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2. A. I. Vogel, "A Textbook of Practical Organic Chemistry", 3rd Edn., Longmans, London, **1956**, pp. 130-132.

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3. W. Leistritz, "Methods of bacterial reduction in spices ", in "Spices: Flavor Chemistry and Antioxidant Porperties" (Ed. S. J. Risch and C-T. Ito), American Chemical Society, Washington, DC, **1997**, Ch. 2.

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4. W. phutdhawong, "Isolation of glycosides by electrolytic decolourisation and synthesis of pentinomycin", *PhD. Thesis*, **2002**, Chiang Mai University, Thailand.

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5. K. Miwa, S. Maeda and Y. Murata, "Purification of stevioside by electrolysis", *Jpn. Kokai Tokkyo Koho 79 89,066* (1979).

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Full Paper

Study on the flexural properties of metallic-hybrid-fibrereinforced concrete

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Abstract: This contribution investigates the flexural properties of metallic-hybrid-fibre-reinforced concrete. Two types of fibres were used: amorphous metallic straight fibre characterised as non-slipping fibre due to its rough surface and large specific surface area, and carbon steel hook-ended fibre characterised as slipping fibre. Three types of concrete: control, single-fibre-reinforced and hybrid-fibre-reinforced were prepared. The fibre was incorporated at 20 and 40 kg/m³ for single-fibre-reinforced concrete, and at 20, 40 and 80 kg/m³ for hybrid-fibre-reinforced concrete. The flexural properties were studied using three-point bending tests. From the experimental results obtained with fibre-reinforced concrete containing single fibre, addition of high-bonding amorphous metallic fibre delays the formation of micro-cracks and results in high peak load whereas carbon steel hook-ended fibres when used in hybrid form result in superior performance compared to their single-fibre-reinforced counterparts. Superior performance as a result of fibre hybridisation is interpreted as a positive synergetic effect between the fibres. The procedure of assessing the positive synergetic effect is also discussed.

Keywords: hybrid-fibre-reinforced concrete, metallic fibres, flexural properties, positive synergetic effect

Introduction

Concrete is characterised as a brittle material with low tensile strength and low strain capacity. To reduce the brittleness and increase the resistance to cracking, reinforcement with short randomly distributed fibres has been successfully used [1-2] and the resulting composite is known as fibre-

reinforced concrete (FRC). The performance of FRC depends on many factors such as fibre material properties, fibre geometry, fibre volume content, matrix properties and interface properties [3].

Most types of FRC used in practice contain only one type of fibre. However, it is known that failure in concrete is a gradual, multi-scale process. The pre-existing cracks in concrete are of the order of microns. Under an applied load, these cracks grow and eventually join together to form macro-cracks. A macro-crack propagates at a stable rate until it attains conditions of unstable propagation and a rapid fracture is precipitated. The gradual and multi-scale nature of fracture in concrete implies that a given fibre can provide reinforcement only at one level and within a limited range of strains [4]. For optimal result therefore different types of fibres may be combined and the resulting composite is known as hybrid-fibre-reinforced concrete (HyFRC).

According to Qian and Stroeven [5], the basic purpose in using hybrid fibre is to control cracks at different size levels in different zones of concrete (cement paste or interfacial transition zone between paste and aggregate) at different curing ages and stress levels or loading stages. The hybridisation of fibres in concrete can be done in different ways such as by combining different aspect ratios, geometry, moduli and tensile strength of fibres [6]. Recently many research studies on HyFRC have been carried out and a brief overview of some of the important studies has been reported [7].

Although different kinds of fibres were used (such as steel, carbon, glass and polypropylene), the majority of research studies on HyFRC seem to focus on steel-polypropylene fibre-reinforced concrete [8]. However, polypropylene fibre has a low Young's modulus and, as a consequence, it cannot prevent the formation and propagation of cracks at a high-stress level, nor can it bridge wider cracks. Therefore, its action is limited to small-crack openings. On the contrary, steel fibre has a considerably higher Young's modulus as compared to polypropylene fibre. This leads to an improved potential for crack control at high-stress level [9].

For the structural application of HyFRC, a suitable combination of two different fibres, in which one fibre can resist the cracks effectively at micro-level as soon as they are initiated and the other fibre can control the crack opening at macro-level, can result in a composite exhibiting properties required for a particular application. In this study, composites containing a high-performance and adhering amorphous stainless metallic fibre and a hook-ended carbon steel fibre both in single and hybrid forms are investigated under flexural loading.

