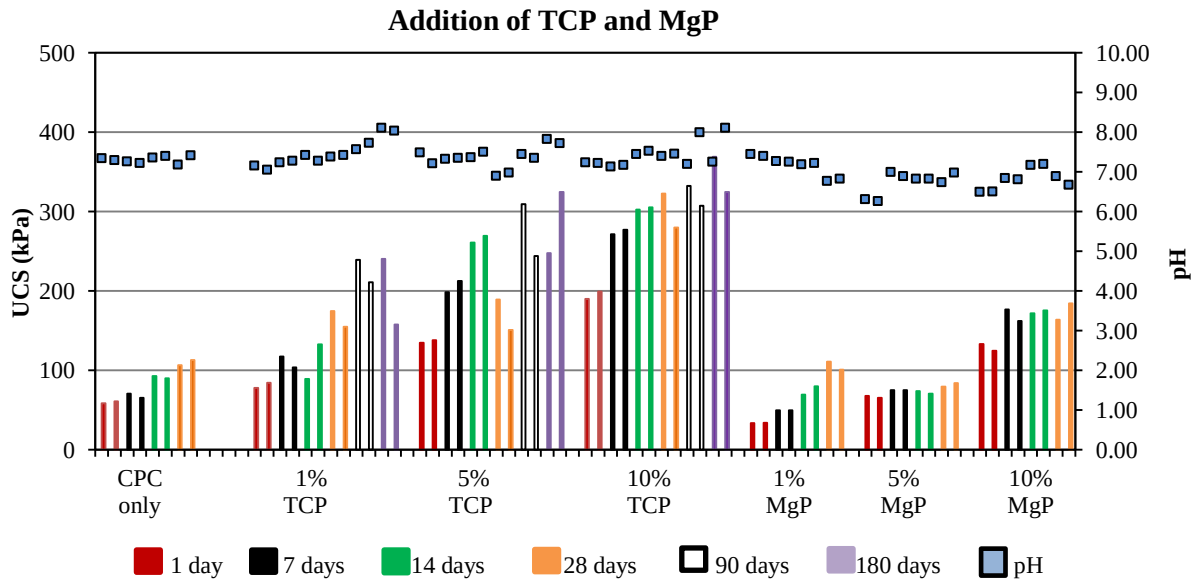


Fig. 3.3: Temporal variation of UCS with the addition of TCP and MgP.

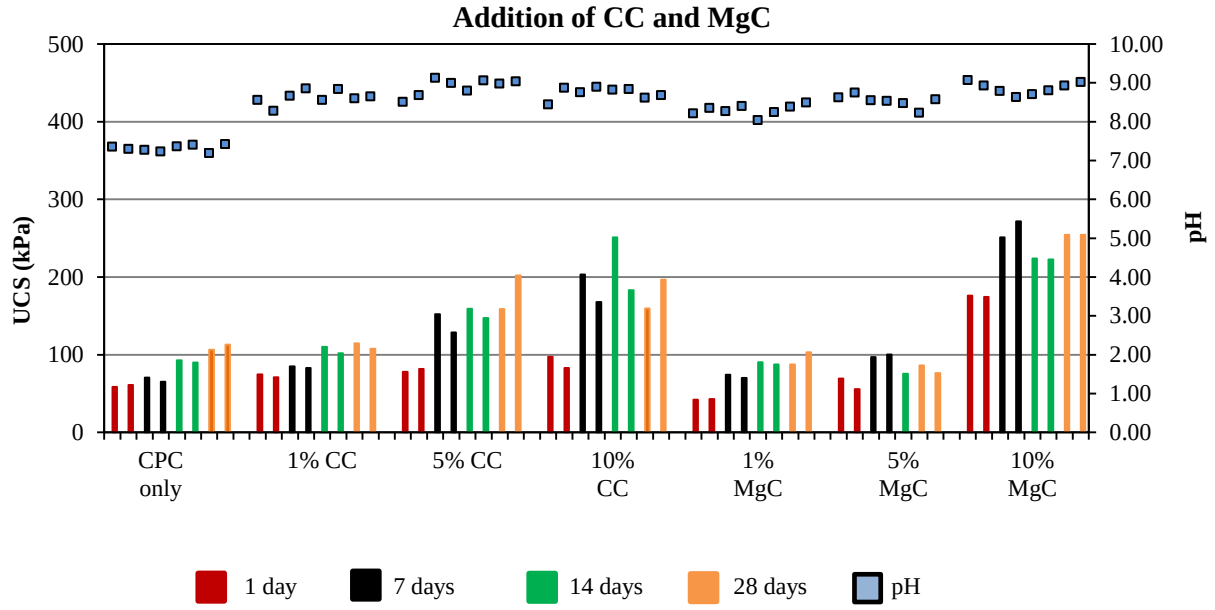


Fig. 3.4: Temporal variation of UCS with the addition of CC and MgC.

3.5.2 Stress–strain curves

As discussed in the Section 3.4.1, two test pieces for each test case were prepared and tested for measuring UCS. According to the compressive stress (σ)–strain (ϵ) curves of the test pieces obtained while measuring of the UCS, the two stress-strain curves obtained for two identical test pieces made for each test case showed almost the same behavior in terms of shape (Figs. 3.5 and 3.6). The stress (σ)–strain (ϵ) curves of TCP-added test pieces after 180 days show peaks at approximately 2.5%, 3.5% and 4% of the failure strain for 1%, 5% and 10% TCP-added test pieces respectively (Fig. 3.5 (b)) and similar to those after 28 days. At the same time, their young's moduli are almost same. The failure strain of the MgP- and MgC-added test pieces showed relative ranges of 2.5%–3.0% and 3.0%– 4.0%, respectively. The stress-strain curves of the TCP-added test pieces showed a higher compressive stress (σ) than MgP-added test pieces at same weight percentage (Fig. 3.5 (a)). The other important thing is the test pieces made from low percentage of MgP and MgC (1% and 5% in this

study) showed compressive stresses less than or similar to the test pieces made only from CPCs.

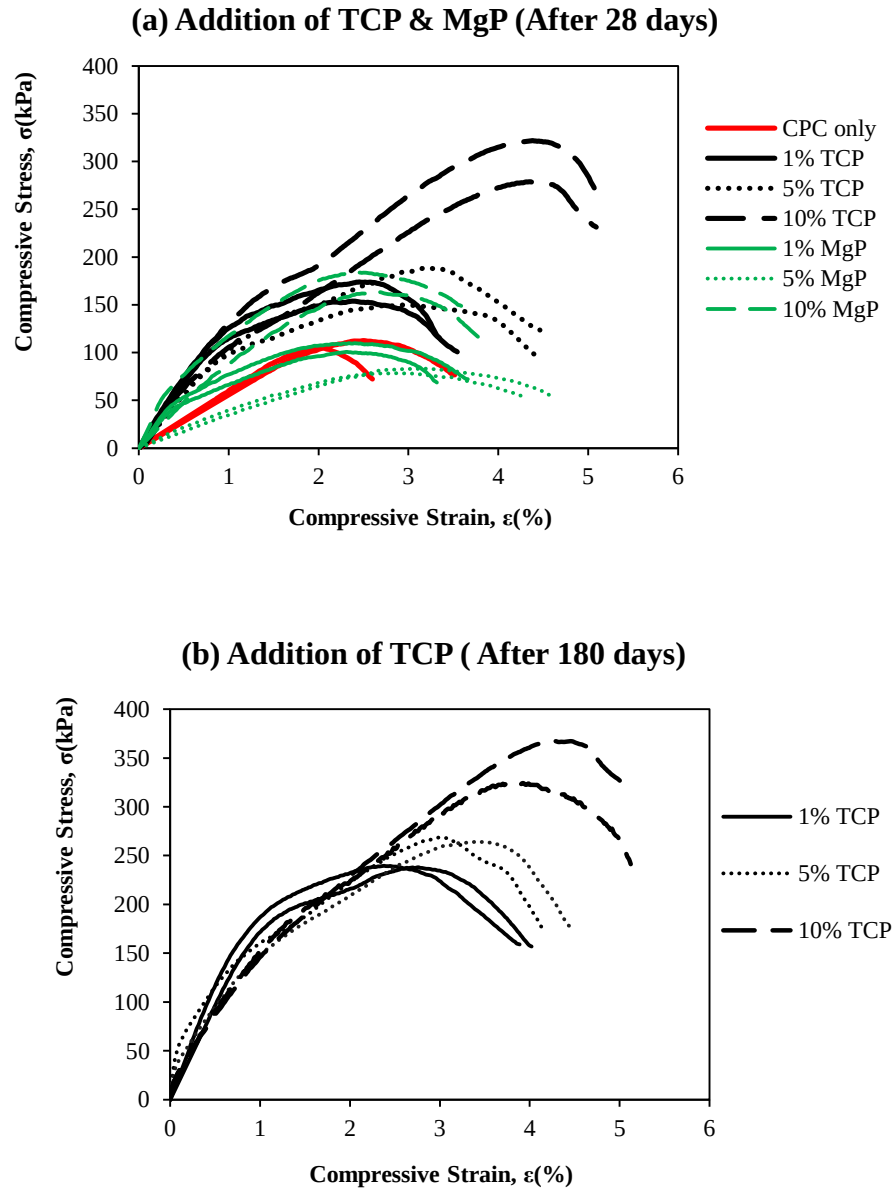


Fig. 3.5: Stress-strain curves the test specimens made using (a) TCP and MgP powders (After 28 days), (b) TCP powder (After 180 days).

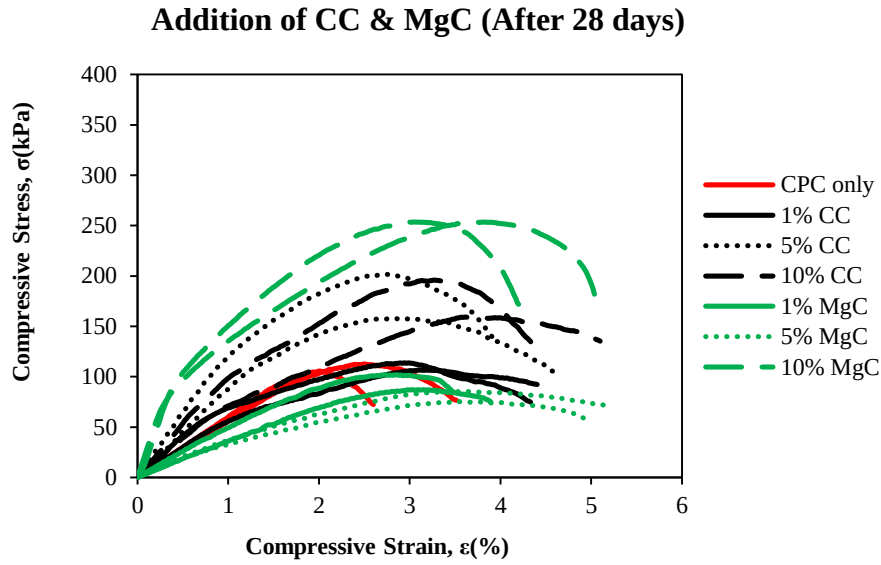


Fig. 3.6: Stress-strain curves of the test specimens made using CC and MgC powders (After 28 days).

3.5.3 Long term stability of sand cemented with CPCs and powder materials

The long term stability of TCP added sand test specimens were examined by measuring the UCS after 90 days and 180 days. The UCS values could be maintained at a level exceeding 200 kPa even after 180 days of curing time. Therefore, we can expect more than 200 kPa value of UCS for 1%, 5% and 10% TCP added test pieces.

3.5.4 Effect of wet density on UCS

According to the wet densities of the test pieces cemented with four kinds of powders and CPC-Chem, the wet density increased with the amount of added powder (Fig.3.7). The wet densities of MgC and MgP added test pieces showed litter lower density values rather than TCP and CC-added test pieces. Furthermore, the UCS was increased with the increase of wet density in TCP and CC- added test pieces. We could not observe such increment of UCS with the increment of wet density in MgP and MgC- added test pieces.

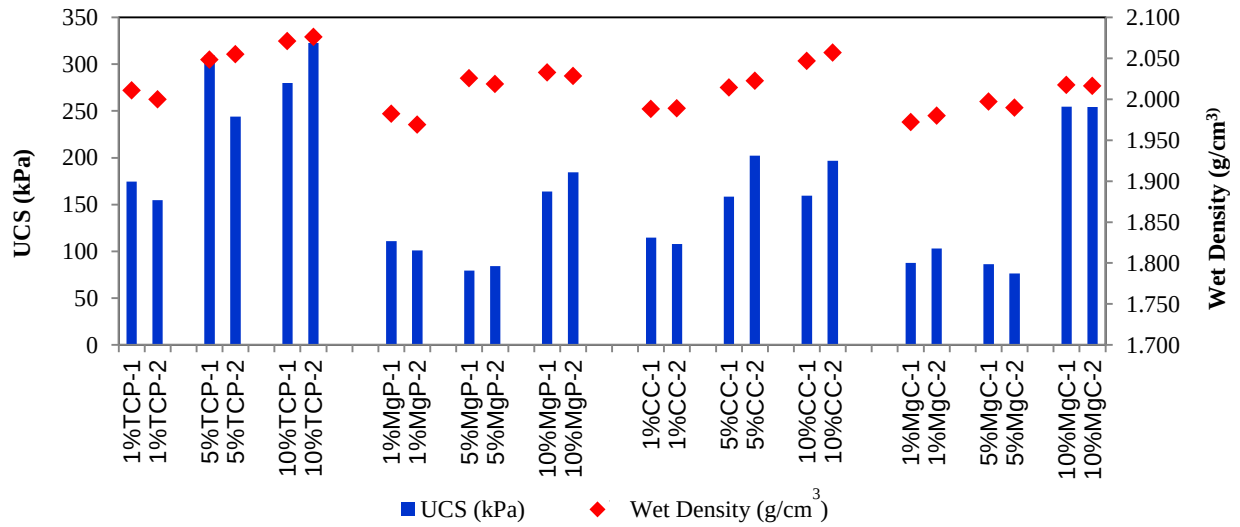


Fig. 3.7: Variation of UCS (kPa) with wet density (g/cm³).

3.5.5 Water impervious property (permeability) of cemented sand test pieces

As shown in Fig. 3.8, the permeability of the Toyoura sand is 2×10^{-4} m/s which considered as very high permeability. After the addition of CPC only, the permeability was reduced to 6×10^{-8} and the addition of only 1% of TCP power to CPC caused to further decrease in permeability by one order of magnitude after 7 days of curing at room temperature (25°C). Cemented material has caused to decrease the permeability of sand by

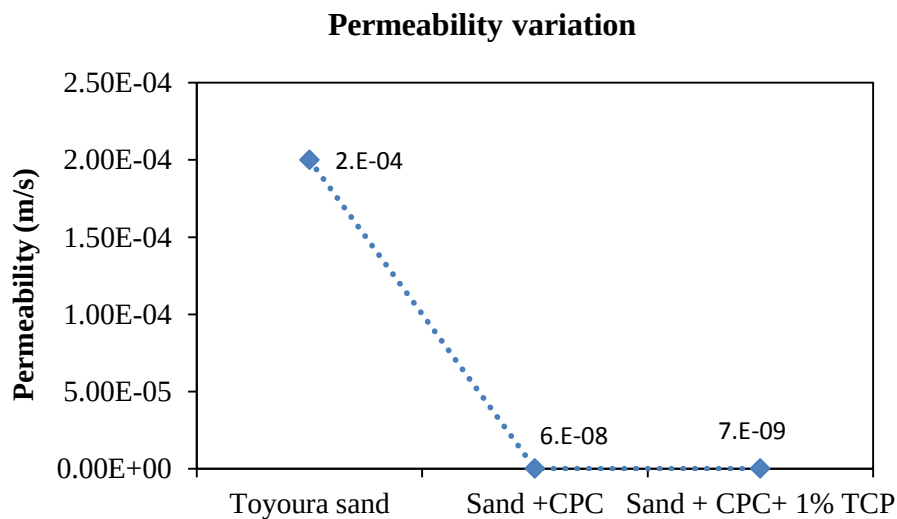


Fig. 3.8: Permeability variation by powder addition.

decreasing the void ratio and, the addition of powder material has caused to further decrease in the void ratio and hence, to further decrease in permeability. The value of decreased permeability by powder addition is almost similar to the permeability of clayey materials. Hence, the permeability can also be controlled by CPC-Powder method.

3.6 DISCUSSION

3.6.1 Variation of UCS of sand test pieces with powder type and powder percentage

According to Figs. 3.3 and 3.4, the result shows that the addition of magnesium compounds is not effective for increasing UCS using CPC-Chem. Previous studies have proved that the magnesium ions are considered to be an inhibiting factor on the self-setting of CPC (Amjad et al., 1984). According to that, magnesium ions in magnesium compounds exchange with the calcium ions in the CPC solution and reduce the precipitation of CPC. At the same time, the SEM images showed that small crystals similar to MgP and MgC were present in MgP- and MgC-added test pieces without bonding with the sand particles (Figs. 3.9 and 3.10). These are the possible reasons to decrease the UCS in MgP- and MgC-added test pieces. Furthermore, a previous research has been shown that TCP, MgP, CC, and MgC do not possess pozzolanic properties with deionized water (Kawasaki and Akiyama, 2013). Therefore, CPC chemical solutions greatly affected for the strength variation of powder added test pieces.

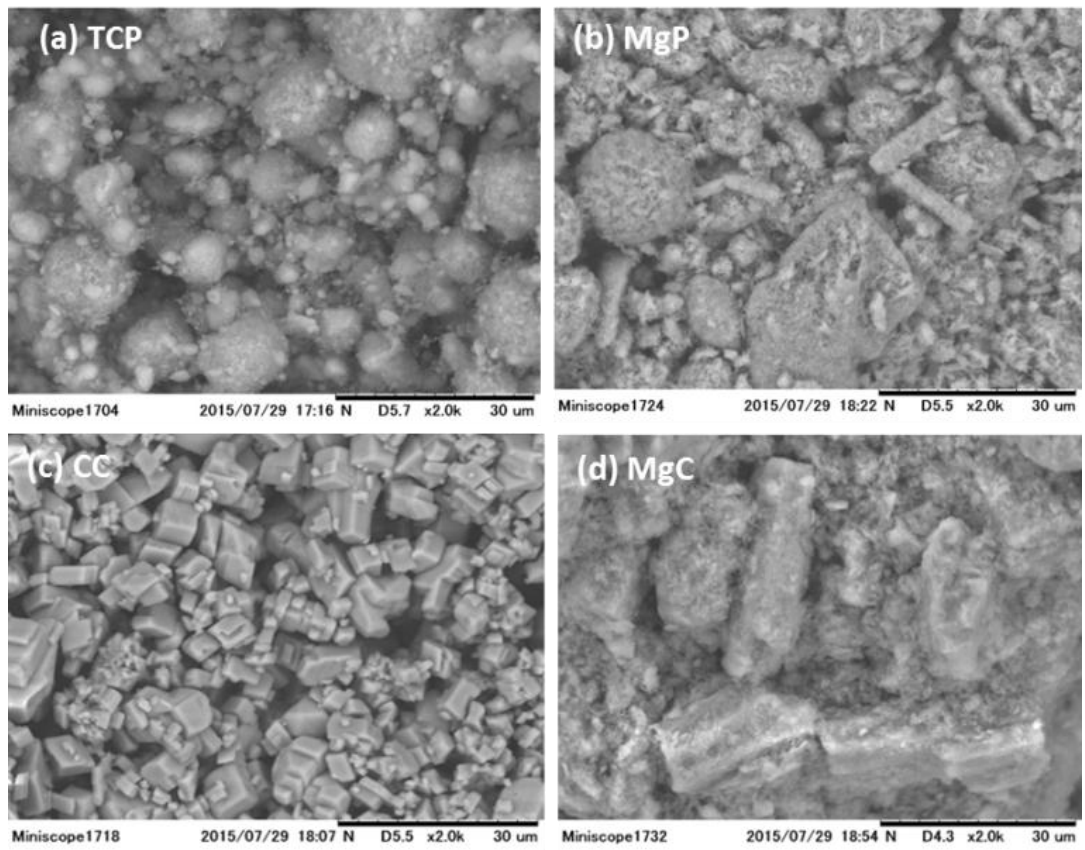


Fig.3.9: SEM images of (a) TCP, (b) MgP, (c) CC and (d) MgC powders.

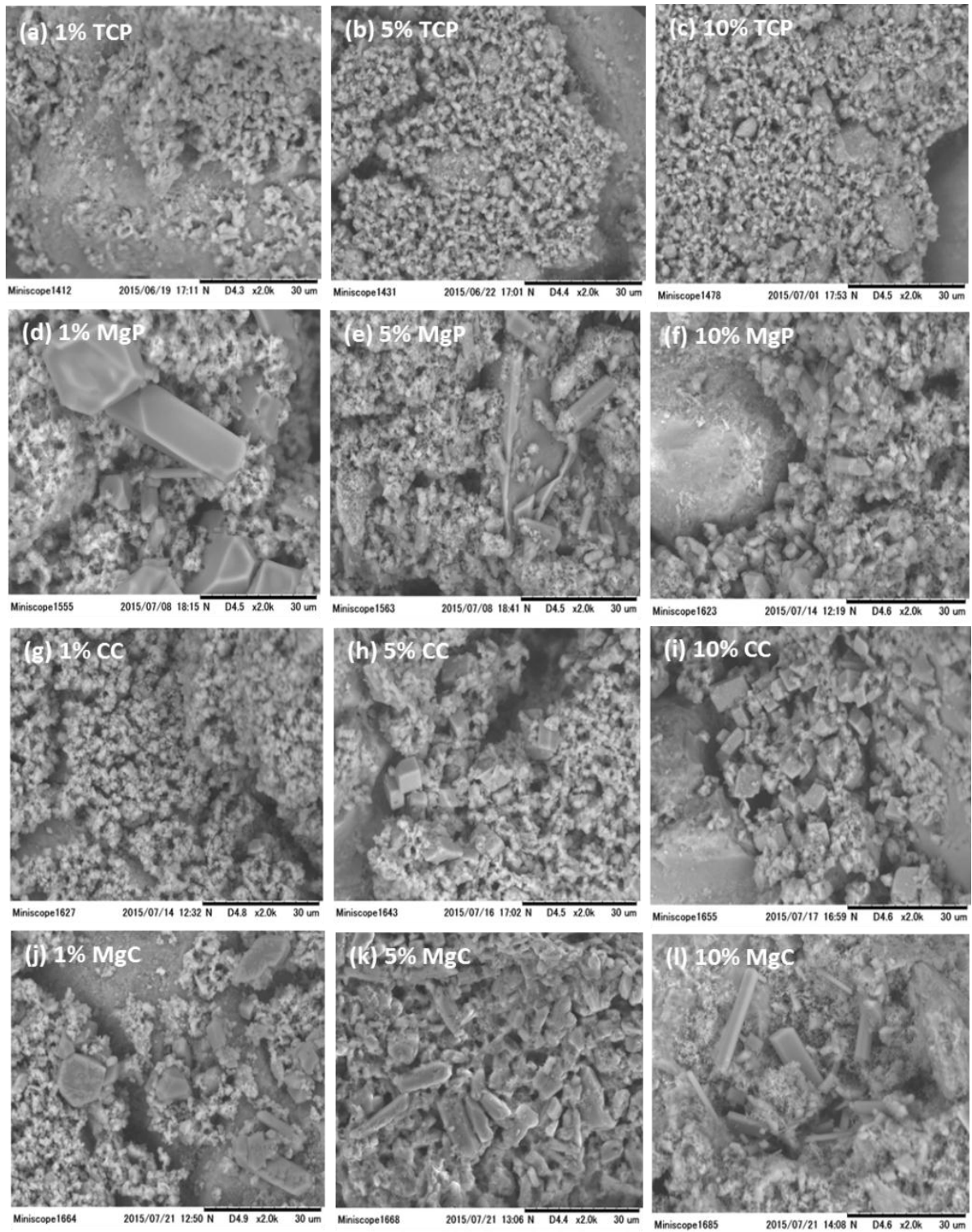


Fig. 3.10: SEM images of TCP-added (a-c), MgP-added (d-f), CC-added (g-i) and MgC-added (j-l) test pieces (28 days).

Two identical test pieces made using TCP and CC showed a little gap between UCS values of those two identical test pieces. The pH values of those identical test pieces were also nearly same. Specially, the upper face of the test pieces cemented with CC and CPC-Chem has swollen slightly after preparing the test pieces. Then, we had to prepare an even surface manually, before conducting UCS test. This is a possible reason to get a little gap in UCS of two identical samples. As we mentioned earlier, because of the upper faces of the test pieces cemented with CC powder and CPC-Chem have swollen slightly after preparing the test pieces, it may provide an underestimate for UCS. The UCS may be improved by the addition of vertical confining pressure. Therefore, it is better to apply CPC-CC Powder method for sub surface environments where considerable overburden pressures exist.