In the present state of the knowledge of FRC, much data are available on the behaviour of hook-ended carbon steel fibre, but very limited data are available on the behaviour of high-modulus, adhering amorphous stainless metallic fibres produced in France. Carbon steel hook-ended fibre is characterised as a slipping fibre due to its smooth surface and are usually pulled out, instead of broken, from the matrix at larger crack openings. Amorphous metallic fibre on the other hand is considered to be a high-performance fibre because of its high-bonding strength with the matrix due to its rough surface and large aspect ratio [10-11]. Moreover, it also has a high elastic modulus compared to polypropylene fibre. Amorphous metallic fibre is considered to be very efficient in the first phase of crack opening. Afterwards, as its high bonding with the concrete matrix prevents it from slipping, it breaks one after another and the through-crack bearing capacity decreases sharply [10]. Moreover, being corrosion-resistant, this fibre has drawn the attention of researchers working on the application of FRC in aggressive environments [12].

Taking into account the non-slipping behaviour of the high-performance, adhering amorphous metallic fibre and the slipping behaviour of the carbon steel hook-ended fibre, the major objective of this work is to make different composites containing these two fibres in both single and hybrid forms and to test their flexural properties. Another objective is to investigate the presence of synergetic effect of these fibres when used in hybrid form.

Materials and Methods

Test series

The experimental study was carried out in two test series. Series I was carried out to investigate the properties of mono-fibre-reinforced concrete. Series II was then carried out to investigate the properties of HyFRC and then to identify the positive synergetic effect of the two fibres if present.

Type of fibre used

Two types of macro-metallic fibre, 30 mm in length were used. Type-I fibre (named in this study as MF1) was an amorphous metallic fibre produced by Saint-Gobain Seva, France. It was composed of Fe and Cr (80%) and P, C and Si (20%) in mass. In two different corrosion tests on this fibre by immersing in HCl (0.1 N) for 24 hours and in FeCl₃ (0.4N) for another 24 hours, no corrosion was observed [13]. Due to its rough surface and large specific surface area, this fibre was characterised by a high degree of bonding with the concrete matrix.

Following is the process of producing the amorphous metallic fibre (MF1) [14]. Molten alloy was placed in a crucible the lower section of which was perforated and fitted with a capillary tube a few millimetres in diameter. Underneath the crucible, a water-cooled wheel with notches at regular intervals rotated at high speed. The alloy fell onto this wheel and underwent hyperquenching. The jet of metal was cut at each notch to form the fibre. The production process is illustrated in Figure 1. The pieces of fibre are shinny, flexible, very thin and ribbon-like as shown in Figure 2.



Figure 1. Production process of amorphous metallic fibre

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Type-II metallic fibre (named in this study as MF2) was made using carbon steel wire produced by Bekaert, Belgium, and was characterised by a weak bond with the matrix owing to its smooth surface and less specific surface area compared to type-I fibre. It was circular with hook-ends. The wire pieces usually adhered to one another in clips of certain number of wires (Figure 2). When these clips were put in the mix, the adhesive dissolved and individual pieces of fibre were distributed evenly throughout the mix. The characteristics of these two types of metallic fibre (MF1 and MF2) are given in Table 1.



Figure 2. Amorphous metallic fibre (MF1) and carbon steel fibre (MF2)

Table 1	Properties of fibres in	nvestigated in this study
	I IUDUIUS UI HUIUS H	investigated in this study

			Dimens	sion (m	m)		Е	Tensile	Density	Cross	
Fibre	Fibre type	L	W	Т	D	Geometry	(GPa)	strength (MPa)	(g/cm ³)	section	
MF1	amorphous metal	30	1.6	0.03	-	Straight	140	2000	7.2	Rectangular	
MF2	carbon steel	30	-	-	0.5	Hook- ended	210	1200	7.8	Circular	

Note: L = length, W = width, T = thickness, D = diameter, E = modulus of elasticity

Concrete constituents

A CEM I 52.5R cement (average particle size, d50: 14 µm), river aggregates, viz. 0/4-mm fine aggregate (sand) and 4/10-mm coarse aggregate (gravel), and a super-plasticiser were used to design the concrete studied in this investigation. The quantity of each constituent of the concrete is given in Table 2.

 Table 2. Constituents of control concrete

Cement	Sand	Gravel	Water	Super-plasticiser
(Kg/m ³)				
322	872	967	193	1.61

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A total of eight concrete mixtures: one control, four mono-fibre reinforced and three hybridfibre reinforced, were investigated. Fibre type and dosage for all the concrete mixtures are given in Table 3 along with the slump value of each mixture. Each concrete mixture was labelled according to the type and quantity of fibre. For example, C20MF1 denotes concrete with 20 kg/m³ of MF1 fibre, and C40HyF stands for concrete with 40 kg/m³ of hybrid fibre. Regarding the slump value, it can be observed that the values for fibre-free concrete and fibred concretes containing only MF2 fibre are not significantly different: the addition of MF2 fibre does not change significantly the fresh properties of the matrix. On the contrary, addition of MF1 fibre reduces significantly the slump value, but, by using a vibrating table, no difficulty in moulding the specimens was encountered even at 40 kg/m³ of MF1 fibre.