3.6.2 Stress–strain curves

According to the results of the stress (σ) - strain (ϵ) curves of TCP-added test pieces after 180 days (6 months) show peaks at approximately 2.5%, 3.5% and 4% of the failure strain for 1%, 5% and 10% TCP-added test pieces respectively (Fig. 3.5 (b)) and similar to those after 28 days. In a previous research that have been used TCP with another CPC solutions DAP and CA, the failure strain of the TCP-added test pieces after about 6 months have been ranged from 0.5% to 1.5% (Kawasaki and Akiyama, 2013). These results can be explained using the formed crystal structures in both studies. We could not observe any specific crystal structure for TCP-added test pieces. However, it has been identified whisker-like structures for TCP-added test pieces at the previous study. Therefore, when compare with the current study, the low strain values have been observed due to brittle behavior in the test pieces of the previous study due to their whisker-like crystal structures.

3.6.3 Effect of wet density on UCS

The differences in wet density between calcium compounds and magnesium compounds- added test pieces can be explained by the differences in density of each powder materials as described in Table 3.1 (TCP and CC are about 3 g/cm³; MgP and MgC are about 2.2 g/cm³). The UCS increased with wet density in TCP- and CC-added test pieces. Even though the wet densities increased with the amount of MgP and MgC powder added, an increment of UCS could not be clearly seen in MgP- and MgC-added test pieces. As discussed in the Section 3.6.1, the reduction of UCS is due to the magnesium ions in magnesium compounds that can be exchanged with the calcium ions in the CPC solution, and finally, due to the reduction of CPC precipitation.

The behavior of the UCS observed under different powder types is similar to the behavior observed from a previous study (Kawasaki and Akiyama, 2013) that has been done using same powder types, same calcium source (CA) and different PO₄³⁻ sources. However, the values of UCS in most of the test cases in this study are little higher than to the same test cases in the previous study. The variation of the density may be one reason. All the density values of the prepared test pieces in this study are in the range of 1.95 g/cm³ to 2.10 g/cm³ and it was in the range of 1.70 g/cm³ to 2.0 g/cm³ in previous study. The variation of density may be due to different PO₄³⁻ sources used in these two studies. The density of DPP used in this study is 2.44 g/cm³ and the density of DAP used in previous study is 1.62 g/cm³ and therefore, the density of the final precipitate may be changed.

3.6.4 Water impervious property (permeability) of cemented sand test pieces

According to Fig. 3.8, the permeability of the Toyoura sand was decreased with the addition of CPC and further decreased with the addition of TCP power to CPC. Cemented material in between sand particles caused to decrease the permeability of sand by decreasing

the voids ratio and, the addition of powder material has caused to further decrease in the void ratio and hence, to further decrease in permeability. The value of decreased permeability by powder addition is almost similar to the permeability of clayey materials. Furthermore, the permeability results were compared with the permeability values obtained after different ground improvement methods in literature (Table 3.3). All the results were graphically represented in Fig. 3.11 for the ease of comparison. According to the results, the permeability reduction obtained from CPC-Powder method is little similar to the permeability reduction obtained from cement grouting under given testing conditions

Table 3.3: Comparison of the permeability obtained after different ground improvement methods in literature with the permeability obtained from this study.

Ground improvement method	Permeability (m/s)		Mean diameter of the particles (mm)	Remarks
	Before treatment	After treatment		
CPC-Powder method (Current study)	2×10^{-4}	$7 \times 10^{-9*}$	0.20	1% TCP powder addition along with CPC. (* after 7 days)
Cement grouting (Rahimi, 1998)	4×10^{-8}	4×10^{-11}	0.00055	10% cement addition
Cement grouting (Rahimi, 1998)	2×10^{-7}	8×10^{-11}	0.0035	10% cement addition
Cement grouting (Rahimi, 1998)	1×10^{-4}	2×10^{-9}	0.26	10% cement addition
Cement grouting (Al-Aghbari and Dutta, 2005)	6×10^{-4}	$7.81 \times 10^{-8**}$	0.21	12% cement addition to desert sand (**after 7 day)
Cement grouting (Santhosh Kumar,2010)	1.86×10^{-4}	$3 \times 10^{-8***}$	0.80	25% cement addition to medium sand (*** after 28 days)
Acrylamide Grouting (Ozgurel and Vipulanandan, 2005)	1×10^{-4}	1×10^{-12}	0.20	-

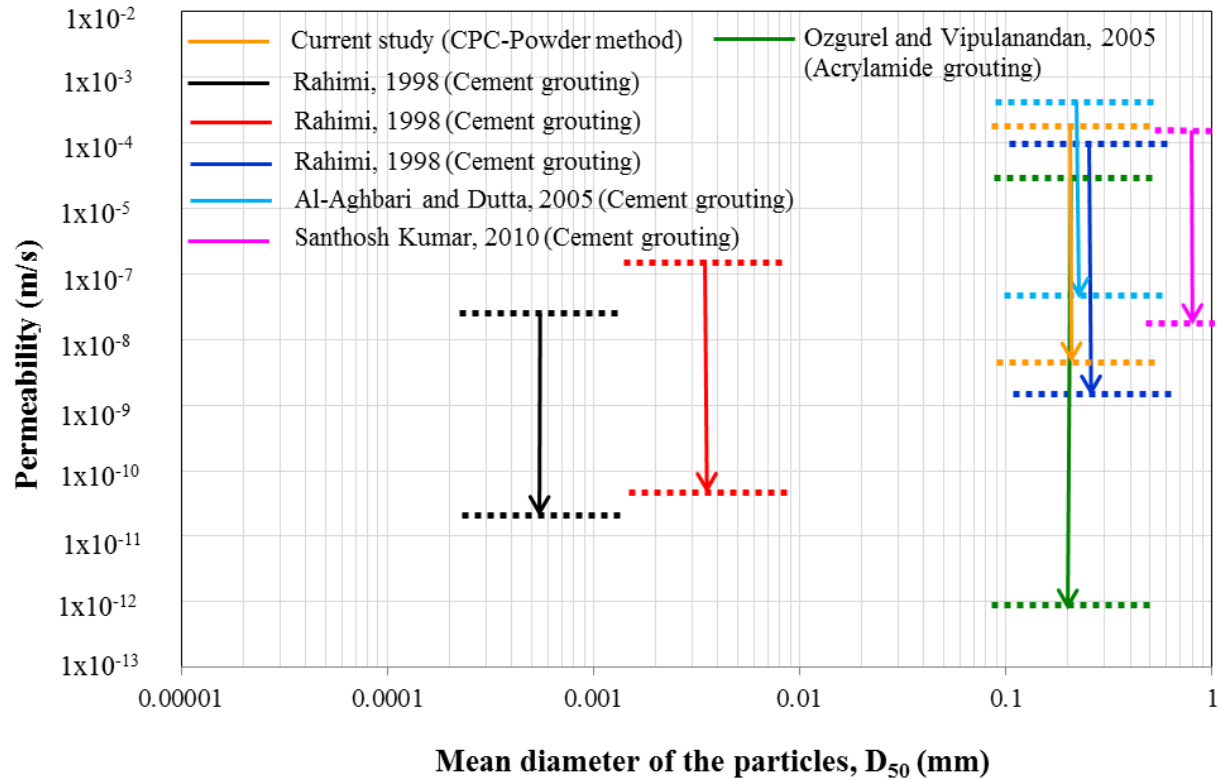


Fig. 3.11: Comparison of the permeability obtained after different ground improvement methods in literature with the permeability obtained from this study.

3.7 CONCLUSIONS

The effectiveness of the CPC-Powder method to improve the UCS of sand more than 100 kPa which was our targeted UCS was examined for small scale, cylindrical samples. The summary of results is as follows.

- The UCS of TCP-added test pieces showed clear increment with the percentage of the powder added and maximum UCS values relevant to 1%, 5% and 10 % TCP were 174.7, 189.1 and 322.6 kPa respectively after 28 days and, 240.4, 269.3 and 368.9 kPa respectively after 180 days. In case of the UCS of CC-added test pieces, it showed 114.6, 202.2 and 196.9 kPa for 1%, 5% and 10 % of CC respectively.

- The UCS of MgP- and MgC-added test pieces showed UCS less than or similar to the test pieces made only from CPC at low percentage of MgP and MgC. At high percentage (eg. 10% in this study) of MgP and MgC, the UCS was dramatically increased. The reason for this increment will be studied in future after conducting Energy Dispersive X-ray Analysis (EDX) and XRD.

All of the TCP- and CC-added test pieces cemented with CPC stock solutions have been showed UCS larger than 100 kPa after 28 days even at the 1% of powder addition. Therefore, according to the strength requirement, there is a possibility to change the powder percentage and/or curing time (days). When time is a crucial factor, it is necessary to increase the powder percentage for early strength requirements. Similarly, when the cost is a crucial factor, it is necessary to use a lower percentage of powder material and allow to a strength improvement with time. This makes the CPC-TCP or CPC-CC method as a suitable ground improvement method for liquefiable areas by keeping some safety factor as required, to use as a cost effective ground improvement practice.

The CPC-Powder method can be applied to produce columns in soft ground as a sand compaction pile (SCP) method or deep mixing method in an actual ground condition. The SCP method is a method of improving soft ground by means of installing well-compacted sand piles in the ground. In sandy ground, the SCP method is often used as a countermeasure against liquefaction and the effectiveness of compaction to prevent liquefaction has been confirmed in past intense earthquakes (Kinoshita et al., 2012) showing the CPC-Powder method to be one of the most reliable improvement method to mitigate liquefaction potential. Furthermore, backfill soil can also be reinforced using this method. The backfill soil tends to subside and manholes and pipelines in the soil tend to be pushed upward due to ground liquefaction during earthquakes. This causes substantial damage to the infrastructure. Hence, backfill soil must be reinforced as a counter- measure against such an occurrence. The Public

Works Research Institute (2008) suggests that a UCS of 100–200 kPa is required for backfill soil reinforcement. The UCS achieved by the addition of TCP and CC powder as shown in this study satisfies this requirement. Furthermore, unlike ground treated with cement-based hardeners, ground treated with the CPC-Powder method can be re-excavated and recycled. This means that the CPC-Powder method can be used as a temporary supplemental hardening method.

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CHAPTER 4: Soil improvement using plant-derived urease induced CC precipitation

4.1 INTRODUCTION

Currently, MICP method is the most widely explored carbonate precipitation method. Microbes having urease activity enhance the hydrolysis of urea and it helps to control the pH and to precipitate carbonate. Therefore, the urease enzyme activity in most of bacteria metabolism process has been used widely as a tool to precipitate calcium carbonate at the presence of Ca^{2+} ions in the system.

One mole of urea is hydrolyzed intracellularly to one mole of ammonia and one mole of carbamate (eq. 4.1), which is spontaneously hydrolyzed to one mole of ammonia and one mole of carbonic acid (eq. 4.2). Ammonia and carbamate subsequently equilibrate in water to form bicarbonate and 2 moles of ammonium and hydroxide ions as described in eqs. 4.3 and 4.4 (De Muynck et al., 2010). The presence of calcium ion in the system will lead to the precipitation of CC once a certain level of super saturation is reached.



Total reaction:



These precipitated carbonate crystals form bonds between sand grains and increase the strength and stiffness of the sand (Van Paassen et al., 2010; Martinez and DeJong, 2009). Although it is a widely explored bio mediated method, it has some disadvantages. The plant-

derived urease may offer many benefits over microbial urease to induce carbonate cementation. Therefore, it is advantageous to explore the knowledge regarding such a technique as an eco-friendly method for different applications in the field of geotechnical engineering as an alternative to the microbial urease.

The extraction of urease enzyme from most urease containing plant species is simple and the enzyme is readily available from laboratory suppliers also. The size of this enzyme is approximately 12 nm in dimension (Blakely and Zerner, 1984) and this small size of urease enzyme gives a distinct advantage over ureolytic microbes which are greater than 300 nm in diameter, with the majority in the range of 500-5000 nm. Therefore, plant-derived urease can be applicable to much finer soils. Furthermore, when urease is used as a free enzyme, it has a limited lifespan and its activity and function decrease with time (Marzadori et al., 1998; Pettit et al., 1976). This limited lifespan may be advantageous in some engineering applications as the enzyme can naturally degrade and eliminate long term impacts to the ecosystem. However, microbial based urease will leave the organisms behind. Therefore, it needs to obtain approvals and licenses from government and require continuous monitoring of microbial ecology for safety (Akiyama et al., 2011). MICP treatment may be limited to deep soil due to limitations of bacterial growth and movement in sub soil. MICP may also be limited to the soils containing fines due to the reduction in pore spaces in fine soils. Based on the size of microorganism, its applicability is limited to well-graded gravel (GW), poorly-graded gravel (GP), well- graded sand (SW), poorly-graded sand (SP), silt (ML), and organic soils (Mitchell and Santamarina, 2005). The symbols within the brackets are according to the unified soil classification system. Bacteria are not expected to enter through pore throats smaller than approximately 0.4 μm . Moreover, the production of urease active bacteria and isolation of pure ureolytic bacteria is one of the main costs for applying to biocementation technology (Van et al., 2011) without enrichment of urease positive bacteria from local

environment (Salwa and Al-Thawadi, 2013). The urease enzyme that is extracted from plant species is little expensive when purchased from lab supply. However, lab grade enzyme is very effective (Knorr, 2014). Furthermore, crude extracts of jack bean (*Canavalia ensiformis*) have the potential to be used as a replacement for commercially available purified urease (Nam et al., 2015). Above mentioned facts are very important to investigate about biocementation of carbonates using plant-derived urease as a promising alternative for microbial urease.

4.2 OBJECTIVES

The objectives of the use of PDUICCP method were to reinforce the small scale sand specimens to an estimated UCS ranging from several kPa to MPa using crude extract of watermelon (*Citrullus lanatus*) seeds urease induced CC precipitation technique and identify the range of applicability of this method using obtained results such as to mitigate the liquefaction and to create artificial soft rocks.

4.3 MATERIALS AND EQUIPMENT

4.3.1 Materials and equipment for Indophenol method

Indophenol method was used to measure the urease activity of the crude extract of watermelon seeds. Following materials and equipment were used for the indophenol method.

(a) Phosphate buffer with pH 7.01

Buffer solution was prepared using 0.1 M potassium phosphate buffer containing 1 mM EDTA).

(b) Substrate

0.1 M urea solution in phosphate buffer was used as the substrate).

(c) Urease enzyme

The enzyme urease solution was prepared using watermelon seeds extract

(d) Phenol-Nitroprusside

The solution was prepared by dissolving 23.5 g of analytical grade reagent phenol in 500 mL of deionized water containing 30 mg of sodium nitroprusside and stored in a brown bottle at 4°C. The reagent is stable for 3 months.

(e) Hypochlorite Reagent

Dissolve 2 g of sodium hydroxide in 100 mL of deionized water containing 7.5 mL of commercially available bleach (5% NaOCl) and store in a brown bottle at 4°C.

(f) Visible spectrophotometer

Visible spectrophotometer was used to measure the intensity of color at 630 nm (OD_{630}).

(g) pH meter (Eutech Instruments Pte., Ltd., Singapore)

4.3.2 Materials and equipment for syringe solidification test

(a) Commercially available sand

Three types of sands with different particle sizes named as Toyoura, Mikawa and Mizunami that can be obtained commercially in Japan were used after drying in an oven up to 110°C about 24 h. The physical properties of each type of sands are presented in Table 4.1.

Table 4.1: Physical properties of different sands used for the current study.

Physical property	Toyoura sand	Mikawa sand	Mizunami sand
Maximum Density (cm^3)	1.645	1.476	1.491
Minimum Density (cm^3)	1.335	1.256	1.348
Particle Density (g/cm^3)	2.64	2.66	2.67
Mean Diameter (mm)	0.2	0.87	1.6

(b) Watermelon seeds extract

(c) Reagents

Calcium chloride (CaCl_2) and urea ($\text{CO}(\text{NH}_2)_2$) was used as reagents.

(d) Incubator

4.3.3 Others materials and equipment

(a) Needle Penetrometer

(b) Ca^{2+} ion meter

(b) Low vacuum SEM machine

SEM machine (TM 3000 Miniscope, HITACHI) was used to observe the microscopic images of cemented sand test pieces.

4.4 METHODOLOGY

4.4.1 Investigation of a new plant-derived urease source from food wastes

Different plant species belong to cucurbit family (*Cucurbitaceae*) such as watermelon (*Citrullus lanatus*), melons (*Cucumis melo*), and pumpkin (*Cucurbita species*) which have urease activity were selected and investigated the best seed suitable for current study. The main purpose of using plant urease enzyme is to catalyze the hydrolysis of urea in to ammonium and carbonate and it helps to increase the pH in the solution. Therefore, we measured the temporal variation of pH in three different solutions prepared from three types of seeds extracts and urea. Each seeds extract was prepared after soaking 1 g of crushed seeds in 10mL of distilled water (soaking time was about 30 min.), filtering and collecting the filtrate. A solution made by dissolving 0.4 M urea in the filtrate was used to measure the pH variation with time. According to the pH variation in each urea-urease solution prepared using watermelon, melon and pumpkin, all of them showed an increment in pH with time (Fig. 4.1). Furthermore, it varies from pH 7 to 9 within 3 h in melon and, pH more than 9

within one hour in watermelon seed extract and urea solutions. The rate of change of pH in pumpkin seed extract and urea solution was low compared with other two types of seeds. Out of them watermelon can be obtained at low price rather than melon. Hence, finally we selected watermelon seeds extract as the urease source for further studies in this chapter.

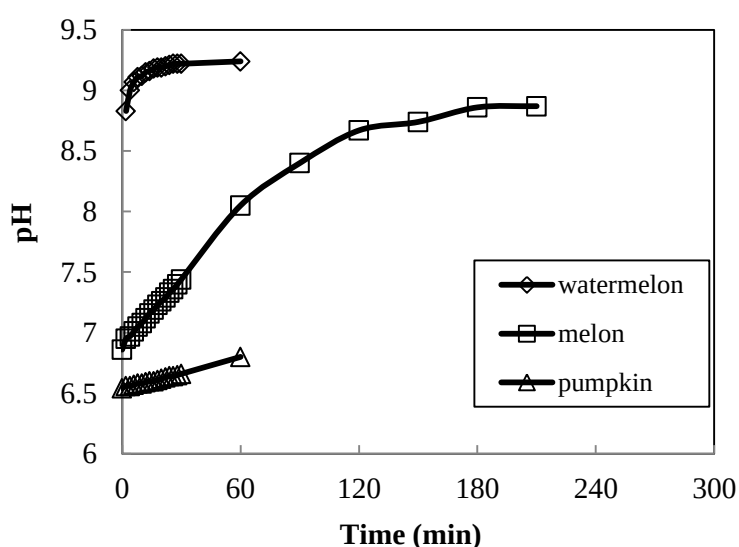


Fig. 4.1: Temporal variation of pH in different seed extracts and urea solution.

4.4.2 Extraction of urease enzyme from watermelon seeds and quantitative measurement of urease activity

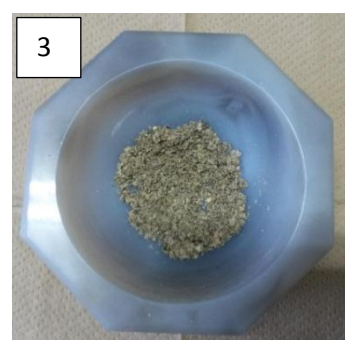
In this study, watermelon seeds were finely ground using a mortar and a pestle. The crushed seeds (0.5 g) were stirred at 500 rpm with 10 mL of distilled water (concentration was represented as 50 g/L) for 1 h. The crude extract was obtained after filtering and collecting the filtrate. One part of the collected crude extract was directly used to measure the urease activity and the other part of the extract was centrifuged at 10,000 rpm for 3 min at 25°C and the supernatant was used to measure the urease activity. The preparation method is shown in Fig.4.2.



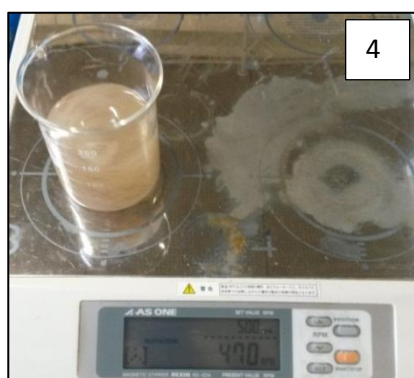
Dry watermelon seeds



Crushing using mortar and pestle



Crushed seeds



Soaking about 1 h with stirring



Remove coarser particles



Filtrate before centrifuge



Centrifuging at 25°C , 10000 rpm, 3 min.

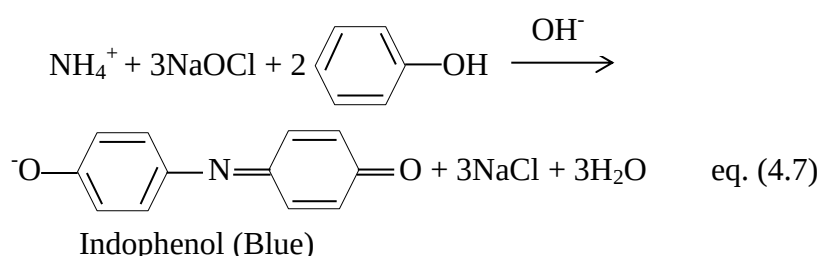


Filtrate after centrifuge

Fig. 4.2: Extraction of urease enzyme from watermelon seeds.

The purpose of measuring urease activity before and after the centrifuge was to identify the effect of solid part on urease activity. Furthermore, the temperature effect on urease activity was examined by measuring urease activity at range of temperatures varying from 25°C to 70°C. Freshly prepared crude extract as described above was used throughout this study and all were after centrifuge. However, centrifuged crude extract was not a fully transparent solution and it also consisted with some suspended fine particles. As mentioned in Section 4.3.1, a 0.1 M urea solution in phosphate buffer with pH around 7.01 was used as the substrate.

A quantitative value for urease activity was obtained using indophenol method (Natarajan, 1995). In this investigation, the enzyme urease is used to catalyze the hydrolysis of urea into CO₂ and NH₃. The formed NH₄⁺ ions react with phenol in the presence of hypochlorite to give the blue dye, indophenol as in eq. (4.7). Intensity of the color produced is proportional to the concentration of NH₄⁺ ion in the sample and is measured at 630 nm. NH₄⁺ ions released as a result of urea hydrolysis is determined by referring to a previously prepared standard curve with relating absorbance at 630 nm for 0.1, 1 and 10 mg/L NH₄⁺ ion solutions prepared from 1000 mg/L NH₄Cl stock solution (Fig. 4.3).



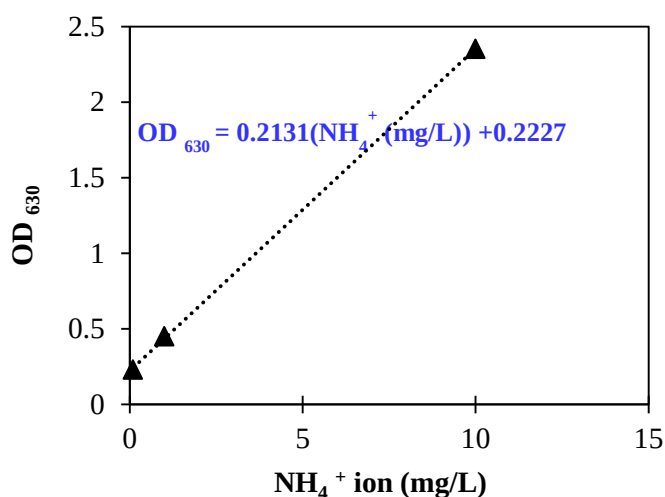


Fig. 4.3: Standard curve for OD₆₃₀ obtained for known NH₄⁺ concentrations.

At the indophenol method, 0.1 M urea solution was prepared using 100 mL of phosphate buffer with pH 7.01 and was heated up to required temperature in a water bath. 1 mL of urease solution was added in to urea solution that was kept in the water bath. 2 mL from the urea- urease reaction mixture was taken out at 0 min, 5 min, 10 min and 15 min after the addition of urease solution. Sampling at 0 min. was done just after adding urease solution in to the urea solution. The reaction was terminated by quickly adding 4.0 mL of phenol reagent followed by 4.0 mL of alkaline hypochlorite reagent in to each sample. After that, the final mixture (10 mL) was incubated at 50-60 °C for 10 min after vortexing. The appearance of the samples after incubating is shown in Fig. 4.4. The absorbance at 630 nm was measured after diluting with distilled water as required. Then a graph like Fig. 4.5 (a) can be drawn and it can be converted to a graph like Fig. 4.5 (b) using the previously prepared standard curve (Fig. 4.3). The rate of NH₄⁺ generation, r_1 (mg/L/min) can be obtained from the graph appear in Fig. 4.5 (b) and urease activity was calculated as shown in below.

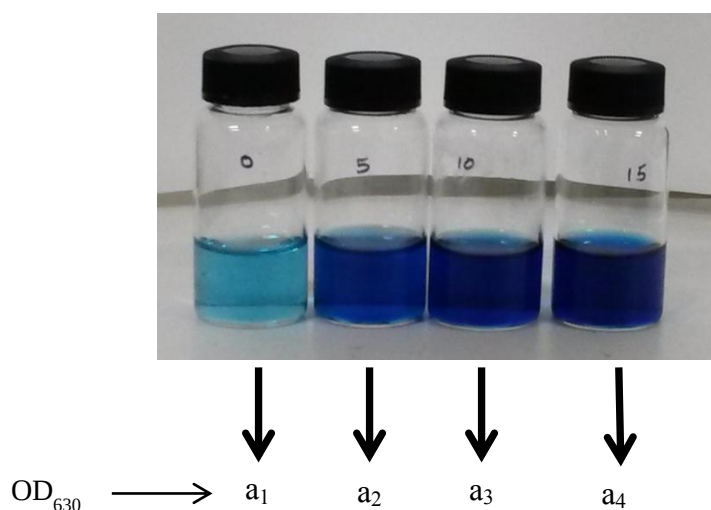


Fig. 4.4: The appearance of the sample used to measure OD_{630} .

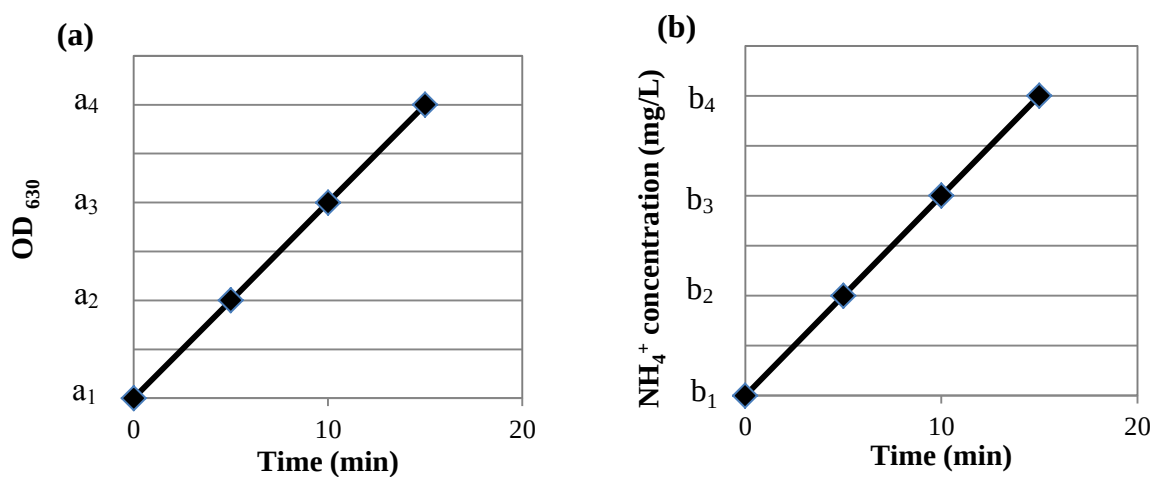


Fig. 4.5: Sample curve for the variation of (a) OD_{630} and (b) NH_4^+ concentrations with time.

The rate of NH_4^+ generation (mg/L/min) = r_1

Rate of NH_4^+ generation- $\mu\text{mol } NH_4^+/\text{min} = r \times 0.1 \times 1/18 \times 10^3 = r_2$

Urease activity = $\mu \text{ mol urea hydrolyzed/min} = [U/\text{mL}] = r_2 \times 1/2$

4.4.3 Test tube experiment

Before starting the syringe solidification test, the rate of precipitation of CC under various combinations of CaCl_2 -urea and urease was evaluated directly in transparent polypropylene tubes by measuring Ca^{2+} concentration with time. As shown in the Fig. 4.6, 10 mL of equimolar solutions of CaCl_2 -urea, with concentrations of 0.3 mol/L (hereafter M), 0.5M and 0.7 M were prepared separately in concentration known watermelon seeds extract.

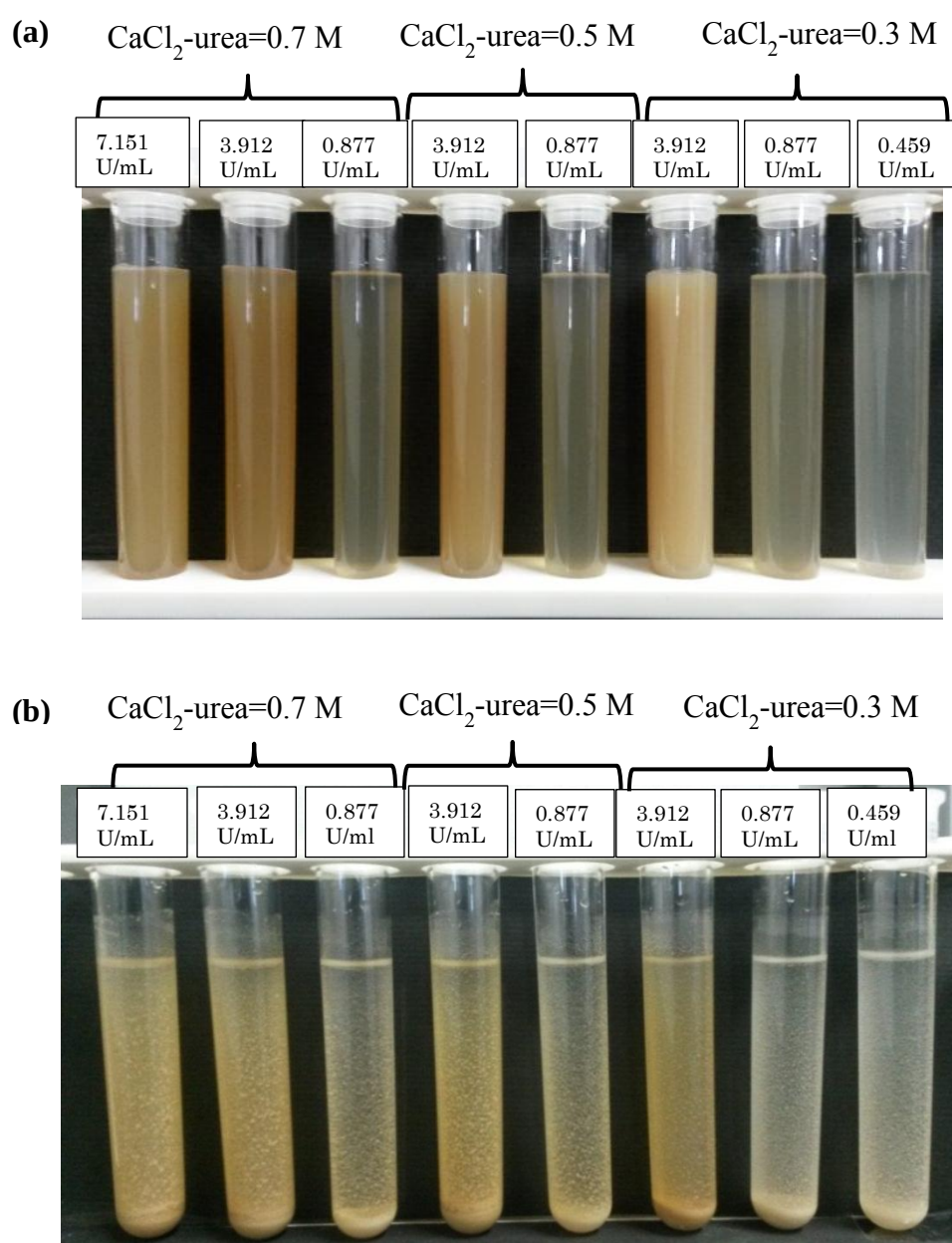


Fig. 4.6: Test tube experiment (a) Just after mixing (b) After 1 day.

Concentrations of seeds extract were changed as 5 g/L (0.459 U/mL), 10 g/L (0.877 U/mL), 50 g/L (3.917 U/mL) and 100 g/L (7.151 U/mL). (Preparation procedure is described in the Section 4.4.2). Values within the brackets are represented the urease activities of each extract at 25 °C). Hereafter, each extract is described using urease activity value instead of the concentration of seeds extract. Similar combination of test tubes were prepared, and measured the temporal variation of Ca^{2+} ions at different temperatures (15, 25 and 35°C) to observe the effect of temperature, effect of urease activity and effect of CaCl_2 -urea concentration on the rate of precipitation of CC.

4.4.4 Syringe solidification test

43.5 g of commercially available Mikawa sand- No. 4 (In case of Toyoura and Mizunami sands, the weights are 48.5 and 44.0 g respectively) (Grain size distribution of each sand is shown in Fig. 4.7) was dried at 110°C for 1 days and then placed into a 35mL

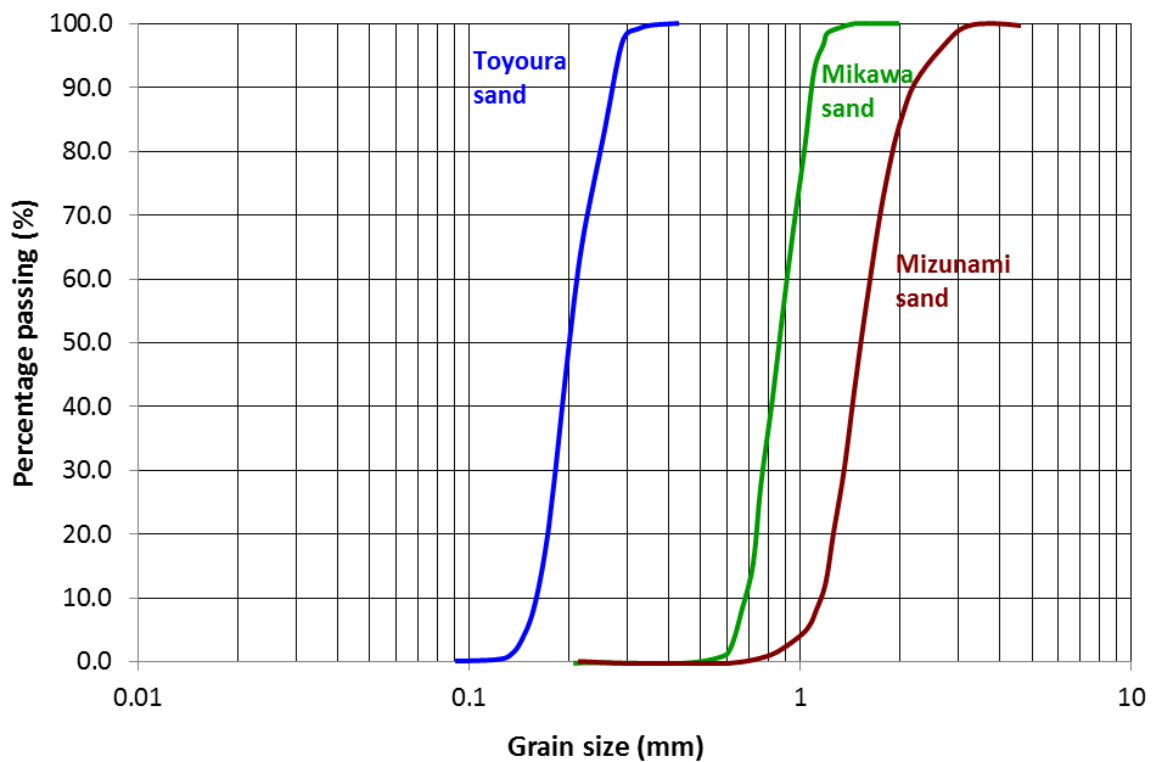


Fig. 4.7: Grain size distribution of Toyoura, Mikawa (No.4) and Mizunami sands.

syringe (diameter, $\phi = 2.3$ cm, height $h = 7.1$ cm) under three equal layers and each layer was compacted, and final density of the specimen was equal to the maximum density of sand. The syringe specimens were kept in an incubator throughout the experiment to control the required temperature. The setting of the syringe solidification test is shown in Fig. 4.8.



Fig. 4.8: The setting of the syringe solidification test.

Subsequently, 13.1 mL of CaCl_2 -urea-urease solution was added to the syringe (calculated initial void volume was 13.1 mL for Mikawa sand. In case of Toyoura sand and Mizunami sand, initial void volumes were 11.1 and 13.0 mL respectively) and kept the final level of the solution as top surface level of the sand specimen. The CaCl_2 -urea-urease solution was prepared by dissolving CaCl_2 and urea in a centrifuged crude extract. The purpose of selecting centrifuged crude extract was to prevent the clogging of specimen with

suspended particles in the crude extract. However, centrifuged crude extract was not a fully transparent solution and it also consisted with some suspended particles. After curing one day, a new CaCl_2 -urea-urease solution with same concentration and volume was added and, at the same time, drained the previously injected solution slowly. While draining out the solution, initially, the rate was maintained at 0.05 mL/s. However, this rate may change (decrease) with curing time due to precipitated CC within the specimen. The Ca^{2+} concentration and pH of the drainage were measured once in every two days to determine temporal variations of these parameters within the specimen. At the same time, two control test specimens were prepared only using CaCl_2 -urea and CaCl_2 -urease. After required curing time, (7, 14, 21 and 28 days in this study) each specimen was carefully removed from the syringe and needle penetration (NP) test was conducted using a needle penetration device to determine a value for estimated UCS. In this way, different syringe solidification tests were conducted by changing different parameters as shown in Table 4.2.

Table 4.2: Different test cases used in syringe solidification test (To be continued...).

Case No.	CaCl ₂ -urea concentration (M)	Urease activity (U/mL)	Curing time (Days)	Temperature (°C)	Type of sand
1	0.7	7.151	7	25	Mikawa
2		3.912			
3			14	15	
4				35	
5				25	Toyoura
6			Mizunami		
7		Mikawa			
8			21		
9			7		
10			14	15	
11				25	
12				35	
13		25	Toyoura		
14			Mizunami		
15			21	Mikawa	
16	0.5	3.912	14	25	Mikawa
17		0.877		15	
18				25	
19				35	
20		0.459		15	
21				25	
22				35	
23	0.3	3.912	14	25	Mikawa
24			21		
25			28		

Table 4.2: Different test cases used in syringe solidification test.

Case No.	CaCl ₂ -urea concentration (M)	Urease activity (U/mL)	Curing time (Days)	Temperature (°C)	Type of sand
26	0.3	0.877	14	25	Mikawa
27			14	15	
28			14	35	
29	0.3	0.877	14	25	Toyoura
30			21		Mikawa
31			28		
32		0.459	14	15	Mikawa
33				25	
34				35	
35			25	25	Toyoura
36					Mizunami
37					Toyoura
38					Mizunami

4.4.5 Needle penetration test to determine a value for estimated UCS

An estimated UCS was obtained from the needle penetration (NP) value given by the needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan) (Fig. 4.9), based on the following regression equation eq. (4.8) (correlation coefficient: 0.941, x : NP (N/mm), y : UCS (MPa)) determined from 114 natural rock samples and 50 improved soils with cement. The chart appears on the needle penetration device has been developed using eq. (4.8). Therefore, we can directly read the UCS value from the chart.

$$\log(y) = 0.978 \log(x) + 2.621$$

eq. (4.8)

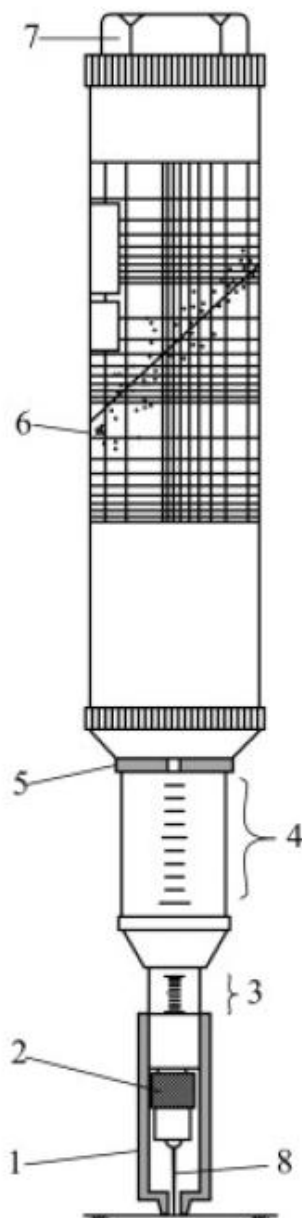


Fig. 4.9: Needle penetration device and its parts: 1. presser, 2. chuck, 3. penetration scale, 4. Load scale, 5. load indicating ring, 6. UCS- Np correlation chart, 7. Removable cap, 8. penetration needle.

The load is applied to the specimen perpendicularly and slowly. The specimen should be fixed to prevent its movement during the application of load (Fig. 4.10).



Fig. 4.10: Conducting needle penetration test.

4.4.6 Evaluation of the theoretical amount of precipitated carbonates within the sand test specimen

The total theoretical amount of precipitated CC content within the sand test specimens were estimated using the equations derived by Danjo and Kawasaki (2016) as shown below by doing small changes as to match it to this study. The Ca^{2+} concentration in the drainage was measured once in every two days. The concentrations at other days were assumed to be the same as the concentration measured at previous day. The daily precipitated Ca^{2+} concentration (C_p (ppm)) was considered to be the difference between the Ca^{2+} concentration in the inlet solution (C_{in} (ppm)) and that of in the outlet solution (C_{out} (ppm)) as shown in eq. (4.9).

$$C_p = C_{in} - C_{out} \quad \text{eq. (4.9)}$$

The initial void volume (V_{v0} (cm^3)) was 13.1 cm^3 , as calculated from the specimen volume (V_t : 29.5 cm^3), the dry mass of sand in the specimen (m_{sd} : 43.5 g), and the sand particle density (ρ_s : 2.66 g/cm^3) (eq. (4.10)).

$$V_{v0} = V_t - m_{sd}/\rho_s = 29.5 - (43.5/2.66) = 13.1 \quad \text{eq. (4.10)}$$

The void volume at n elapsed days was determined by eq. (4.11). For this equation, the density of CC (ρ_{CaCO_3} (g/cm^3)) was considered to be equivalent to that of calcite (2.71 g/cm^3) because the precipitate was calcite.

$$V_{vn} = V_{v0} - \sum_{i=0}^n \frac{m_{pi}}{\rho_{\text{CaCO}_3}} \quad \text{eq. (4.11)}$$

The daily amount of CC precipitation (m_{pn} (g)) between $n-1$ and n elapsed days were calculated by eq. (4.12). The molecular masses of CC and Ca^{2+} were 100.1 g and 40.1 g , respectively.

$$m_{pn} = (C_{pn} / 1000 \times V_{vn-1} / 1000) \times 100.1 / 40.1 \quad \text{eq. (4.12)}$$

Moreover, the CC precipitation from $n-1$ to n days (X_{pn} (g/g sand)) was calculated as shown in eq. (4.13)

$$X_{pn} = m_{pn} / m_{sd} \quad \text{eq. (4.13)}$$

4.4.7 Observation of the segments of cemented test pieces by SEM

Segments of the UCS test pieces were observed by SEM (TM 3000 Miniscope, HITACHI) just after the UCS tests.

4.5 RESULTS

4.5.1 Quantitative value for urease activity of watermelon seeds urease

The obtained urease activity (U/mL) values for different concentrations of seeds extracts are graphically represented in Fig. 4.11.

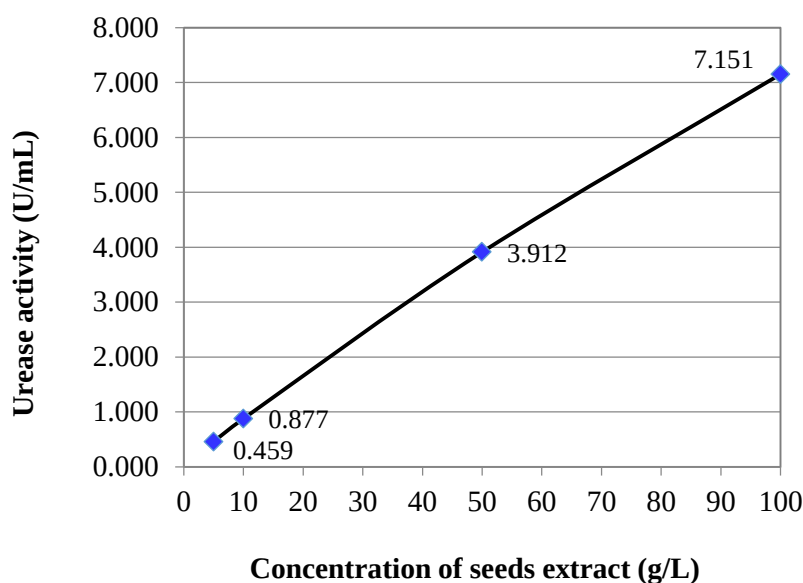


Fig. 4.11: Urease activity for different concentrations of seeds extracts at 25°C reaction temperature.

According to the results, the urease activity was increased with the increase of the concentration of seeds extracts and the increment was not linear. Furthermore, Fig. 4.12 shows the activity before and after centrifuge as well as the variation of the activity with temperature. The crude extracts before and after centrifuge showed different activity values. The urease activity before centrifuge was little higher than after centrifuge. Furthermore, the urease activity values at different reaction temperatures ranging from 25°C to 70°C were examined and the maximum activity was obtained around 50 °C (Fig. 4.12). Afterwards, the activity abruptly declined with further increase of temperature. When compare with the urease activities of other plant urease sources obtained from previous studies, maximum

urease activity of jack bean has been occurred at 65°C (Sumner, 1951) and inactivated at temperatures above 70°C (Frankenberder and Tabatabai, 1982). Maximum urease activity of chickpea seeds has been obtained at temperature of 40°C and beyond that, the enzyme has been denatured rapidly and thus, lost its activity (El-Hefnawy et al., 2014). This result is closely related to those reported by Das et al. (2002) and Srivastava et al. (2001) for pigeon pea urease but differs from that stated by El-Shora (2001).

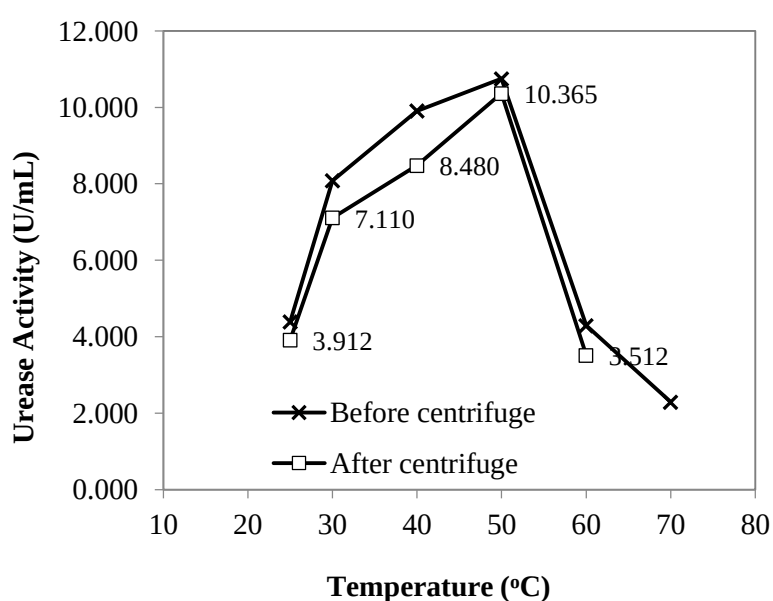


Fig. 4.12: Variation of urease activity with different reaction temperatures.

4.5.2 Results of the test tube experiment

Temporal variation of Ca^{2+} ion concentration in the polypropylene test tubes under different urease activities are shown in Fig. 4.13.

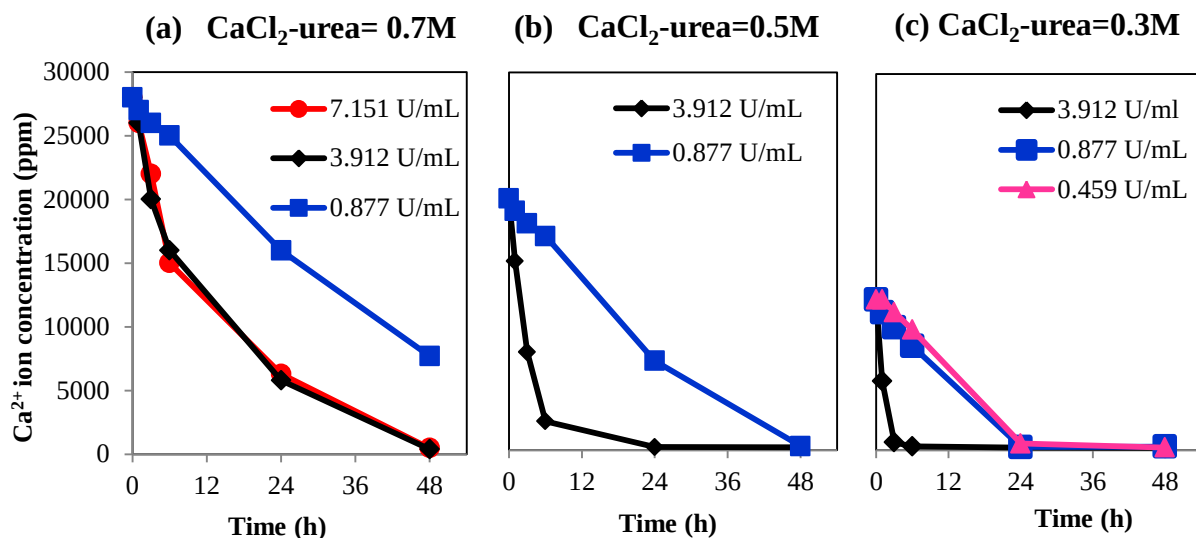


Fig. 4.13: Variation of Ca^{2+} ion with urease activity.

The rate of change of Ca^{2+} ion in the solution was changed with the urease activity. The rate of decrease of Ca^{2+} ions in the solution increases with the increase of urease activity, at each concentration of CaCl_2 -urea as shown in Fig. 4.13. The rate of change of Ca^{2+} ion in the solution is directly proportional to the precipitation of CC.

The temperature effect on CC precipitation under different urease activity was investigated. The variation of Ca^{2+} ion concentration with temperature at each concentration of CaCl_2 -urea is shown in Figs. 4.14, 4.15 and 4.16. The rate of decrease of Ca^{2+} ion in the solution was increased with temperature regardless of the urease activity. However, in Fig. 4.16, the rate of decrease of Ca^{2+} ion at 25°C and 35°C were almost same. Although there were some Ca^{2+} ions remained in the solution after 24 h in some testing cases relevant to 35°C as in Figs. 4.14 and 4.15, further decrement of Ca^{2+} ions could not be seen in 35°C like in 25°C and 15°C.

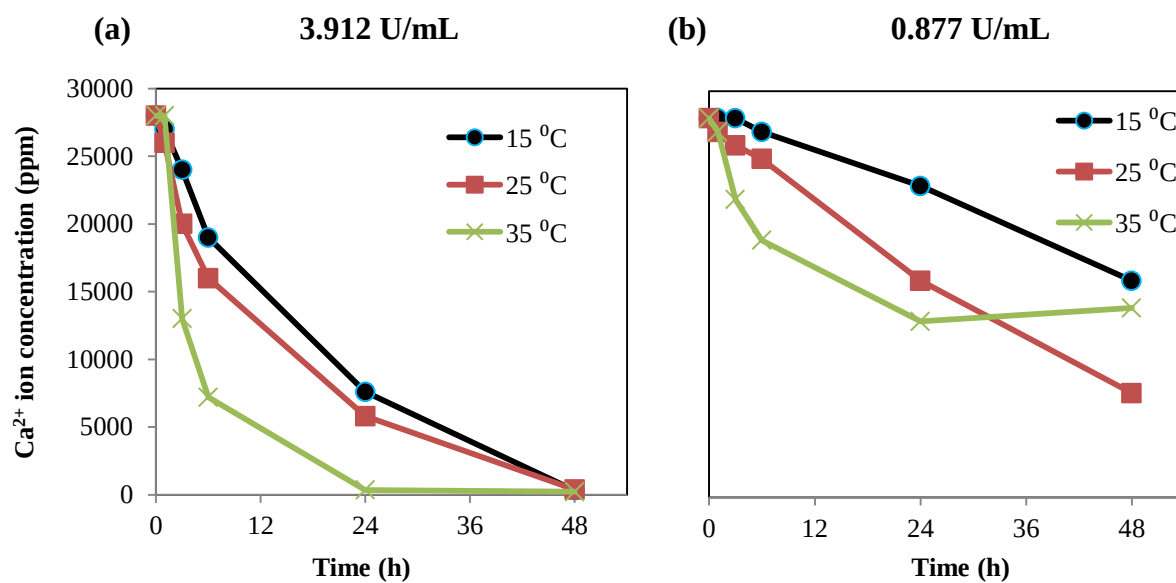


Fig. 4.14: Variation of Ca^{2+} ion with temperature (CaCl_2 -urea=0.7M).

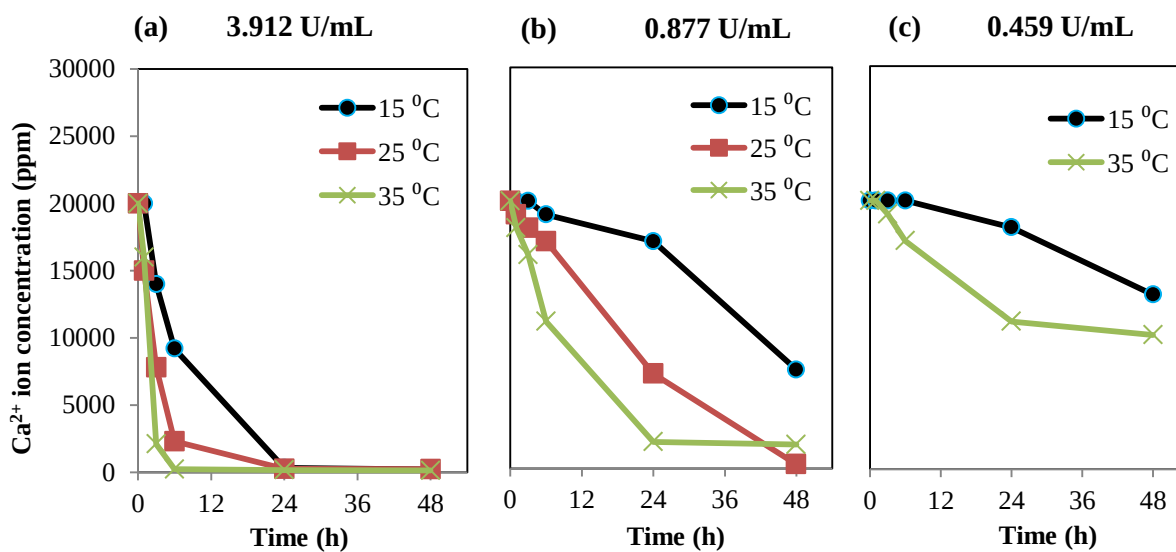


Fig. 4.15: Variation of Ca^{2+} ion with temperature (CaCl_2 -urea=0.5M).

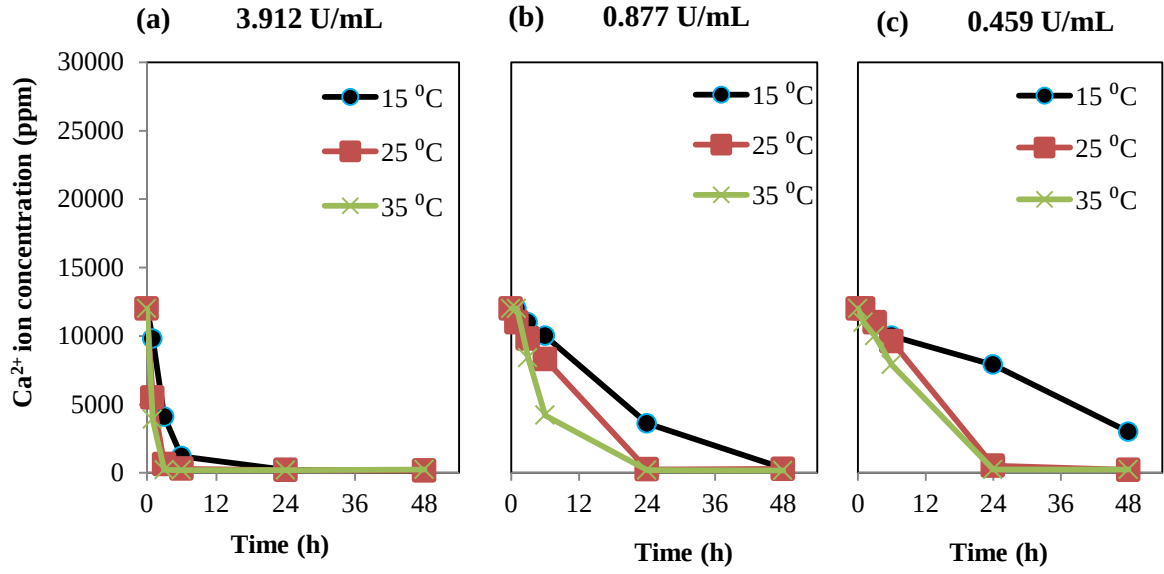


Fig. 4.16: Variation of Ca^{2+} ion with temperature (CaCl_2 -urea=0.3M).

4.5.3 Results of the syringe solidification test

(a) Relationship between CaCl_2 -urea concentration and estimated UCS

After decided curing time, the NP values of each sample were measured using needle penetration device as described in the methodology section and the estimated UCS was obtained by using eq. (4.8) or directly using the chart on needle penetration device. Estimated UCS values obtained for different concentrations of CaCl_2 -urea are shown in Fig. 4.17. The minimum estimated UCS value that can be obtained from the needle penetration device was 200 kPa. Therefore, the estimated UCS values less than 200 kPa were reported as 0 kPa for the ease of represent it graphically. The appearance of some syringe test specimens just before the needle penetration test is shown in Fig. 4.18.

According to Fig. 4.17, the estimated UCS values were increased with CaCl_2 -urea concentrations under constant urease activity.

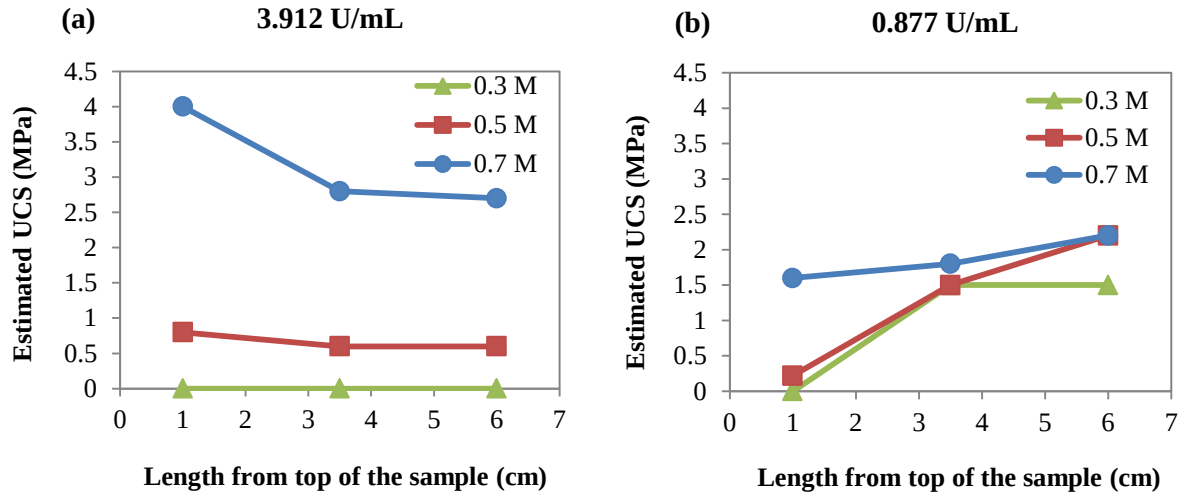


Fig. 4.17: Effect of CaCl_2 -urea concentration on estimated UCS after 14 days of curing time

(a) Urease activity = 3.912 U/mL and (b) Urease activity = 0.877 U/mL.

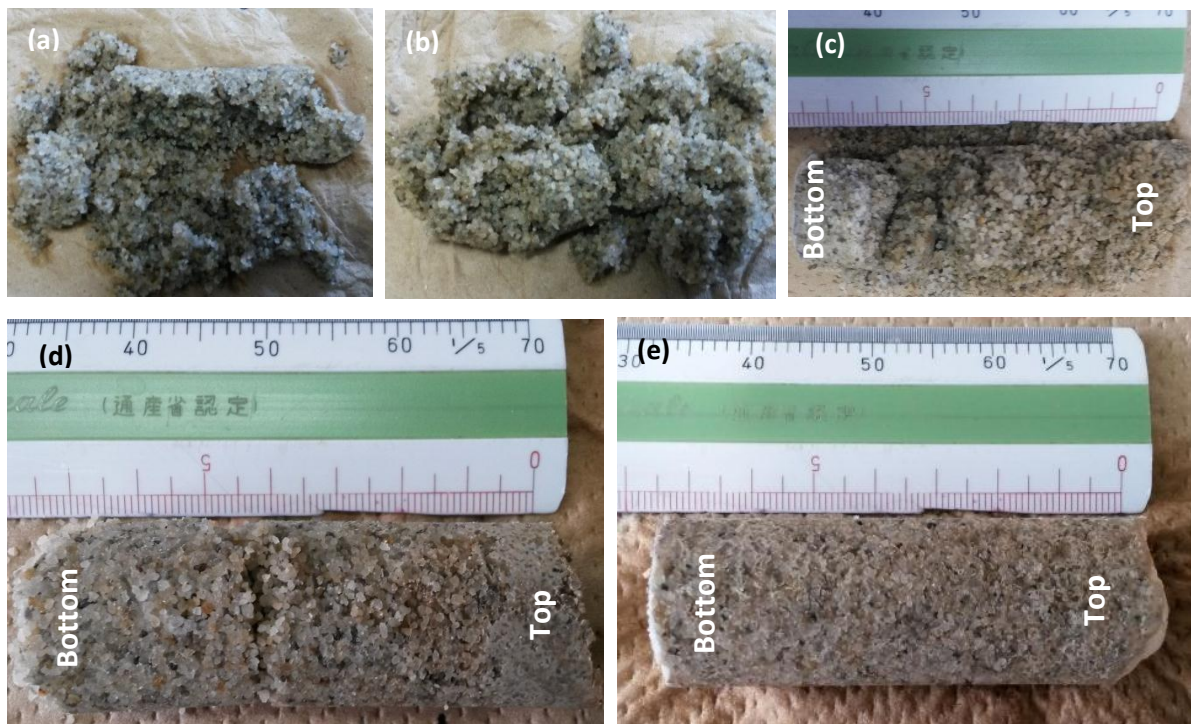


Fig. 4.18: Appearance of Syringe test specimens after 14 days of curing time (a) 0.7 M

CaCl_2 + urea, No urease (b) 0.7 M CaCl_2 + urease-3.912 U/mL, No urea and (c) 0.3 M CaCl_2 + urea +urease-3.912 U/mL, (d) 0.5 M CaCl_2 + urea +urease-3.912 U/mL and (e) 0.7 M CaCl_2 + urea +urease-3.912 U/mL.

(b) Relationship between urease activity and estimated UCS

According to Fig. 4.19, in the case of 0.7 M CaCl_2 -urea, the estimated UCS was increased with the increase of urease activity. Similarly, the estimated UCS was also increased with the increase of urease activity up to certain level in the cases with 0.5M and 0.3 M CaCl_2 -urea and afterward further increment of urease activity caused to decrease the estimated UCS value.

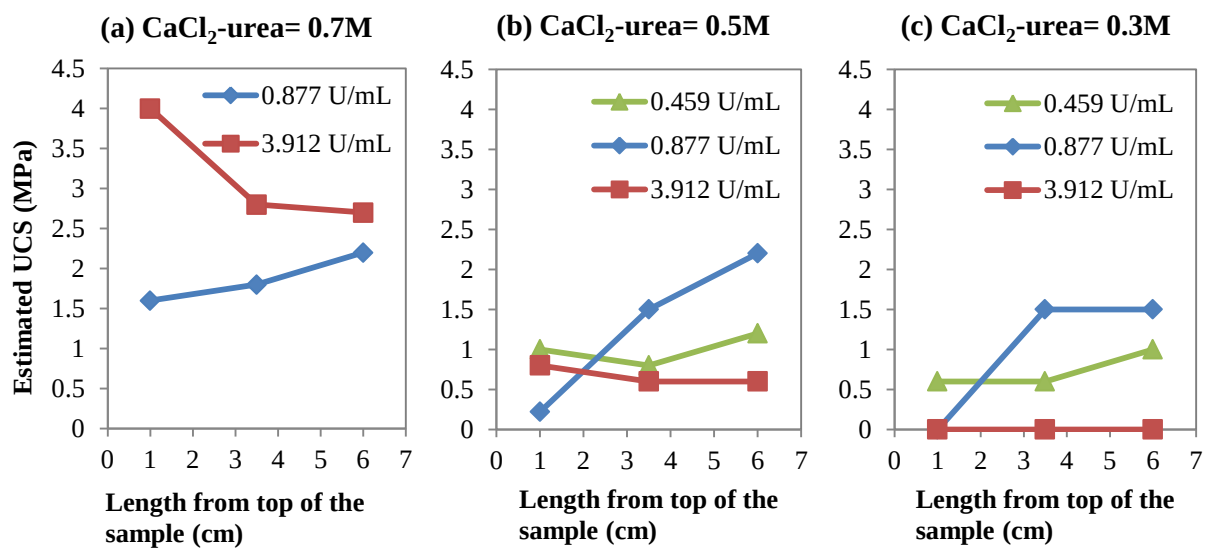


Fig. 4.19: Effect of urease activity on estimated UCS after 14 days of curing time.

(c) Relationship between curing time and estimated UCS

As shown in Fig. 4.20, the estimated UCS values were increased with curing time (days) regardless of the CaCl_2 -urea concentration and urease activity.

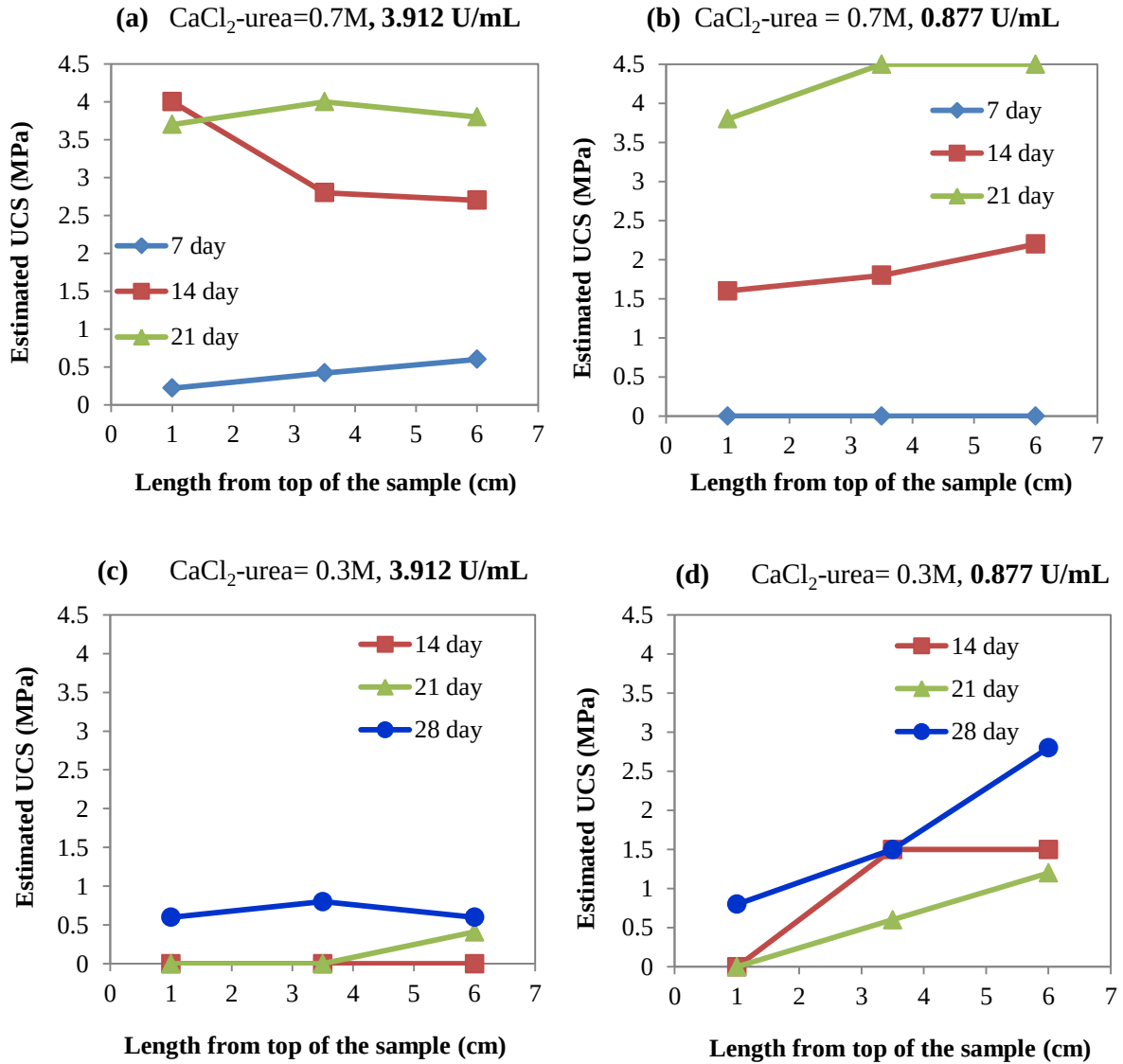


Fig. 4.20: Effect of curing time on estimated UCS with different concentrations of CaCl₂-urea and urease activity.

(d) Relationship between temperature and estimated UCS

In order to identify the relationship between temperature and estimated UCS of solidified sand test specimens, different testing cases were also conducted by changing the temperature as 15, 25 and 35°C. CaCl₂-urea concentration and urease activity also changed to investigate the effect of temperature on estimated UCS more clearly. The obtained results are

graphically represented in Fig. 4.21. According to the results, a clear pattern of estimated UCS (after 14 days of curing time) with temperature could not be observed.

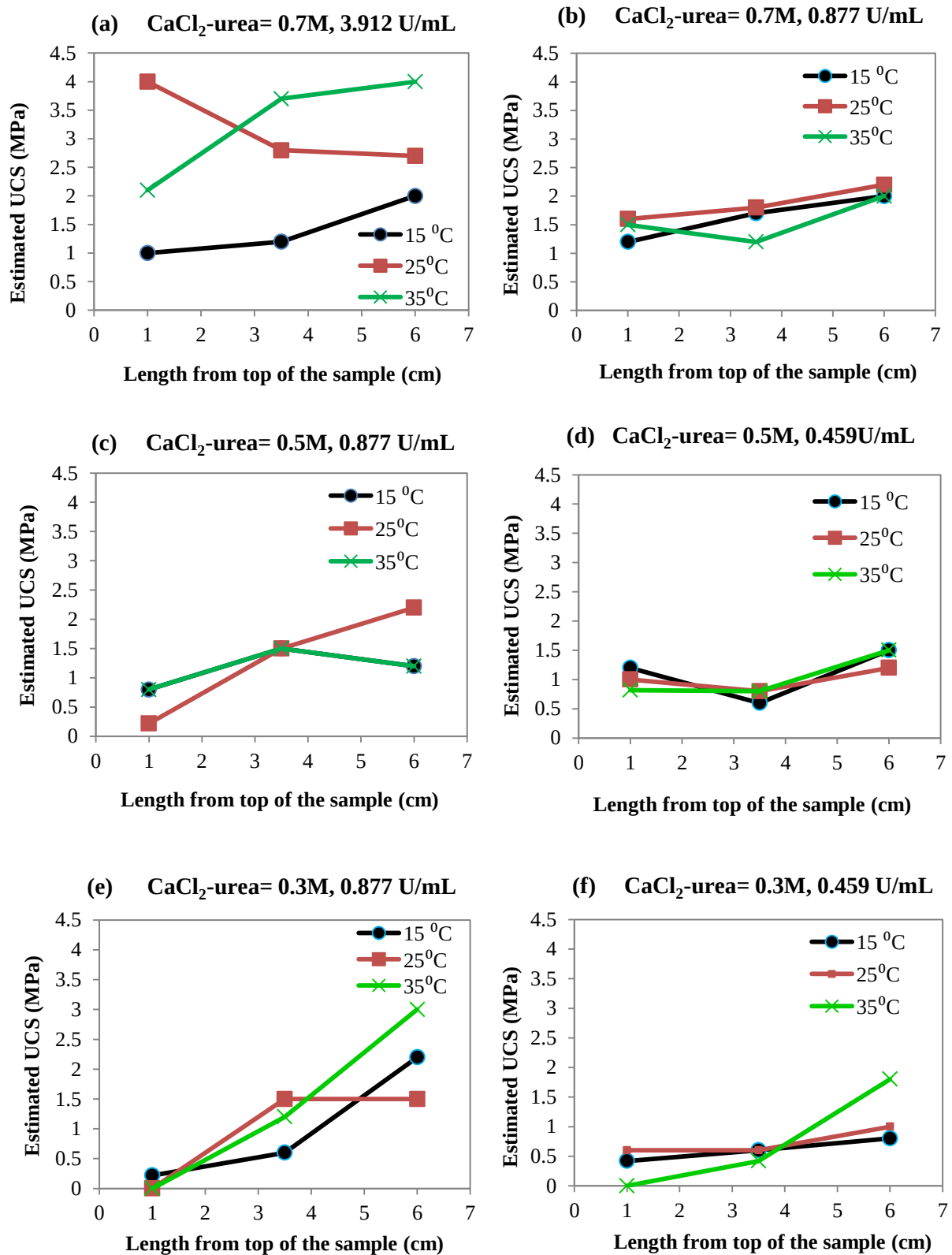


Fig. 4.21: Effect of temperature on estimated UCS.

(e) Relationship between particle size of sand and estimated UCS

Fig. 4.22 shows the effect of particle size on estimated UCS after 14 days of curing time. As mentioned in the Section 4.3.2, mean diameters of Toyoura, Mikawa and Mizunami sands are $200\ \mu\text{m}$, $870\ \mu\text{m}$ and $1600\ \mu\text{m}$ respectively. According to the results, the pattern of strength distribution of Mikawa and Mizunami sands is different from Toyoura sand. The top of the Toyoura sand test pieces were more solidified than middle and bottom of the test pieces. Conversely, the bottom of the Mikawa and Mizunami sand test pieces were more

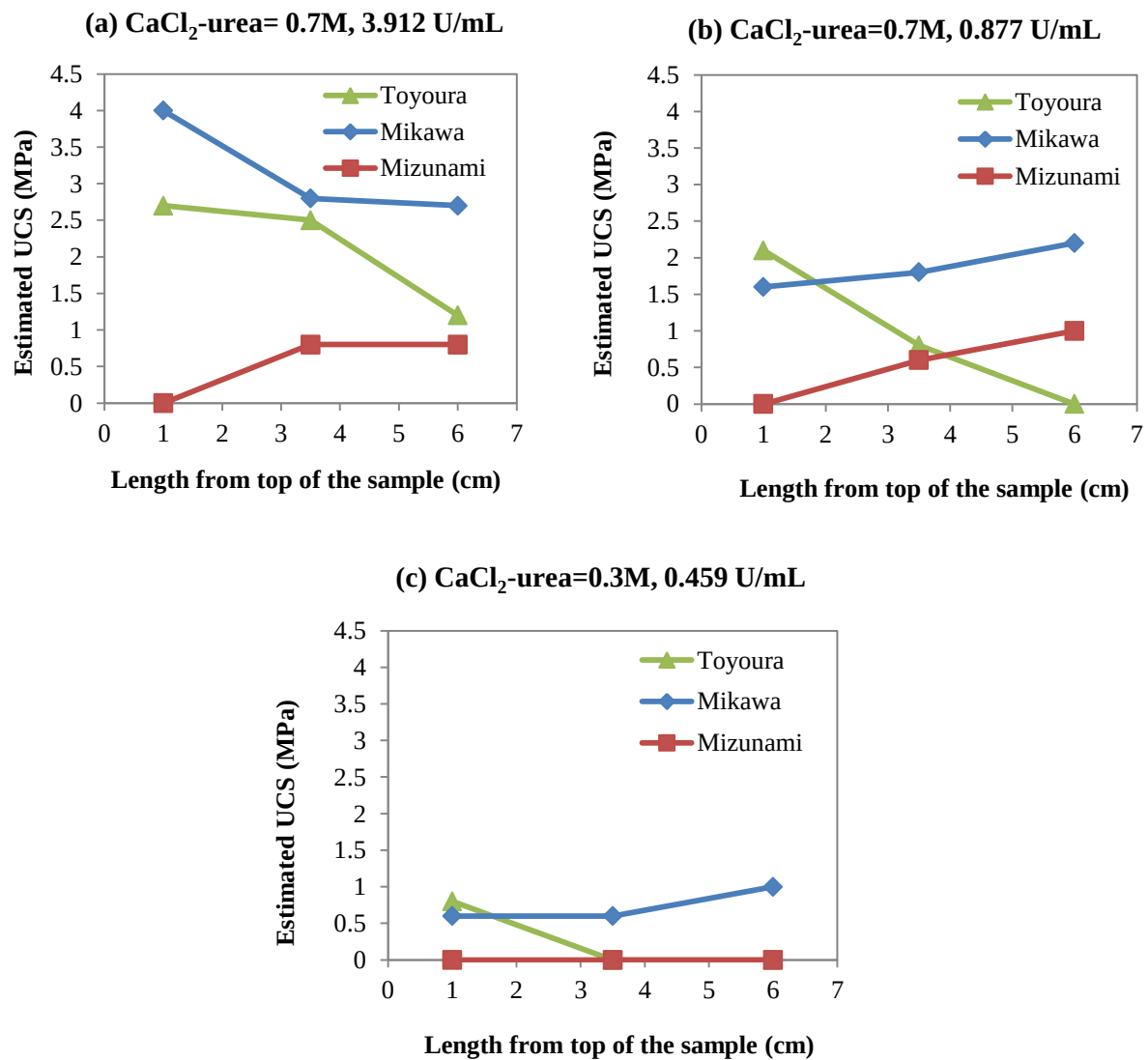


Fig. 4.22: Effect of particle size on estimated UCS.

solidified than middle and top of the test pieces. Furthermore, when compare with Toyoura and Mikawa sand test pieces Mizunami sand test pieces show lower value of estimated UCS. The appearance of some solidified test specimens made using three different sands is shown in Fig. 4.23.



Fig. 4.23: Solidified sand test specimens (a) Toyoura (b) Mikawa and (c) Mizunami sand.

(f) SEM images

SEM images show the bonding of precipitated CC on the surface of sand particles. Furthermore, calcite polymorph was observed for all the test cases as similar to Fig. 4.24 (b) regardless of the urease activity and CaCl_2 -urea concentration, and these appeared in the form of rhombohedra. Intergrowth and steps may indicate some disturbances during the growth process of the crystal. Similarly, in some previous studies related to commercially available plant urease induced CC precipitation, calcite polymorph has been observed rather than other polymorphs of CC (Hamdan et al., 2013; Yasuhara et al., 2012). Sondi I and Sondi BS (2005) explain that the crystal phase formation and growth morphology of CC precipitates is mainly due to the primary structure (amino acid sequence) of each urease. According to their physicochemical surface properties, they may interact with the inorganic phase at the solid/liquid interface and thus direct the growth of inorganic structures.

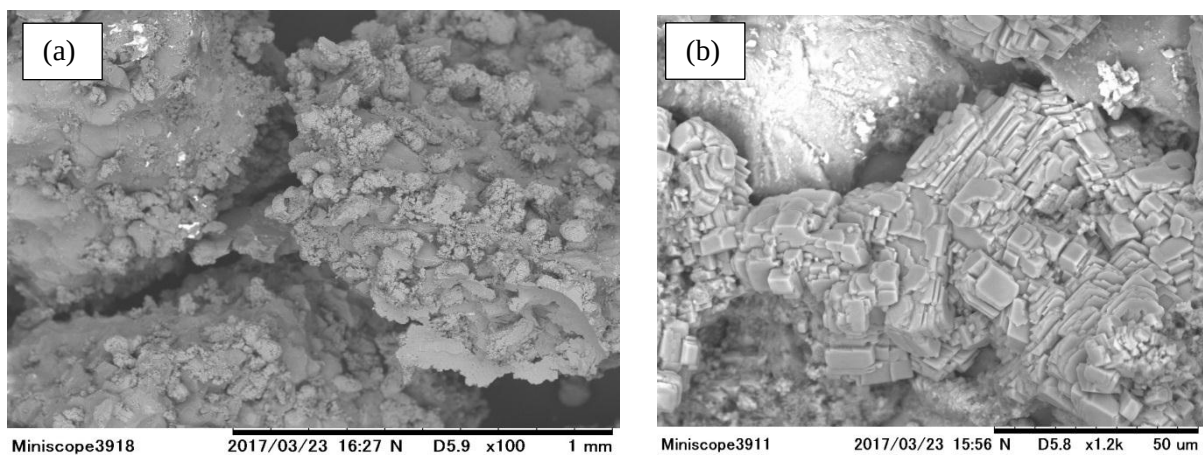


Fig. 4.24: SEM images of CC precipitated Mikawa sand test specimen (CaCl_2 -urea= 0.3M, 0.877 U/mL, 14 days of curing time and 25°C temperature), (a) x 100, (b) x 1200.

4.6 DISCUSSION

4.6.1 Effect of reaction temperature on urease activity

According to the results, the urease activity before centrifuge was little higher than after centrifuge. This may be due to the presence of some urease activity in the fine solid particles in the crude extract that cannot be dissolved in distilled water during 1 hour soaking period. Moreover, the maximum activity was obtained around 50 °C (Fig. 4.12). Afterwards, the activity abruptly declined with further increase of temperature. That means they are no longer active and cannot function properly and reach to the denaturation at higher temperatures more than 50°C.

4.6.2 Effect of urease activity and temperature on CC precipitation in test tubes

(a) Effect of urease activity on CC precipitation

According to the results of Fig. 4.13, when the urease activity increases, the rate of decrease of Ca^{2+} ion in the solution increases and therefore, the rate of precipitation of CC increases. The reason is the increase of the rate of urea hydrolysis while increasing the urease activity. It caused to release more CO_3^{2-} ions to the solution and finally to precipitate CC more rapidly. Moreover, when the amount of urea in the solution is small, the amount of urease required to hydrolyze the small amount of urea is small. Therefore, when the CaCl_2 -urea concentration decreases the amount of urease required to precipitate CC decreases.

(b) Effect of temperature on CC precipitation

According to Figs. 4.14, 4.15 and 4.16, the rate of decrease of Ca^{2+} ion in the solution was increased with temperature regardless of the urease activity. This can be explained using Fig. 4.12. When the temperature increases, urease activity increases within the temperature

range we selected for test tube experiment (15°C-35°C). Then the rate of hydrolysis of urea increases with temperature. It caused to release more CO_3^{2-} ions to the solution and finally to precipitate CC more rapidly. Although there were some Ca^{2+} ions remained in the solution after 24 h, in some testing cases in Figs. 4.14 and 4.15, further decrement of Ca^{2+} ions could not be seen in 35°C as in 15°C and 25°C. As shown in Fig. 4.25, store temperature caused to change the urease activity. According to that, we can expect some urease activity even after 24 h in the tubes experiment done at 15°C and 25°C. Hence, further decrement of Ca^{2+} ions could be observed after 24 h in 15°C and 25°C temperatures. However, further decrement of Ca^{2+} ions could not be seen in 35°C after 24 h due to loss of urease activity after 24 h as shown in Fig. 4.25.

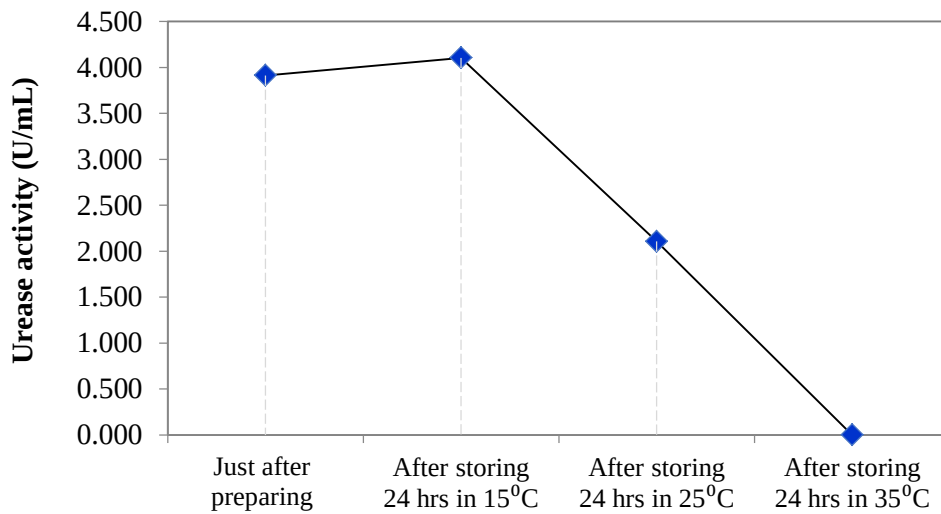


Fig. 4.25: Effect of store temperature on urease activity (Testing temperature was 25 °C).

4.6.3 Syringe solidification test

(a) Effect of CaCl_2 - urea concentration on estimated UCS

According to Fig. 4.17, the estimated UCS values were increased with CaCl_2 -urea concentrations ranged from 0.3 M to 0.7 M under constant urease activity. It can be simply

explained using the amount of precipitated CC. According to eq. (4.5) and eq. (4.6), 1 mole of urea and 1 mole of CaCl_2 would react to form 1 mole of CC. Hence, when we increase the equimolar concentrations of CaCl_2 -urea from 0.3 M to 0.7 M, the amount of precipitated CC increases and it helps to reduce the voids between sand grains. As a result of that, estimated UCS increases.

(b) Effect of urease activity on estimated UCS

According to the results in Fig.4.19, a higher estimated UCS was obtained at low urease activity rather than high urease activity for 0.5M and 0.3 M CaCl_2 -urea. The rate of hydrolysis of the urea tends to decrease with decreasing the urease activity and it may be favorable for precipitating CC within the specimen in the test cases relevant to 0.5 M and 0.3 M concentrations of CaCl_2 -urea.

The low concentration of urea (0.3 M) was rapidly hydrolyzed by the urease with 3.912 U/mL rather than the high concentration of urea (0.7 M). Therefore, CC was started to precipitate before injecting the solution into the syringe specimen in 0.3 M CaCl_2 -urea solution. As a result of that, the voids between sand particles are filled with pre-precipitated CC (PPCC) instead of precipitating within the specimen. It may not be favorable for effective bond between sand particles. Furthermore, some of the PPCC was remained at the top surface of the specimen. However, further decrease of urease activity caused to decrease the estimated UCS value, which may be due to an insufficient amount of urease to hydrolyze 0.3 M and 0.5 M urea and hence, insufficient amount of CO_3^{2-} to precipitate CC within one day. Similar observation could be seen in the test cases which consist of 0.7 M CaCl_2 -urea. The estimated UCS decreased with decreasing the urease activity. As mentioned before, the reason may be due to an insufficient amount of urease to hydrolyze 0.7 M urea. This can be clearly understood using the Ca^{2+} ion concentrations in the outlet solutions (Fig. 4.26).

According to Fig. 4.26 (a), at low urease activity, the Ca^{2+} ion concentration in the outlet was higher than the high urease activity. That means the rate of urea hydrolysis is low at low urease activity and the produced CO_3^{2-} ions are not sufficient to bind with free Ca^{2+} ions in the solution. This is the reason to get low estimated UCS for the urease activity of 0.877 U/mL rather than 3.912 U/mL in Fig.4.19 (a). Therefore, it is needed to select best urease concentration appropriate to each concentrations of CaCl_2 -urea solution.

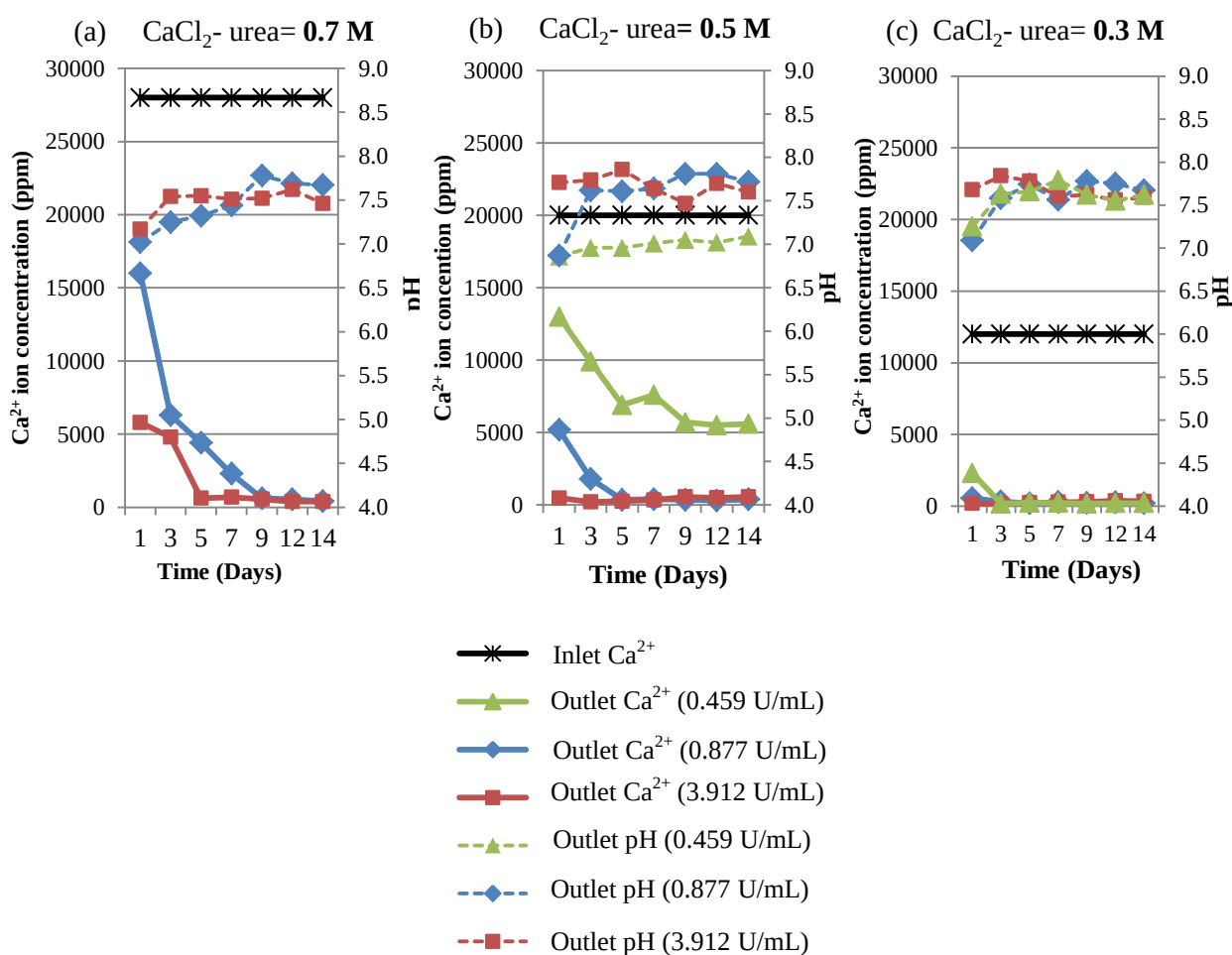


Fig. 4.26: Temporal variation of Ca^{2+} ion and pH with different urease activity values.

Furthermore, the Ca^{2+} ion concentration in the outlet solution decreased and became constant with curing time in all testing cases in Fig. 4.26. The fine particles remain in the crude extract showed some urease activity during urease activity determination. After

draining out the previously injected solution, those particles may remain within the specimen and it may also help to hydrolyze the urea and hence to precipitate more Ca^{2+} ions than previous day. In addition to the fine particles, the urease adsorbed by sand particles may also help to hydrolyze the urea.

(c) Effect of curing time on estimated UCS

According to the Fig. 4.20, estimated UCS values increased with curing time. It can be simply explained using the amount of precipitated CC. The amount of precipitated CC increases with the increase of curing time. The total CC precipitation within the syringe specimen relevant to CaCl_2 -urea concentration of 0.7 M was calculated using the method described by Danjo and Kawasaki (2016) and it was 0.11, 0.21 and 0.23 g/g sand after 7, 14 and 21 days of curing time respectively. The precipitated CC relevant to CaCl_2 -urea concentration of 0.3 M was 0.10, 0.14 and 0.17 g/g sand after 14, 21 and 28 days of curing time respectively. The amount of precipitated CC after 7 days of curing time with 0.7 M CaCl_2 -urea is nearly same to the precipitated CC after 14 days of curing time with 0.3 M CaCl_2 - urea. However, estimated UCS values were different in those two testing cases as shown in Fig. 4.20. As mentioned before, because of the rapid urea hydrolysis, CC precipitation takes place before injecting the solution into the sand specimen. Some of the PPCC remained at the top surface of the specimen and hence, the total amount of calculated CC was not effectively precipitated within the specimen in 0.3 M CaCl_2 -urea-urease solution. This is the reason to change the estimated UCS even at the same amount of calculated CC.

(d) Effect of temperature on estimated UCS

Before understanding the effect of temperature on estimated UCS, it is important to study the effect of temperature on urease activity. As described in Fig. 4.12, the urease activity increases with temperature up to about 50°C and afterward, abruptly declines with further increase of temperature. During urease activity test, the urease extract is kept about 15 min in required temperature. However, during syringe solidification test, the extract is remained 24 h in required temperature as we select 24 h injection interval. The storage of urease about 24 h under different temperatures causes to change the activity in different ways (Fig. 4.25). As shown in Fig. 4.27, a conceptual image of temporal variation of urease activity within the sand specimen during the injection periods was prepared by using the Fig. 4.25 as a supportive figure.

As mentioned in the Section 4.6.3 (b), fine particles consist in the crude extract and/or urease adsorbed by sand particles may remain after draining the previous day injected solution from the outlet. Therefore, after next day injection, the amount of urease involve for the hydrolysis of urea is a summation of urease remains within the specimen. However, the quantity of urease remains within the specimen depends on temperature. Fig.4.27 shows the possible change in urease activity within the specimen with time as well as with temperature. At the temperature of 35°C, it shows constant urease activity at each curing day, and no contribution from previously injected ureases. After keeping 24 h in 35°C temperature, urease deactivates. However, it does not happen for 15°C and 25°C temperatures used in this study (Fig. 4.25). Therefore, the conceptual image of urease activity within the sand specimen at later stages of injection can be either one of (iii), (iv), (v) and (vi) in Fig. 4.27. The way of this change in urease activity affected for the CC precipitation within sand specimen. The variations of Ca^{2+} ions in the outlet at different temperatures are shown in Figs.4.28 and 4.29.

The effect of temperature on estimated UCS is unclear due to uneven change in urease activity with temperature.

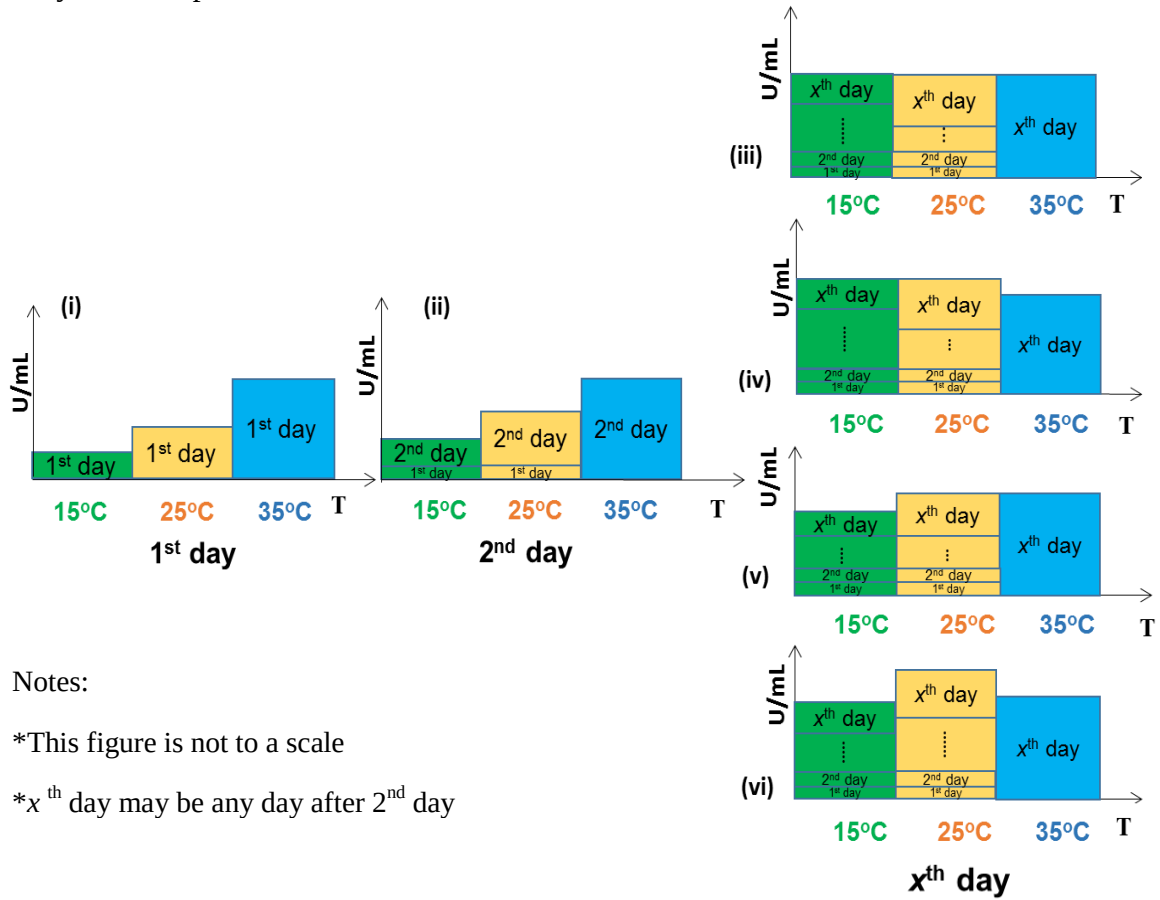


Fig. 4.27: Conceptual image of temporal variation of urease activity within the sand specimen.

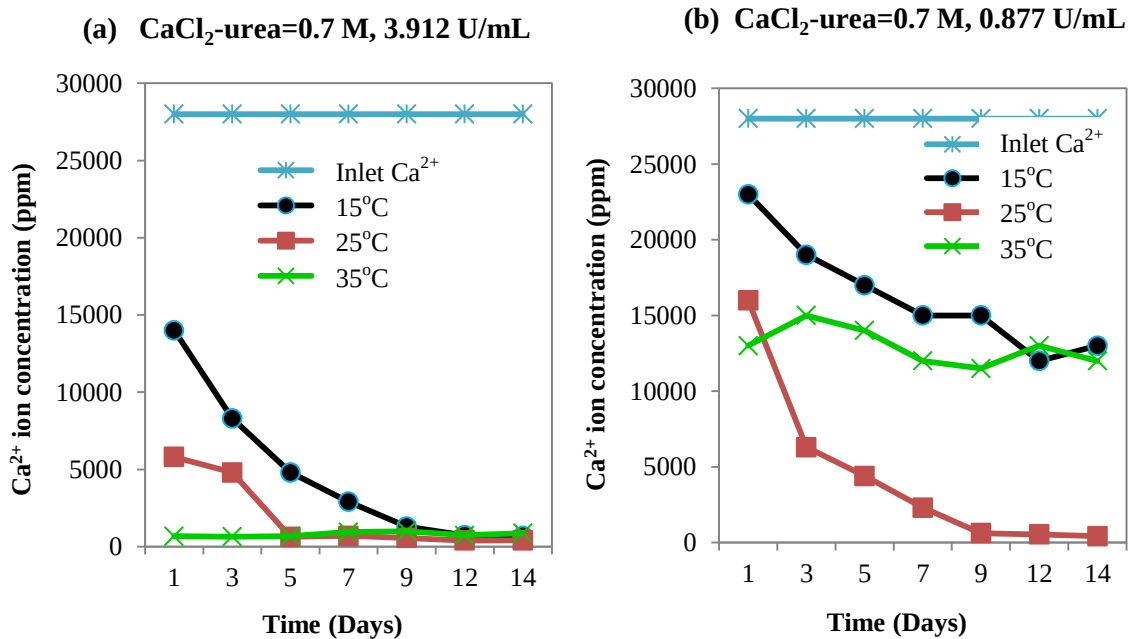


Fig. 4.28: Temporal variation of Ca^{2+} ion with temperature ($\text{CaCl}_2\text{-urea}=0.7 \text{ M}$).

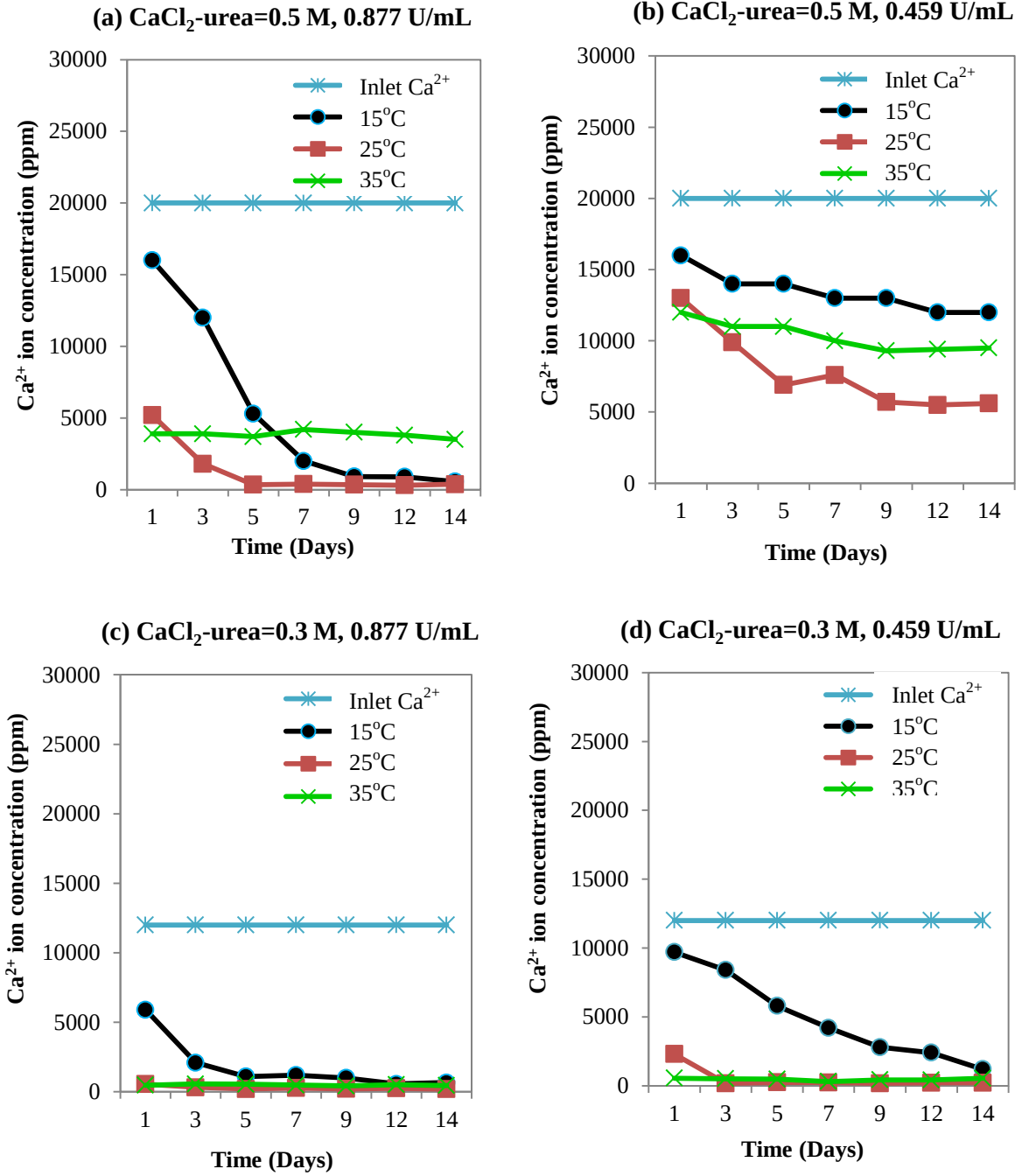


Fig. 4.29: Temporal variation of Ca^{2+} ion with temperature ((a) and (b) $\text{CaCl}_2\text{-urea}=0.5 \text{ M}$, (c) and (d) $\text{CaCl}_2\text{-urea}=0.3 \text{ M}$).

(e) Effect of particle size of sand on estimated UCS

When compare with the sand test pieces made using Toyoura, Mikawa and Mizunami sands, Mizunami sand test pieces show lower values of estimated UCS. Out of all three types of sand, Mizunami sand is the largest in particle size. Although it is largest in particle size, the void volumes of Mizunami and Mikawa sand test pieces are almost same. Therefore, the precipitated CC should be same within these two types of sand specimens under same testing conditions. Even though the precipitated amount of CC is same, the surface area of Mizunami sand particles is smaller than the same volume of Mikawa sand particles. Then, the bond between Mizunami sand particles may not be strong like in Mikawa sands with larger surface area. This may be a possible reason to get low estimated UCS value for Mizunami sand when compare to Mikawa sand. If we consider the same reason to compare the estimated UCS of Toyoura and Mikawa sand test specimens, the estimated UCS of Toyoura sand should be larger than Mikawa sand due to larger surface area in Toyora sand. According to the results, it was not happen. This may be due to the change in precipitated CC within these two sand test specimens. The penetration rate of CaCl_2 -urea-urease solution was low especially at later stages of curing time due to very small particle size of Toyoura sand. Then, most of the CC precipitated at the top surface of the specimen instead of within the specimen. This may be a possible reason to get low estimated UCS for Toyoura sand rather than Mikawa sand.

The other important observation is the pattern of strength distribution of three types of sands. As an average of the results, the top of the Toyoura sand test pieces was more solidified than middle and bottom of the test pieces. Conversely, the bottom of the Mikawa and Mizunami sand test pieces was more solidified than middle and top of the test pieces. When CaCl_2 -urea-urease solution was injected, the solution was uniformly penetrated through the sand specimen at initial stages of curing time. However, the rate of penetration

was decreased with the curing time due to gradual deposition of CC. When the rate of penetration becomes slow, CC was started to precipitate before penetrating the solution into the syringe specimen. Some of the PPCC were penetrated to upper portion of the specimen and some of them were remained on top surface of the specimen and hence, the total amount of CC was not precipitated within the specimen. The quantity of PPCC penetrated to middle and lower portion was small due to trapping the PPCC in pore spaces in the upper portion. Therefore, in-situ precipitation of CC can be expected at middle and lower portion instead of filling the pores with PPCC. Furthermore, the PPCC was not properly bond to the sand particles. Therefore, the bond between sand particles was not strong. This may be a possible reason to get low estimated UCS value at the upper portion of the specimen made using Mikawa and Mizunami sand. The bond between sand particles in middle and lower portion was stronger than the upper portion due to in-situ precipitation of CC at middle and lower portion. In case of Toyoura sand, there was no possibility to penetrate PPCC due to very small pore size in Toyoura sand, and PPCC was remained only at the top surface of the specimen. Therefore, in-situ precipitation of CC was taken place. However, the penetration of the solution to bottom of the specimen was low due to small pore size. It caused to get higher estimated UCS value at upper portion rather than middle and lower portion of the specimen.

4.6.4 Comparison of the relationship between UCS and CaCO_3 content with previous studies

The relationship between estimated UCS and total amount of precipitated CaCO_3 obtained from this study is shown in Fig. 4.30.

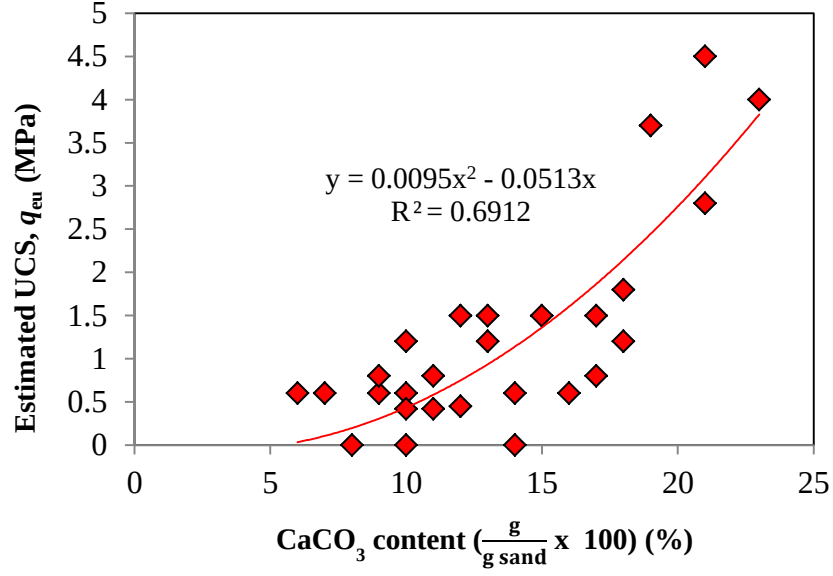


Fig. 4.30: The relationship between estimated UCS and CaCO₃ content (Current study).

According to Fig. 4.30, following relationship between estimated UCS and total CaCO₃ precipitated was obtained from this study (eq. (4.14)).

$$q_{eu} = 0.0095x^2 - 0.0513x \quad \text{eq. (4.14)}$$

Where, q_{eu} = Estimated UCS (MPa)

$$x = \text{CaCO}_3 \text{ content } \left(\frac{g}{g \text{ sand}} \times 100 \right), \%$$

Moreover, the relationship between total amount of precipitated CaCO₃ within the sand specimen and the UCS obtained in this work was compared with similar relationships available in the literature in which MICP and PDUICCP methods have been adopted to obtain a strength improvement of sand (Fig. 4.31). Testing conditions of the selected previous studies and this study are also shown in Table 4.3.

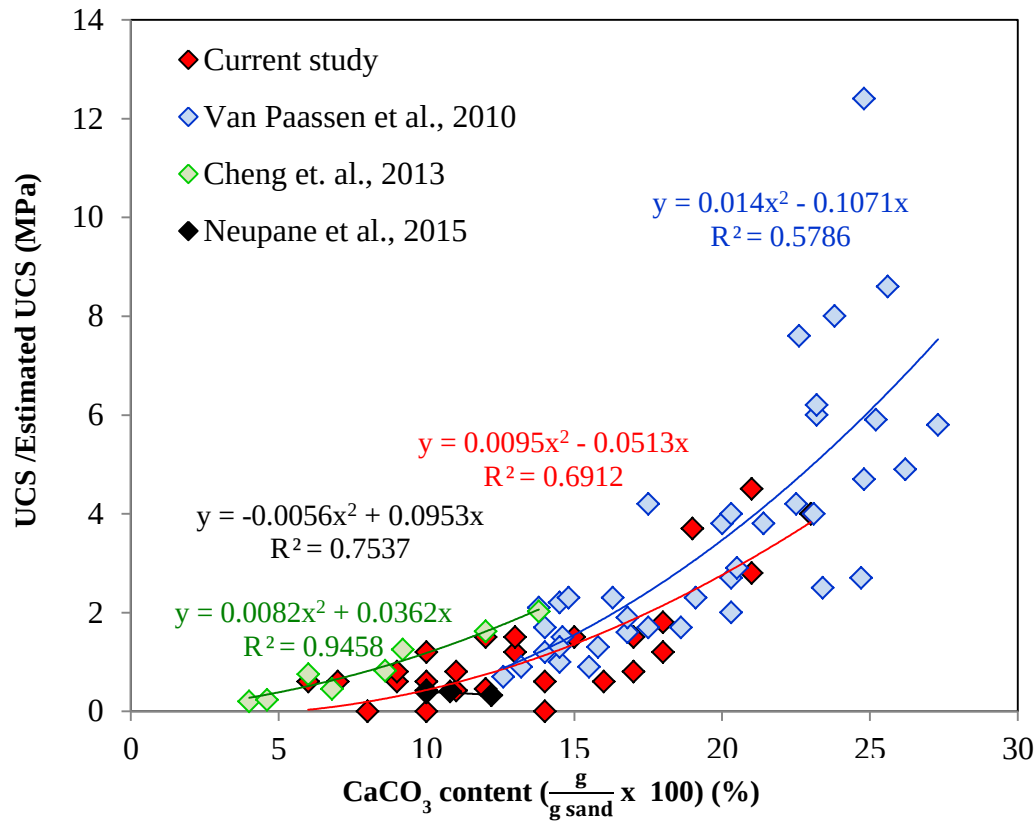


Fig. 4.31: Comparison of the relationship between UCS and CaCO_3 content with previous studies.

Table 4.3: Test conditions of each study.

Reference	Type of urease	Dry Density of the sand specimen (g/cm^3)	Type of sand
Current study	Plant urease - Watermelon (<i>Citrullus lanatus</i>) seeds urease (crude extract)	1.47	Poorly graded medium to coarse sand
Van Paassen et al., 2010	Bacterial urease- <i>Sporosarcina pasteurii</i>	1.56	Poorly graded fine to medium sand
Cheng et al., 2013	Bacterial urease- <i>Bacillus sphaericus</i>	1.62-1.63	Poorly graded medium to coarse sand
Neupane et al., 2015	Commercially available plant urease	1.57	Poorly graded fine to medium sand

According to the results of the current study, similar relationship between UCS and CaCO_3 content was observed with previous studies in which MICP and PDUICCP methods adopted. However, all the UCS values obtained from the current study are estimated UCS values of the middle of sand specimen and the UCS values in previous researches are exact UCS values. At the same time, all the CC content given in this study is theoretical amount of precipitated CC.

4.6.5 Comparison of PDUICCP technique with MICP technique

Although the plant urease and bacterial urease hydrolyze urea in the same way, the formation mechanism of CC within the sand specimen is slightly different. In the MICP process, the surface of bacterial cell plays a major role in adsorption of cell connected urease activity on solid surface. Bacterial cell surfaces have negatively charged groups and therefore, divalent cations like Ca^{2+} and Mg^{2+} bind onto their cell surfaces easily at neutral pH and the bound cation (Ca^{2+} ions) reacts with anions (CO_3^{2-}) to form CC (Fig. 4.32). Therefore,

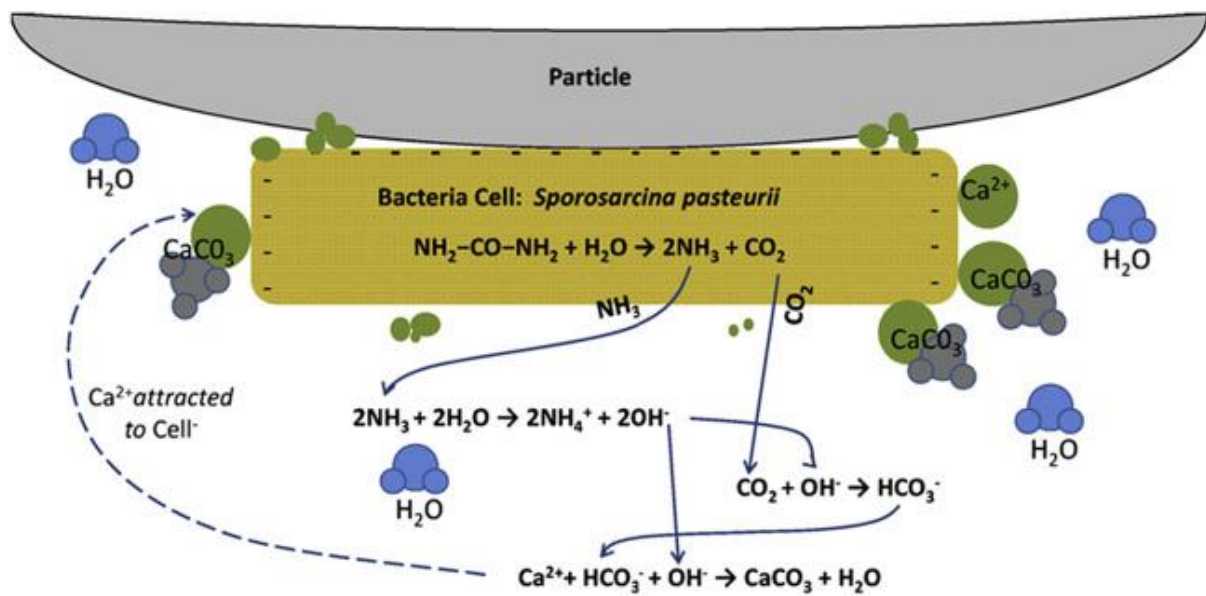


Fig. 4.32: Schematic diagram of MICP process using ureolysis (DeJong et al., 2010).

bacterial cells are not just a stock of enzyme but also a point of crystallization due to high urease activity on the site of cell adsorption and hence, it makes ideal nucleation sites for carbonate deposition (Ferris et al., 1996; Stocks-Fischer et al., 1999; Ramachandran et al., 2001). However, in case of plant-derived urease, the adsorption of enzyme molecules cannot create such density of urease activity on solid surface and therefore, it does not provide a proper nucleation site and just act as a catalyst for urea hydrolysis and liberate NH_4^+ and CO_3^{2-} ions. It helps to provide a neutral pH environment as well as CO_3^{2-} ions for CC deposition. According to this, there is a possibility to get higher UCS from microbial urease rather than the plant-derived urease. However, plant and bacterial urease also differ from their impact on phase formation, crystallization processes, and the morphological properties of CC particles due to distinct differences in their primary structures (amino acid sequences) (Sondi I and Sondi BS, 2005). Some factors influencing for optimal urease activity such as temperature, pH and substrate concentration may also differ from one urease source to another urease source. Therefore, the UCS value obtains for same concentration of CaCl_2 , urea and urease under same temperature may be different.

As mentioned in the Section 4.1, the size of commercially available purified plant urease enzyme is approximately 12 nm in dimension (Blakely and Zerner, 1984) and this small size of urease enzyme gives a distinct advantage to penetrate the cementation solution in to deeper area over ureolytic microbes which are greater than 300 nm in diameter, with the majority in the range of 500-5000 nm. However, we could not achieve this advantage using crude extract of watermelon seeds extract. Even though a centrifuged extract was used, it was not a fully transparent solution and it consisted with some suspended particles.

4.6.6 Comparison of the cost of crude enzyme of plant urease and commercially available plant urease use for PDUICCP method

The major merit of using crude enzyme of watermelon seeds urease over commercially available plant urease for PDUICCP method is the reduction of cost. Following calculation may help to get an idea about the cost of commercial urease and amount of seeds needed for crude enzyme of urease to achieve same amount of solidification.

- Let's assume 1m^3 area in the ground consists with Mikawa sand, and the area is treated 14 days with a solution having 3.912 U/ml of urease activity. (1mL of the centrifuged crude extract obtained from a solution having 50g/L of crushed seeds gives urease activity of 3.912 U).
- Maximum density of Mikawa sand= 1.476 g/cm^3
- Particle density= 2.66 g/cm^3

Weight of sand in 1m^3 (kg) = $(1.476/10^3) \times 10^6 = 1476$

The total volume of the sand (m^3) = $(1476 \times 1000/2.66)/10^6 = 0.55\text{ m}^3$

The total void volume in between sand= $1 - 0.55 = 0.45\text{ m}^3$

Amount of required seeds, (kg) = $50 \times 0.45 \times 10^6 \times 14 / (10^3 \times 10^3) = 315\text{ kg}$

Amount of required commercial urease with 150 U/mg, (g)

$$= (1 \times 3.912 \times 0.45 \times 10^6 \times 14/150)/10^3 = 164.3\text{ g}$$

Cost of 1 g of commercial urease with 150 U/mg = 14,500 JPY

Total cost for commercial urease= $164.3 \times 14,500 = 2,382,408\text{ JPY}$

If there is a possibility to collect large quantity of seeds, the total cost for commercial urease can be saved in PDUICCP method.

4.7 CONCLUSIONS

Chapter 4 presented about the use of PDUICCP method as an alternative method for MICP. The crude extract of crushed watermelon seeds was applied for the current study and small scale test specimens made from commercially available Toyoura, Mikawa and Mizunami sands were cemented and achieved satisfactory estimated UCS values. According to the results of this study, following conclusions were obtained.

- Estimated UCS of several kPa to MPa was obtained by changing the concentration of CaCl_2 -urea, urease as well as curing time. The increase of curing time caused to increase in estimated UCS value. The increase of CaCl_2 -urea concentration from 0.3 M to 0.7 M also caused to increase the estimated UCS value. However, the effect of urease activity on estimated UCS is different for different concentrations of CaCl_2 -urea solutions. It is better to keep low urease activity for low concentrations of CaCl_2 -urea solutions and high urease activity for high concentrations of CaCl_2 -urea solutions to maintain the rate of urea hydrolysis and finally to maintain the rate of carbonate precipitation. Otherwise, CC starts to precipitate before injecting the solution into the syringe specimen and then, the voids between sand particles especially in the upper portion of the sand specimen fill with PPCC instead of precipitating within the specimen. It may not be favorable for effective bond between sand particles. Furthermore, some of the PPCC was remained at the top surface of the specimen. In this study, the urease activity of 3.912 U/mL is suitable for 0.7 M CaCl_2 -urea solutions and 0.877 U/mL for 0.3 M and 0.5 M CaCl_2 - urea solutions. The average estimated UCS obtained after 14 days of curing time for 0.7 M CaCl_2 -urea and 3.912 U/mL urease was around 3.0 MPa and for 0.3 M and 0.5 M CaCl_2 - urea and 0.877 U/mL urease was around 1.5 MPa - 2.0 MPa for Mikawa sand at 25°C temperature.

- Although the urease activity was increased with the temperature up to about 50°C, we could not obtain a clear increment of UCS with the increment of temperature from 15 °C to 35 °C. As we selected 1 day injection interval, there is possibility to change the activity of urease solution during that period. The change rate is depending on temperature. As an example, even though the urease activity is low at temperature of 15 °C, the storage of urease about 24 h in 15°C temperature does not cause to change the activity. Although the urease activity is high at 35 °C temperature, the storage of urease about 24 h in 35 °C causes to deactivate the urease. However, the storage of urease about 24 h in 25 °C causes to decrease the activity but not deactivate the urease. Therefore, the effect of temperature on estimated UCS was unclear due to uneven change in urease activity with temperature. Out of 15°C, 25°C and 35°C temperatures, 25°C temperature was favorable for this study.

- Out of three types of sands, the practical difficulties such as penetration of injection solution were less with Mikawa sand and considerable strength could be achieved. Although the injection was not a problem in Mizunami sand, the strength gain was low compare with Mikawa sand. Therefore, Mikawa sand was suited for this study. However, a well-controlled distribution of CC should be achieved to use this method from laboratory to large scale applications. According to this study, the control of the rate of CC precipitation is one solution. Then, the amount of PPCC can be reduced.

- By changing each and every parameters mentioned above, there is a possibility to apply this method for some applications such as strength improvement of weak unconsolidated soil, specially saturated loose sand to mitigate the liquefaction, protection and restoration of limestone monuments and statuarys, artificial stone formation (like sandstone), etc.

- The urease obtained from the crude extract of crushed watermelon seeds has a potential to be applied instead of commercially available urease for carbonate precipitation.

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CHAPTER 5: Conclusions and future research works

5.1 Summary of the current study and main conclusions

This thesis discussed about low environmental impact type ground improvement techniques those are managed by science of biology, chemistry and physics together, and the potential of applying this knowledge to geotechnical engineering applications under two main topics named as soil improvement using CPCs precipitation and soil improvement using PDUICCP.

In Chapter 1, the research background, literature review, objectives of this study and originality and usefulness of the thesis were discussed.

Chapter 2 described about the soil improvement using CPCs precipitation (CPC-Chem method). During this study, the UCS of sand test pieces made using CA and DPP as Ca^{2+} and PO_4^{3-} sources respectively was analyzed by changing Ca^{2+} and PO_4^{3-} concentrations, Ca/P ratio, curing time and sand type. According to the results of the UCS values obtained, the UCS values were varied according to the Ca^{2+} and PO_4^{3-} concentrations. When the concentrations of both sources increased, the UCS increased regardless of the Ca/P ratio. The Ca/P ratio changed the final pH in the CPC mixture. When Ca/P=0.5, pH was neutral or weakly alkaline and when Ca/P=1, pH was weakly acidic, and when Ca/P ratio was increased further, pH remained in acidic range. However, by considering previous researches, the behavior of pH with Ca/P ratio is mainly dependent on the Ca^{2+} and PO_4^{3-} sources. The rate of self-setting of CPCs was changed with Ca^{2+} and PO_4^{3-} concentrations. At low concentrations of Ca^{2+} and PO_4^{3-} , the rate of self-setting was low or almost zero compare with high concentrations.

According to the results of UCS test carried out for Toyoura sand test pieces, a UCS more than 100 kPa which was our objective value was obtained after 28 days of curing time

from the test case with $CA = 0.75M$ and $DPP = 1.5M$ ($Ca/P = 0.5$). Furthermore, the best concentration range to achieve 100 kPa at early stage rather than 28 days is $CA = 0.75M - 1.0M$ and $DPP = 1.5M - 2.0M$. For $Ca/P = 1$, the above range is $CA = DPP = 0.75 - 1.0M$. These ranges are also suitable for Mikawa sand. However, it gives little higher values rather than Toyoura sand during selected ranges of concentration.

However, continued research is needed to identify the process or processes that link crystal precipitation to the increase in strength more clearly. Furthermore, shearing and permeability tests using test pieces cemented by CPC should be conducted to evaluate the applicability of CPCs for purposes such as permeability control and reinforcement of soil and rock.

In Chapter 3, the soil improvement using CPCs along with some powder materials (CPC-Powder method) was investigated. Our aim was to improve the strength of CPC-Chem by using two kinds of phosphate powders (TCP; MgP) and two kinds of carbonate powders (CC; MgC) as seed crystals, and exceed the UCS more than 100 kPa. All of the TCP- and CC-added test pieces cemented with CPC solutions have been showed UCS larger than 100 kPa after 28 days even at the 1% of powder addition. Therefore, according to the strength requirement, there is a possibility to change the powder percentage and/or curing time (days). When time is a crucial factor, it is necessary to increase the powder percentage for early strength requirements. Similarly, when the cost is a crucial factor, it is necessary to use a lower percentage of powder material and allow to a strength improvement with time. This makes the CPC-TCP or CPC-CC method as a suitable ground improvement method for liquefiable areas by keeping some safety factor as required. We can use this method by using low cost TCP and CC materials instead of chemical reagents of TCP and CC to use as a cost effective ground improvement practice. The addition of magnesium compounds was not

effective for increasing the UCS by using CPC-Chem. The Mg^{2+} in magnesium compounds exchange with the calcium ions in the CPC solution and reduce the precipitation of CPC.

The CPC-Powder method using TCP and CC can be applied to producing columns in soft ground as a sand compaction pile method or deep mixing method in an actual ground condition. The sand compaction pile (SCP) method is a method of improving soft ground by means of installing well-compacted sand piles in the ground. In sandy ground, the SCP method is often used as a countermeasure against liquefaction and the effectiveness of compaction to prevent liquefaction has been confirmed in past intense earthquakes showing the CPC-Powder method to be one of the most reliable improvement methods to mitigate liquefaction potential. Furthermore, backfill soil can also be reinforced using this method. The Public Works Research Institute suggests that a UCS of 100–200 kPa is required for reinforcing backfill soil. Because of ground liquefaction during earth quakes, backfill soil tends to subside and manholes and pipelines in the soil tend to be pushed upward due to ground liquefaction during earthquakes. This causes substantial damage to the infra- structure. Hence, backfill soil can also be reinforced as a counter- measure against such an occurrence.

The Chapter 2 and Chapter 3 of this thesis discussed about the soil improvement using artificial phosphate biomineral formation. Furthermore, the Chapter 4 presented about the soil improvement using artificial CC biomineral formation. The plant-derived urease was used along with $CaCl_2$ -urea for CC formation as an alternative method for MICP. The crude extract of crushed watermelon (*Citrullus lanatus*) seeds was used for the current study and small scale test specimens made from commercially available Toyoura, Mikawa and Mizunami sands were cemented and achieved satisfactory estimated UCS values. According to the results of this study, following conclusions were obtained.

Estimated UCS of several kPa to MPa was obtained by changing the concentration of $CaCl_2$ -urea, urease as well as curing time. The increase of curing time and $CaCl_2$ - urea

concentration from 0.3 M to 0.7 M caused to increase the estimated UCS value. The effect of urease activity on estimated UCS was different for different concentrations of CaCl₂-urea solutions. It is better to keep low urease activity for low concentrations of CaCl₂- urea solutions and high urease activity for high concentrations of CaCl₂-urea solutions to maintain the rate of urea hydrolysis and finally to maintain the rate of carbonate precipitation. Otherwise, CC starts to precipitate before injecting the solution into the syringe specimen and then, the voids between sand particles especially in the upper portion of the sand specimen fill with PPCC instead of precipitating within the specimen. It may not be favorable for effective bond between sand particles. Furthermore, some of the PPCC was remained at the top surface of the specimen. In this study, the urease activity of 3.912 U/mL was suitable for 0.7 M CaCl₂-urea solutions and 0.877 U/mL for 0.3 M and 0.5 M CaCl₂-urea solutions. The average estimated UCS obtained after 14 days of curing time for 0.7 M CaCl₂-urea and 3.912 U/mL urease was around 3.0 MPa and for 0.3 M and 0.5 M CaCl₂-urea and 0.877 U/mL urease was around 1.5 MPa - 2.0 MPa for Mikawa sand at 25°C temperature.

Although the urease activity was increased with the temperature up to about 50°C, we could not obtain a clear increment of UCS with the increment of temperature from 15 °C to 35 °C. As we selected 1 day injection interval, there is a possibility to change the activity of urease solution (decrease) during that period. The change rate is depending on temperature. As an example, even though the urease activity was low at the temperature of 15 °C , the storage of urease about 24 h in 15 °C temperature does not cause to change the activity. Although the urease activity is high at 35 °C temperature, the storage of urease about 24 h in 35 °C causes to deactivate the urease. However, the storage of urease about 24 h in 25 °C causes to decrease the activity but not deactivate the urease. Therefore, the effect of temperature on estimated UCS could not be predicted due to uneven change in urease activity

with temperature. Out of 15°C and 35°C temperatures, 25°C temperature was favorable for this study.

Out of three types of sands, the practical difficulties such as penetration of injection solution were less with Mikawa sand, and considerable strength could be achieved. Although the injection was not a difficult problem in Mizunami sand, the strength gain was low compare with Mikawa sand. Therefore, Mikawa sand was suited for this study. However, a well-controlled distribution of CC should be achieved to use this method from laboratory to large scale applications. According to this study, the control of the rate of CC precipitation is one solution. Then, the amount of PPCC can be reduced.

By changing each and every parameters mentioned above, there is a possibility to apply this method for some engineering applications such as strength improvement of weak unconsolidated soil, specially saturated loose sand to mitigate the liquefaction, protection and restoration of limestone monuments and statuarys, artificial stone formation (like sandstone), etc.

The urease obtained from the crude extract of crushed watermelon seeds has a potential to be applied instead of commercially available urease for carbonate precipitation.

5.2 Future research works

5.2.1 Suggestions for future research works in CPC method

As mentioned in Chapter 1, CPC has two unique characteristics: its solubility is dependent on its pH (Fig. 1.1) and it exhibits a self-setting mechanism (Fig. 1.2) (Tung, 1998). From Chapter 2, we discovered that grout comprising only CPC (CPC-Chem) increased the UCS of sand test pieces with time at high concentrations of Ca^{2+} and PO_4^{3-} due to its self-setting property. At low concentrations of Ca^{2+} and PO_4^{3-} , the increment of UCS was low or almost zero. However, the use of high concentrations of Ca^{2+} and PO_4^{3-} solutions to obtain higher

UCS is not economical. As a future investigation, we can propose a new attempt to increase the precipitation volume of CPCs by changing the pH artificially using a plant-derived urease enzyme and urea induced pH regulator. However, according to the Fig. 1.1, there is an optimal pH value for each CPC for their maximum precipitation. Therefore, it is necessary give an attention while increasing pH artificially to overcome the dissolution of CPCs at higher pH than the optimal pH. The **APPENDIX B** may be helpful to initiate such a CPC biogROUT in future. Such a CPC biogROUT would enable the control of solidification speed; furthermore, the strength of the ground can be increased by using a combination of CPC chemical grout, for rapid solidification, and CPC biogROUT, for long-term solidification.

5.2.2 Suggestions for future works in plant-derived urease induced CC method

In this study, we followed very simple method to extract the urease enzyme from watermelon seeds. Finally, a centrifuged extract was used for the study. However, it was not a fully transparent solution and it consisted with some suspended particles. Although the urease activity in these suspended particles contributes to the urea hydrolysis and finally to the CC precipitation, these suspended particles may clog between sand particles. This may be a disadvantage for penetrating cementation solution into deeper areas. Therefore, it is important to investigate a new, low cost extraction method to extract almost all urease into the solution. In addition to that, a well-controlled distribution of CC should be achieved to use this method from laboratory to large scale applications. We could not achieve it from our current study. The control of the rate of CC precipitation is one solution. Then, the amount of PPCC can be reduced. Therefore, it is necessary to consider those matters more clearly from future research works.

The major challenges in this novel area for field applications include assessment of subsurface soil condition including soil type, pH, mineralogy and their interaction with the available fluids and minerals, ground water flow characteristics and available minerals in the

ground water. The control and predictability of the in-situ distribution of CC and related engineering properties in the subsurface are also not yet sufficient. Furthermore, there is a possibility to release NH_3 during urea hydrolysis process. The ratio of urea/ CaCl_2 can be maintained as low as possible to diminish the production of NH_3 . However, this is not a permanent solution to overcome the problem with NH_3 . Therefore, these form the greatest challenge for further optimization. It is also necessary to investigate the durability, longevity and reversibility of the carbonate precipitation process under economical point of view.

The potential of the use of artificial bio minerals has brought a new revolution in various engineering applications. However, it is needed to explore deeply about this novel area in order to bring it environmentally safe, cost effective and to develop this technology from lab to field scales.

APPENDIX A: Methods for evaluating urease activity

Table A.1: Methods for evaluating urease activity (To be continued...).

Test Method	Test Item	Measurement	Remarks	References
Titrimetric Method	Soybean oil meal	Volume of NaOH required to finish the titration	*Application :In mixed-feed industry to identify the low concentration of urease in soybean oil meal (when urea and soybean oil meal are combined in cattle feeds)	Croston et al., 1955
Titrimetric Method	Soybean meals	Volume of NaOH required to finish the titration	*Application : To determine urease in soybean meals of high activity *Recommended for all highly active meals	Smith et al., 1956
Titrimetric Method	Jack Bean	Color change of the p-Nitrophenol indicator from yellow to colorless during the titration	*Limitation: This is not for determining the activity of Urease, from <i>Bacillus pasteurii</i> , U7127 *Urease activity is reported as Units/g solid.	http://www.sigmaaldrich.com/technicaldocuments/protocols/biology/enzymatic-assay-of-urease-from-jack-beans.html
Spectrophotometric Stop Rate Determination	-	Absorbance is taken at 480 nm wavelength using a spectrophotometer	*Urease activity is reported as Units/g enzyme (One unit will liberate 1.0 μ mole of NH_3 from urea per minute at pH 8.2 at 30°C.) *Nessler's reagent is used to determine NH_3 generated from hydrolysis of Urea	https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/2/urease.pdf

Table A.1: Methods for evaluating urease activity (To be continued...).

Test Method	Test Item	Measurement	Remarks	References
Caskey-Knapp method	Soybean meal	Color change due to variation in pH	*pH indicator: phenol red solution (If sufficient urease is present color change to deep red) *Application : Food processing Industry to detect inadequately heated soybean oil meal *Limitation: Cannot indicate excessive heat treatment	Caskey et al., 1944
Modified Caskey-Knapp method /pH Change method (potenciometric method)	Soybean oil meal	pH value using pH meter with glass electrode	*Application : In food processing Industry (detecting inadequately heated soybean oil meal)	Bird et al., 1947
“	Soybean meal and byproducts	pH value using pH meter with glass electrode	*Application : In Soybean Industry (Detecting urease present in soybean products like soy-bean meals, soy flour, and mill feeds)	http://www.scribd.com/doc/220182129 /Actividad-Ureasica-22-90
“	Soybean meal and byproducts	pH value using pH meter with glass electrode	*Application: Soybean Industry (Detecting urease present in soybean meal and its by-products)	http://www.fao.org/docrep/field/003/ab479e/AB479E05.htm

Table A.1: Methods for evaluating urease activity (To be continued...).

Test Method	Test Item	Measurement	Remarks	References
Conductimetric Method	Soybean	Resistance in ohms (Specific conductance is reciprocal ohms)	Application: To study urease isolated from soybeans	Croston et al., 1955
Wiffin's conductimetric Method	Bacteria (<i>proteus vulgaris</i> and <i>Sporosarcina pasteurii</i>)	Conductivity	Urease activity is reported as mM urea hydrolysed.min ⁻¹	Whiffin, 2004
Wiffin's conductimetric Method	Bacteria (<i>Sporosarcina pasteurii</i>)	Conductivity	-	Mortensen et al., 2011
Colorimetric Method	Soybean meal	The absorbance (optical density) is measured at 430 nm wavelength using the spectrophotometer	*Application: To determine urease activity of soybean meal *Urease activity was reported as mg/L urea decomposed. (An unit of urease activity is defined as one milligram per liter of urea decomposed)	Schramm and Aines, 1959

Table A.1: Methods for evaluating urease activity (To be continued...).

Test Method	Test Item	Measurement	Remarks	References
Colorimetric Method	plant seeds	Color change due to variation in pH	*pH indicator: red cabbage extract (At pH 7, the solution is violet/blue and in the acidic range it turns red and in alkaline range it turns green *1% or 2% phenolphthalein solution and bromothymol blue are alternatives for pH indicators. (it turns from yellow to blue in pH 7.6)	http://www.scienceinschool.org/print/607 (http://www.eurovolvox.org/Private/Meetings/Napoli/Urease)
Colorimetric Method	Soybean	Absorbance is taken at 405 nm wavelength using a spectrophotometer	*Nessler's reagent is used to determine NH_3 generated from hydrolysis of Urea *One unit of urease activity is defined as the amount of enzyme required to liberate $1.0 \mu\text{M}$ of NH_3 from urea per min at pH 8.0 and temperature 30°C)	Khan et al., 2013
Colorimetric Method	Jack bean meal	Optical density is measured at 630 nm (Phenol Hypochloride solution was added to develop color.)		Selvamurugan et al., 2007

Table A.1: Methods for evaluating urease activity.

Test Method	Test Item	Measurement	Remarks	References
Colorimetric Method	Soil	optical density is measured at 690 nm using the spectrophotometer	*Urease activity is expressed as $\mu\text{g N}$ hydrolysed/g dry soil per 2 hours at 37°C .	Kandeler and Gerber, 1988
Colorimetric Method	Soil	Absorbance (optical density) is measured at 690 nm wavelength, using the spectrophotometer	*Urease activity is calculated as μmole of ammonium released per hour per gram of soil.	Allison S, 2001
Colorimetric Method	<i>Pararhodobacter</i> sp.	Color change due to variation in pH	*pH indicator: Cresol red solution (yellow to purple has been observed from pH 7.2 to pH 8.8) *Medium used to isolate microorganism is ZoBell2216E medium	Danjo and Kawasaki, 2014
Colorimetric Method	Bacteria	-	*Christensen's urea medium was used	Christensen, 1946
Colorimetric Method	Bacteria	optical density is measured at 430 and 560 nm wavelengths,	*Urease activity is reported as Units/ml enzyme (One unit of urease activity corresponds to the amount of enzyme that hydrolyzes $1\mu\text{M}$ of urea per minute)	Okyay and Rodrigues, 2013

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APPENDIX B: Precipitation of CPC homo-gel using a plant-derived urease enzyme and urea induced pH regulator

APPENDIX B.1 INTRODUCTION

As mentioned in chapter 1, CPC has two unique characteristics: its solubility is dependent on its pH (Fig. 1.1) and it exhibits a self-setting mechanism (Tung, 1998). From chapter 2, we discovered that grout comprising only CPC (CPC-Chem) increased the UCS of sand test pieces with time due to its self-setting property. Furthermore, the UCS increased with the increment of Ca^{2+} and PO_4^{3-} concentrations. However, the use of high concentrations of Ca^{2+} and PO_4^{3-} solutions to obtain higher UCS is not economical. Therefore, this chapter presents another attempt to increase the precipitation volume of CPCs by changing the pH artificially using a plant-derived urease enzyme and urea induced pH regulator. Such a CPC biogROUT would enable the control of solidification speed; furthermore, the strength of the ground can be increased by using a combination of CPC chemical grout, for rapid solidification, and CPC biogROUT, for long-term solidification.

According to the literature findings related to this study, the volume of precipitated CPC crystal has been increased with the pH (Akiyama and Kawasaki, 2012a) (Fig. B.1). However, in this study, the pH change was obtained by mixing two different PO_4^{3-} sources. Different mixing proportions of monoammonium phosphate (MAP, pH 4.2) and diammonium phosphate (DAP, pH 8.0) have been used to prepare PO_4^{3-} solutions with different pH values. These results indicate the possibility of developing a CPC biogROUT in which an increase in biological pH brings about crystal precipitation of CPC from a low-pH injection solution. Moreover, another set of experiments have been conducted to observe the precipitation status. The proportion of the CPC precipitation volume to the reaction mixture volume (PPV) has been measured (the maximum PPV has been 1.0) after 3 months (Fig. B.2). According to Fig. B.2, it clearly shows the amount of precipitated volume is closely related to

the pH of the reaction mixture. At the same time, the type of Ca^{2+} and PO_4^{3-} sources also change the pH of the reaction mixture. These results give an indication to increase the precipitation volume by increasing pH in the system. However, according to the Fig. 1.1, there is an optimal pH value for each CPC for their maximum precipitation. Therefore, it is necessary give an attention while increasing pH artificially to overcome the dissolution of CPCs at higher pH than the optimal pH.

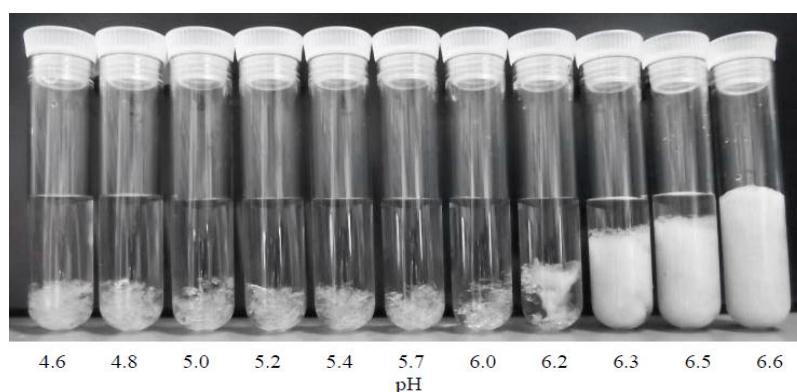
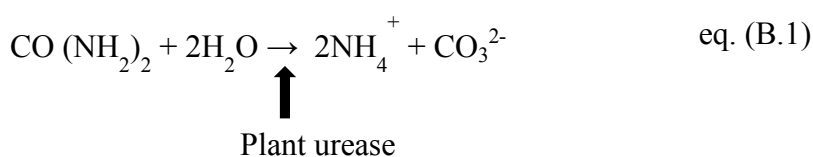


Fig.B.1: Effect of pH on the precipitation volume of CPC.

APPENDIX B.2 MECHANISM OF pH INCREMENT BY USING UREA AND UREASE

Urease enzyme is used to accelerate the hydrolysis of urea. The NH_4^+ and CO_3^{2-} produced from this reaction (eq. B.1) actually represent the final products of series of reactions. The NH_4^+ ions actually start out as NH_3 . When the ammonia reacts with water, it creates OH^- ions, which raise the pH of the system. The resulting pH increment helps to precipitate different types of CPCs at the presence of Ca^{2+} and PO_4^{3-} sources as they are highly pH dependent.



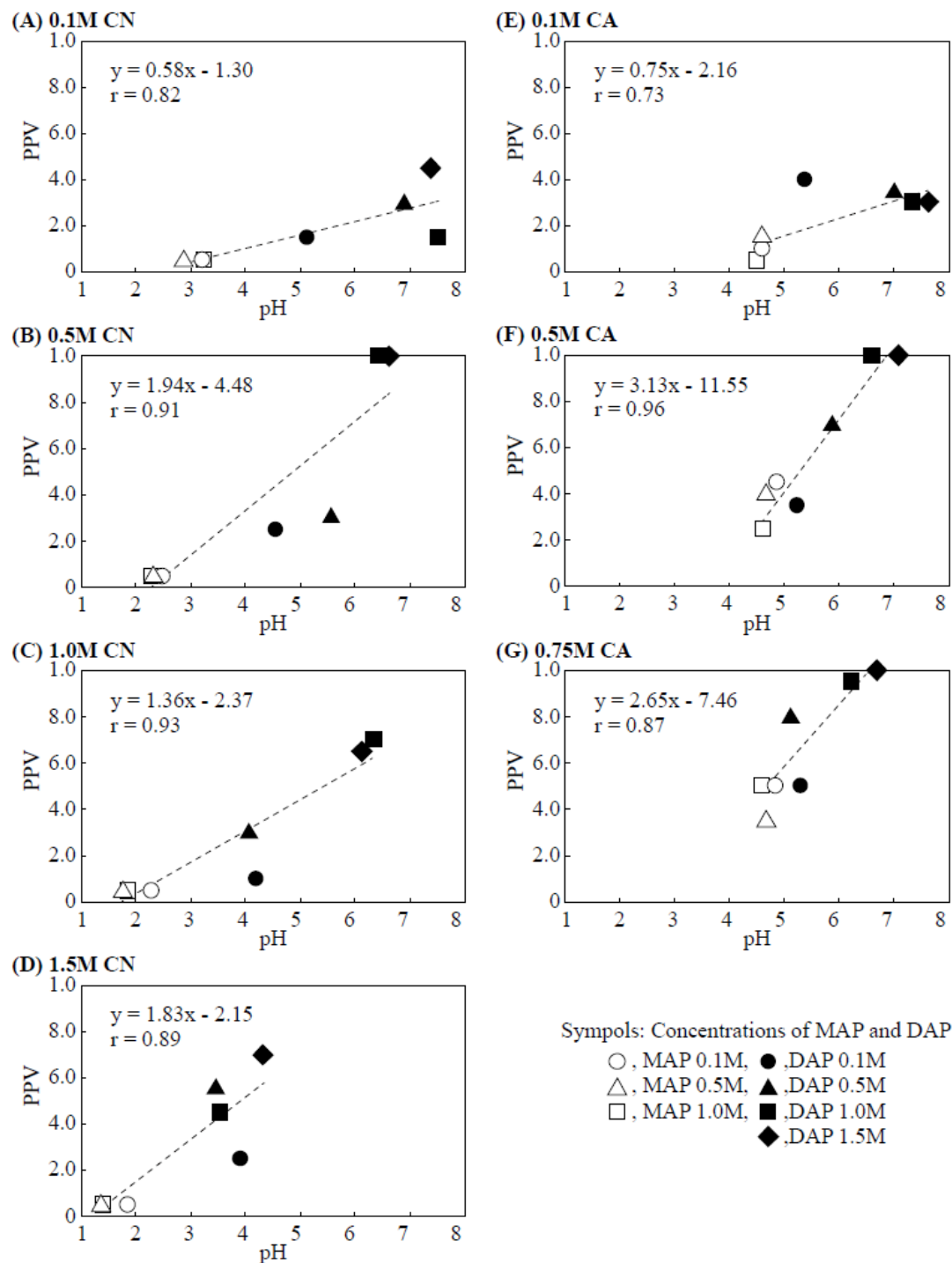


Fig.B.2: Relationship between the proportion of CPC precipitation volume of reaction mixture (PPV) and pH for calcium nitrate (CN) and calcium acetate (CA) (Akiyama and Kawasaki, 2012a).

APPENDIX B.3 OBJECTIVES

Our aim was to promote the precipitation volume of CPC homo-gel using a plant-derived urease enzyme and urea induced pH regulator and evaluate the potential utility of a new CPC biogroup.

APPENDIX B.4 MATERIALS

APPENDIX B.4.1 Materials

(a) Reagents

Different Ca^{2+} and PO_4^{3-} sources which are having different initial pH values were selected for this study as shown in Table B.1.

Table B.1: Different Ca^{2+} and PO_4^{3-} sources used in the current study.

Ca^{2+} source		PO_4^{3-} source	
Name	Initial pH	Name	Initial pH
1. $\text{Ca}((\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O})$ - (CA)	6.5-8.5	1. K_2HPO_4 -(DPP)	8.7-9.4
2. $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ - (CN)	4-6	2. $\text{NH}_4\text{H}_2\text{PO}_4$ -(MAP)	4-4.5
3. $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ - (CL)	4.5-8.5		

(b) 10 M NaOH and 2 M HCl solutions

(c) Polypropylene tubes

(d) Commercially available jack bean urease

APPENDIX B.4.2 Equipment

(a) pH meter (Eutech Instruments Pte., Ltd., Singapore).

APPENDIX B.5 METHODOLOGY

APPENDIX B.5.1 pH adjustment of CPCs for test tube experiment by using NaOH and HCl

Before using a urease enzyme and urea induced pH regulator, test tube experiment was done with the help of 2 M HCL and 10 M NaOH solutions. A series of polypropylene test tubes were taken and 2 mL of Ca^{2+} source was measured into each test tube. Before adding PO_4^{3-} source, a series bottles having PO_4^{3-} source with different pH values was prepared by adding HCL or NaOH as required. Finally, the reaction mixtures were prepared by mixing PO_4^{3-} source with Ca^{2+} source at a 1:1 volume ratio (2 mL each). After adding both sources together, sealed test tubes were stored under 25°C, and after 1 day, the pH was measured in each test tube. Similarly, five different series of test tubes were prepared by changing Ca^{2+} and PO_4^{3-} sources as shown in Table B.2.

Table B.2: Testing cases used for test tube experiment.

Case No	Ca^{2+} source	PO_4^{3-} source
Case 1	Basic (CA-0.75M)	Basic (DPP-0.5M)
Case 2	Basic (CA-0.75M)	Acidic (MAP-0.5M)
Case 3	Acidic (CN-0.75M)	Basic (DPP-0.5M)
Case 4-1	Acidic (CN-0.75M)	Acidic (MAP-0.5M)
Case 4-2	Acidic (CL-0.75M)	Acidic (MAP-0.5M)

APPENDIX B.5.2 Measurement of temporal variation of pH

For the measurement of temporal variation of pH in different CPC mixtures, we selected case 1, case 2 and case 3 described in Appendix B.5.1. Instead of adding NaOH, we added urea and commercially available jack bean urease to increase the pH. Different

reaction mixtures with different concentrations of urea and urease were prepared to observe the pH variation with changing both concentrations.

At first, required amount of commercially available urease powder was dissolved in Ca^{2+} source and urea was dissolved in PO_4^{3-} source. After preparing both sources separately, the reaction mixtures were prepared by mixing PO_4^{3-} source with Ca^{2+} source at a 1:1 volume ratio (2 mL each). After adding both sources, the mixture was mixed well about 2 min using a small chemical spoon. Subsequently, pH variation was measured using a pH meter.

APPENDIX B.6 RESULTS AND DISCUSSION

APPENDIX B.6.1 pH adjustment of CPCs for test tube experiment by using NaOH and HCl

The appearance of the test tubes in each testing case after 1 day and 14 days are shown in Figs. B.3 and B.4. The test tubes marked using blue circles are control samples (without adding urea and urease). After 1 day, it was difficult to identify a clear increment or decrement of precipitated volume in case 1, case 2 and case 3. Afterward, test tubes were kept under sealed condition as stored before and clear change of the precipitated volume with pH was observed after 14 days.

According to the results of case 1, the precipitated volume seems to be decreased with pH. This may be due to exceed the optimal pH as described in the section APPENDIX B.1. Hence, it has become to dissolve the precipitate at high pH. This shows the range of pH we can use to increase the pH is very narrow in this case. Therefore, we cannot expect considerable increase in precipitation volume. Hence, it is suitable to select different combinations of Ca^{2+} and PO_4^{3-} sources that give relatively low initial pH values like in case 2, case 3 and case 4. Otherwise, it is suitable to select high Ca/P ratio using same Ca^{2+} and PO_4^{3-} used in case 1 rather than Ca/P=1.5 used in this experiment because, in chapter 3, we

showed the decrement of pH with the increment of Ca/P ratio. Although we selected low pH values, it has given very low precipitation volume at highly acidic pH in case 4-1 and case 4-2. These combinations are satisfied our requirement of increasing precipitation volume by increasing pH. However, the purpose of doing this test tube experiment was to apply this method for sand solidification test in future. Therefore, we cannot expect such solidification with low amount of precipitation as seen in case 4-1 and case 4-2. By concerning all requirement, case 2 and case 3 can be selected as possible cases for sand solidification using a plant-derived urease enzyme and urea induced pH regulator.

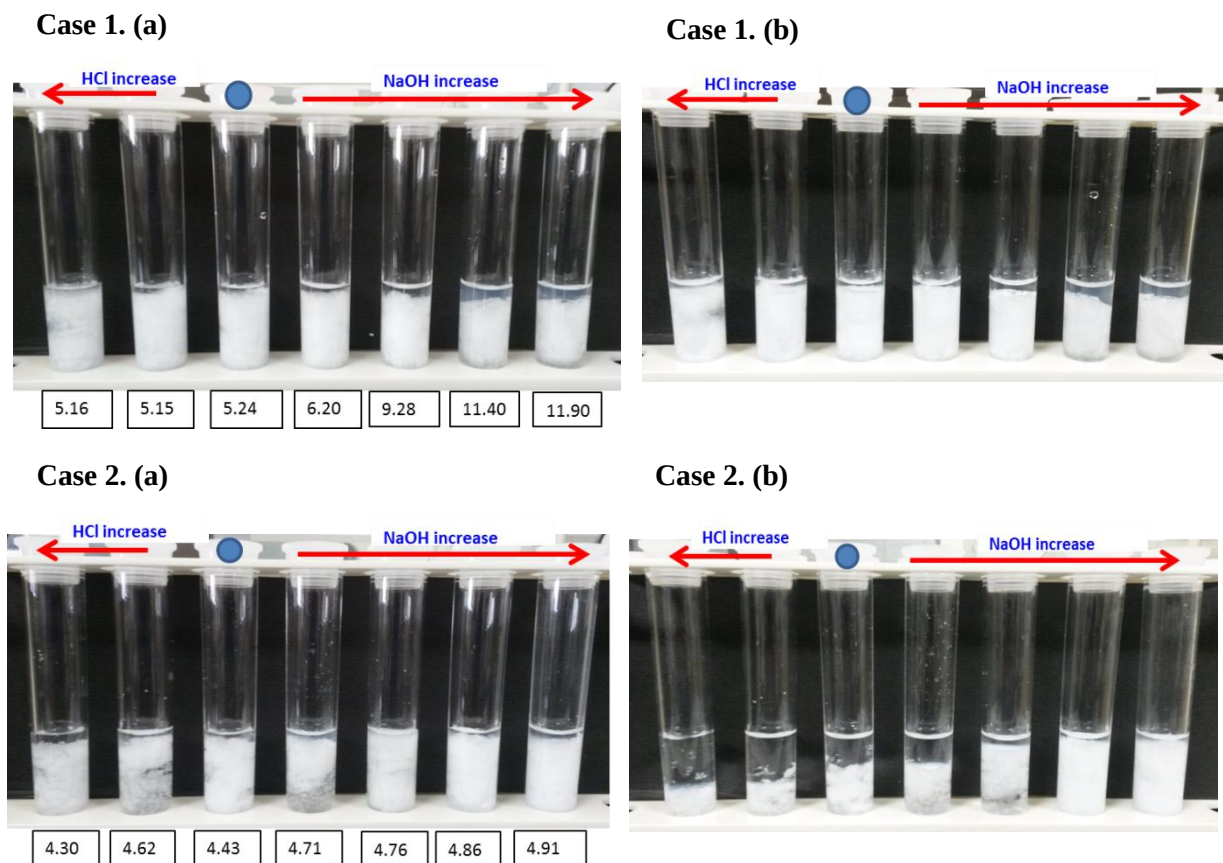


Fig.B.3: Test tubes experiment- Case 1 and Case2 (Ca/P=1.5) (a) after 1 day (b) after 14 days.



Fig.B.4: Test tubes experiment- Case3 and Case 4 (Ca/P=1.5) (a) after 1 day (b) after 14 days.

APPENDIX B.6.2 Measurement of temporal variation in pH

Temporal variation of pH under different concentrations of urea and urease are shown in Fig. B.5. The pH was increased in all sub cases in case 1 without in control samples which were made using only from CPC and, CPC with urea. Therefore, urease has positive effect on increasing pH for case 1.

Furthermore, the amount of urease facilitated to change the rate of increment of pH. The increase of the amount of urease (mg/L) helped to increase the rate of increment of pH (Fig.B.6 (a)). At the same time, the amount of urea (M) helped to maintain ultimate pH in the mixture (Fig.B.6 (b)).

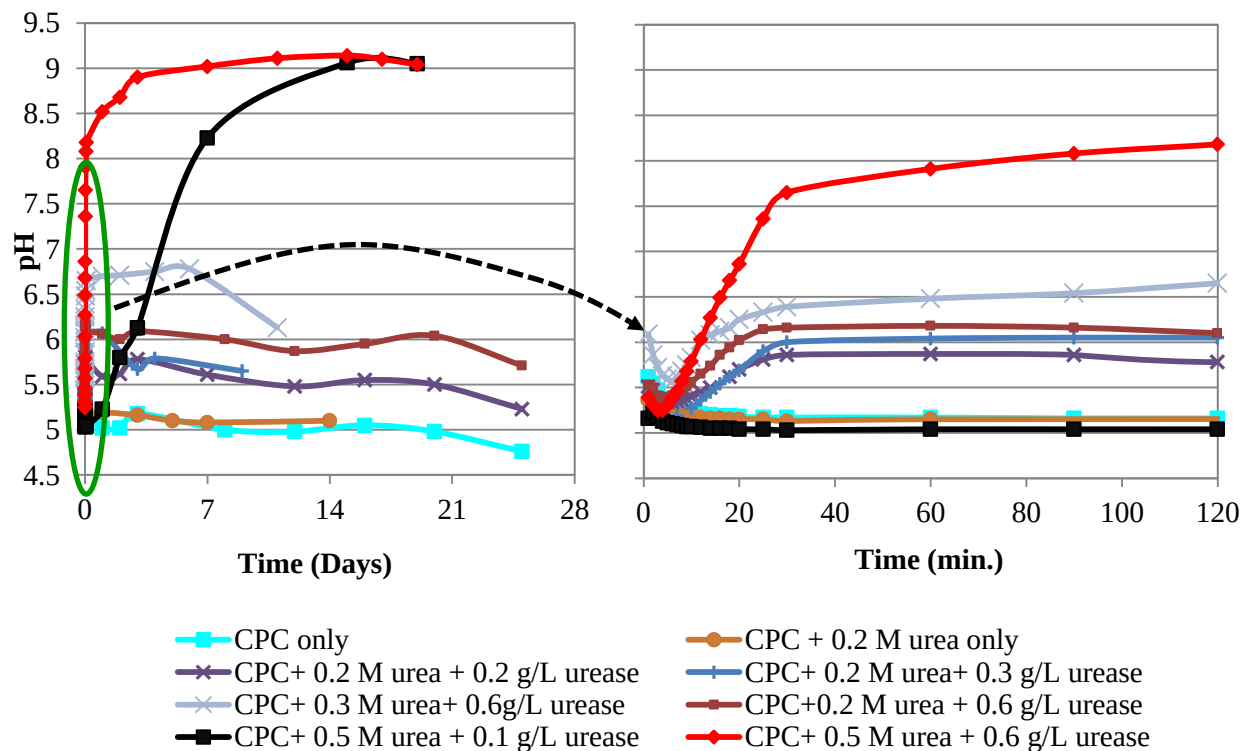


Fig.B.5: Temporal variation of pH– Case 1 (CA=0.75 M, DPP=0.5 M, Ca/P=1.5).

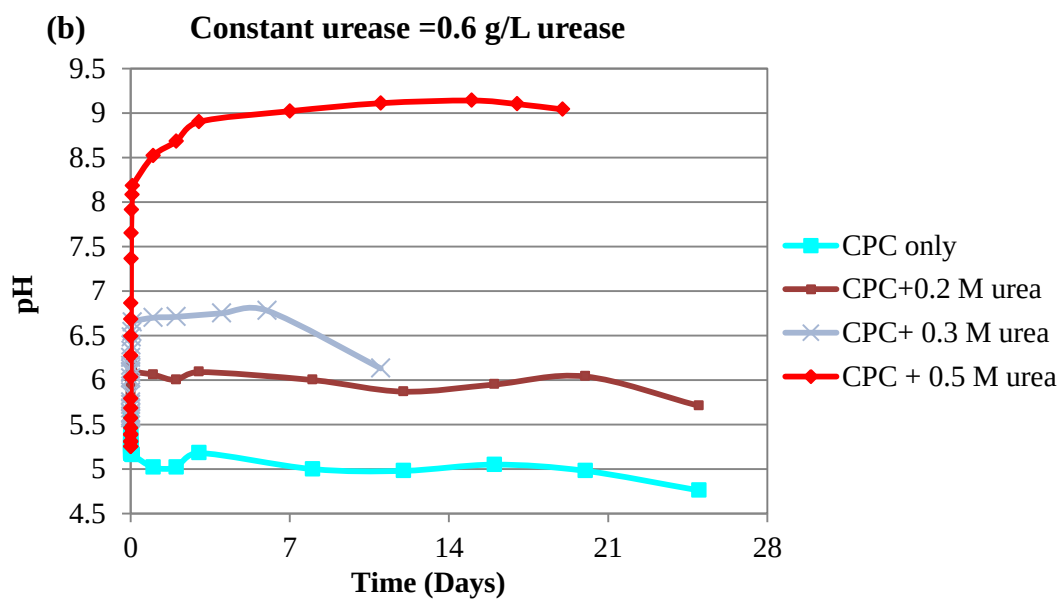
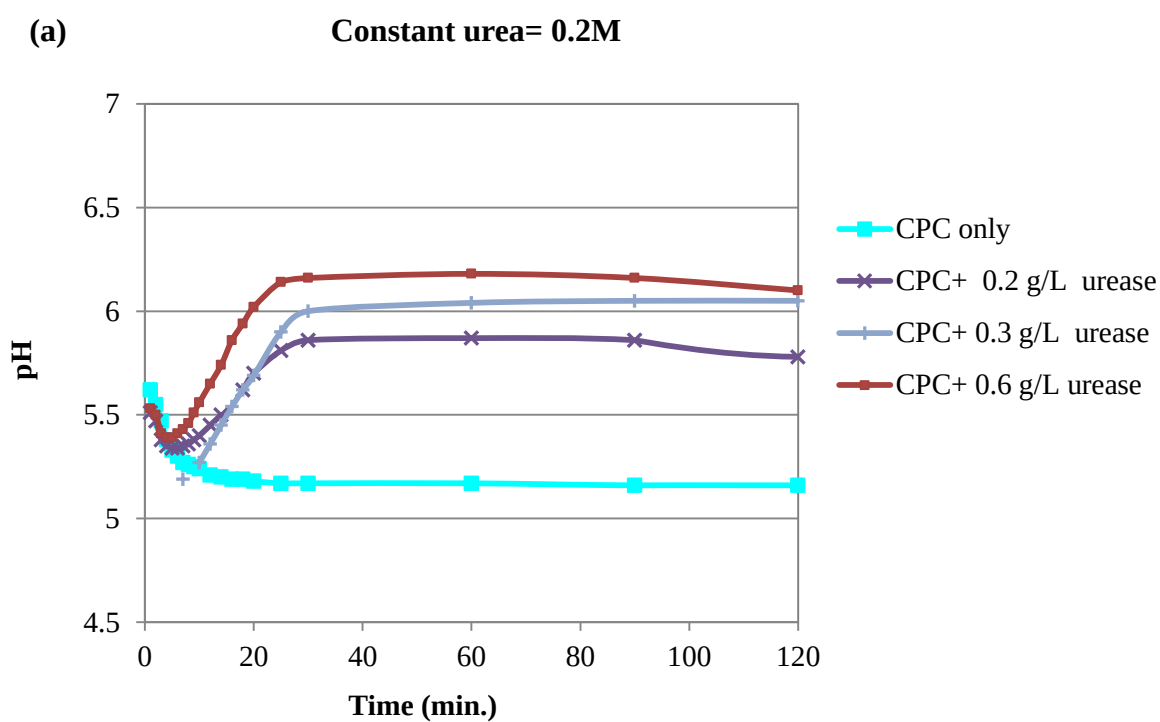


Fig.B.6: Temporal variation of pH– Case 1 (CA=0.75 M, DPP=0.5 M, Ca/P=1.5) (a) after changing urease concentration (b) after changing urea concentration.

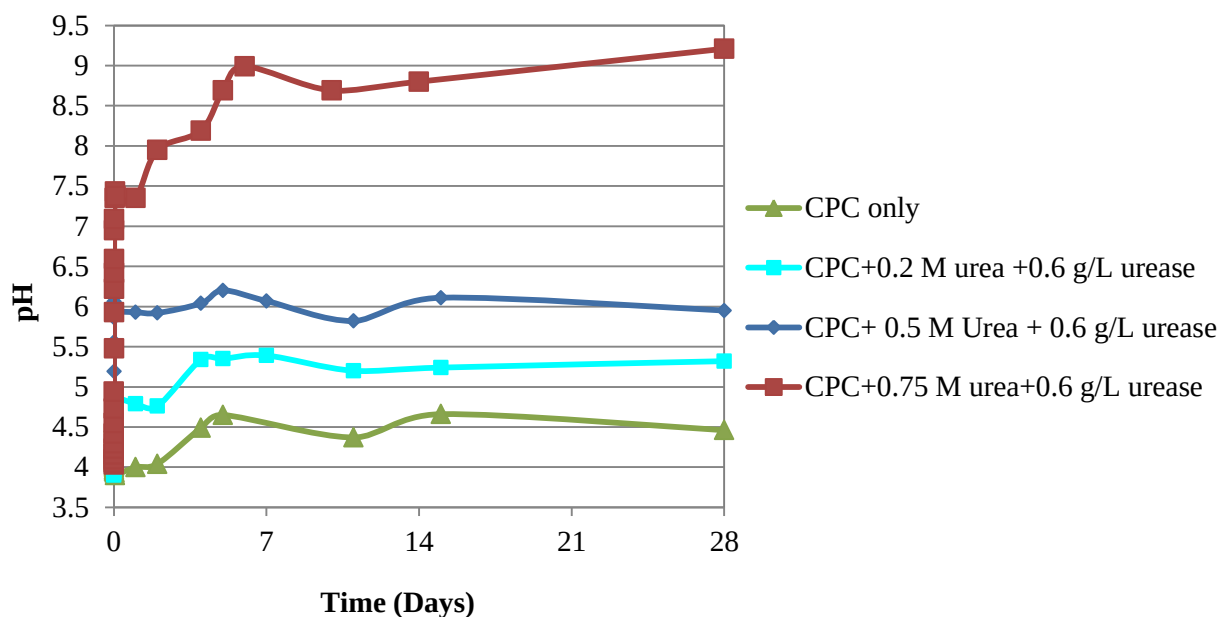


Fig.B.7: Temporal variation of pH– Case 2 (CA=0.75 M, MAP=0.5 M, Ca/P=1.5).

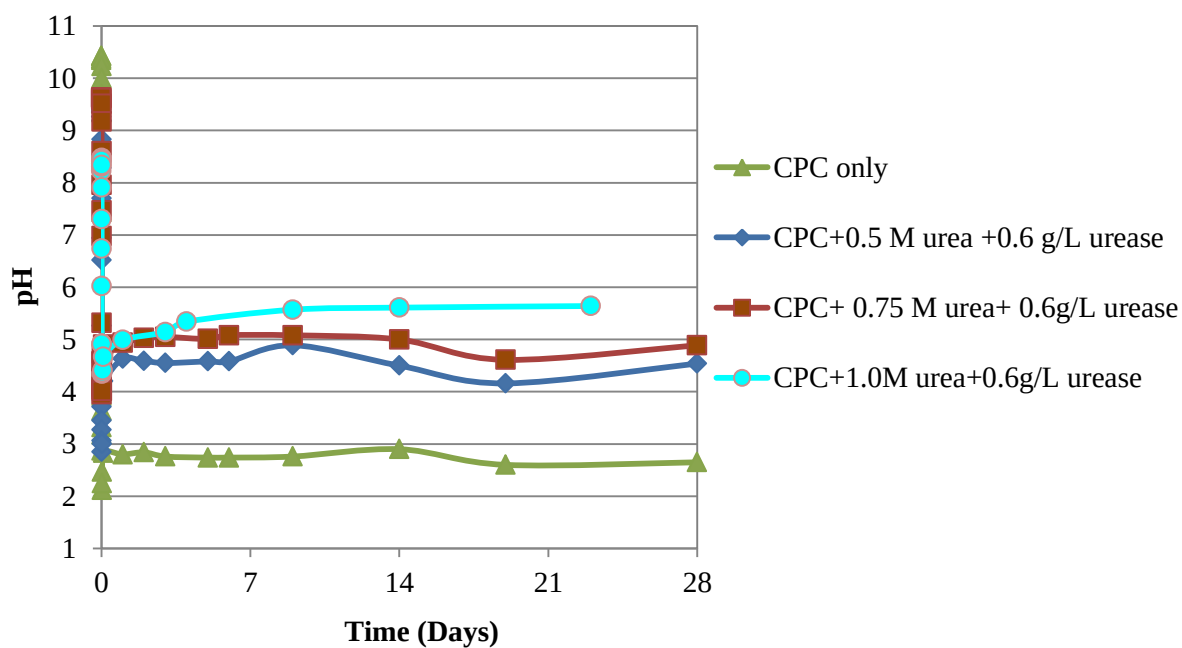


Fig.B.8: Temporal variation of pH– Case 3* (CN=2.25 M, DPP=1.5 M, Ca/P=1.5).

All of the test cases we used to measure the temporal variation of pH showed an incremental behavior of pH with time (Figs.B.5, B.7 and B.8). According to the type of Ca^{2+} and PO_4^{3-} sources, original pH of the mixture was changed. In case 1, original pH in the

mixture prepared only from CPC was around 5 and it was around 4.5 and 3 for case 2 and case 3* respectively. Only difference between case 3 and case 3* was the concentration of each sources. Type of Ca^{2+} and PO_4^{3-} sources and Ca/P ratio were same. We could observe same behavior of pH for case 3 and case 3* because as we discussed in chapter 02, same Ca/P ratio indicate same pH. However, the amount of precipitated CPCs was changed in both cases and it was increased with the increase of concentration.

In addition to that, we observed another important thing in case 3*. Bubbling was started after about 20 min. of initial mixing of Ca^{2+} and PO_4^{3-} sources and it remained about 10 min. in all the sub cases in case 3* without in control sample. This may be due to CO_2 gas that can be released during urea hydrolysis. Therefore, we should give attention while preparing sand test specimen for the determination of UCS in future. The preparation of sand test specimen immediately after mixing may result a swelling of the specimen and therefore, an underestimate of UCS.

APPENDIX B.7 CONCLUSIONS

In this study, we investigated the potential utility of a plant-derived urease enzyme and urea induced pH regulator to increase the volume of precipitate of CPCs and hence to use a new CPC biogROUT for sand solidification in future. According to the results, we made following conclusions.

- There is a positive effect on increasing pH in CPC mixture by using urea and urease enzyme. The amount of urease facilitated to change the rate of increment of pH. The increase of the amount of urease (mg/L) helped to increase the rate of increment of pH as well as the amount of urea (M) helped to maintain ultimate pH in the mixture. However, it is necessary to consider about optimal pH for maximum precipitation at each Ca/P ratio

(Fig. 1.1) while increasing pH using urea and urease. Otherwise the precipitate may dissolve at high pH rather than optimal pH.

- According to the type of Ca^{2+} and PO_4^{3-} sources, original pH of the mixture was changed. Hence, the amount of urea and urease need to achieve required pH is depend on the Ca^{2+} and PO_4^{3-} sources we use.

In this study, we considered only the change in volume of precipitate with pH. However, future researches are needed to identify the types of crystal in the precipitate because crystal type may change the mechanical properties of CPCs such as stress- strain behavior. After knowing these details clearly, there is a possibility to apply this new CPC biogROUT for sand solidification in future to enhance the strength of sand rather than in CPC-Chem method.

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