Concrete	Mixture	Content of f	ibres (kg/m ³)	Total quantity		
mixture	type	type MF1 MF2		of fibre (kg/m ³)	Slump (mm)	
CCONT	Control				170	
C20MF1		20		20	70	
C40MF1		40		40	45	
C20MF2	Mono-fibre		20	20	155	
C40MF2			40	40	130	
C20HyF		10	10	20	100	
C40HyF	Hybrid fibre	20	20	40	60	
C80HyF		40	40	80	40	

Table 3. Fibre content in different concrete mixtures and their slump values

Specimen preparation and test method

For each concrete mixture, five 100x100x500-mm prismatic specimens were cast. The concrete was placed in each mould in two layers. After each layer, the mould was placed on a vibrating table for compaction. There was no difficulty in moulding the specimens; all mixes flowed easily under an external vibration (vibrating table). Specimens were demoulded after 24 hours and then placed in a curing room with 100% relative humidity and 20°C temperature until the day of testing. These prismatic specimens were used to determine the flexural properties, i.e. modulus of rupture (MOR), residual flexural tensile strength (RFTS) and flexural toughness (FT). Three-point bending tests were performed on notched beams as shown in Figure 3, using a universal testing machine manufactured in Laboratory of Materials and Durability of Structures, Toulouse, France. The maximum loading capacity of the machine is 50 kN.

A notch, 17.5 mm deep and 3 mm wide, was cut in the centre of each prismatic specimen with a concrete saw. All tests were controlled by crack mouth opening displacement (CMOD) using linear variable differential transducer (LVDT). CMOD rate was kept at 0.01mm/min up to 0.1 mm crack opening, and then it was increased to 0.2 mm/min until the completion of the test. The mid-span deflection was also measured using LVDT. CMOD, force and deflection were automatically recorded

using data acquisition system. The testing setup and arrangement of displacement sensors (LVDT) is shown in Figure 3.



Figure 3. Testing setup for three-point bending test

Results and Discussion

Representative curves of load-CMOD and load-deflection behaviour of all the concrete mixtures are shown in Figures 4 and 5 respectively, where it can be observed that reinforced matrices exhibit high strength and toughness compared to un-reinforced matrix. It is important to mention here that each representative curve shown here is not an average of five samples. In fact, after plotting the curves of all samples of each composition, a single representative curve was selected. However, the values of each flexural property (i.e. MOR, RFTS and FT) given in the following sections are the average of those from five samples of each composition.

Modulus of rupture (MOR)

The modulus of rupture (MOR) of all concrete mixtures of series I (mono-fibre-reinforced) and II (hybrid-fibre-reinforced) was calculated from the maximum load attained in the test using elastic analysis. The average value for each mixture is shown in Figure 6 along with scatter of test results. It can be observed in series-I testing that the addition of MF1 metallic fibre provides appreciable increase in MOR, whose value also increases with increase in fibre content as expected. On the other hand, addition of MF2 metallic fibre does not provide any significant increase in MOR at the dosage of 20 kg/m³ (C20MF2), although at 40 kg/m³ (C40MF2) the MOR is increased by 35.3% compared to control concrete (CCONT). In series-II testing (Figure 6), the highest MOR is exhibited by C80HyF concrete mixture. In the case of C40HyF, MOR increases appreciably compared to that of C40MF2, but it is lower than the value attained by C40MF1. A similar trend is observed in the case of concrete mixtures containing fibre at 20 kg/m³ dosage in hybrid and single forms: MOR of C20HyF is higher than that of C20MF2 but lower than that of C20MF1. Percentage increase in MOR for each FRC compared to fibre-free concrete is shown in Figure 7. It should be mentioned here that the comparison of C40HyF with C20MF1 and C20MF2, and that of C80HyF with C40MF1 and C40MF2 are more important for the investigation of synergetic effect which is discussed later in this paper.



Figure 4. Load versus CMOD curves



Figure 5. Load versus deflection curves



Figure 7. Percentage increase in MOR compared to CCONT (control)

Residual flexural tensile strength (RFTS)

FRC has the potential of exhibiting higher strength and ductility in comparison with unreinforced mortar or concrete, which fails in tension immediately after the formation of the first macrocrack [3]. In this study, the procedure proposed in European standard NF EN 14651 [15] is used to calculate the RFTS of FRC using the expression:

$$f_{R,j} = \frac{3F_j l}{2bh_{sp}^2} \tag{1}$$

where $f_{R,j}$ is RFTS corresponding to CMOD = CMOD_j (*j*=1,2,3,4); F_j is the load corresponding to CMOD_j; *l* is the span length; *b* is the width and h_{sp} is the distance between the tip of the notch and the top of the specimen. In this study, RFTS has been determined at CMOD values of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mm. Average values of RFTS of different concrete mixtures are shown in Figure 8.



Figure 8. RFTS of different concrete mixtures as a function of CMOD

Figure 8a shows a comparison of different quantities of MF2 metallic fibre in the mix. When the quantity is increased from 20 to 40 kg/m³, the RFTS is greater at all CMOD values as expected. With both volume fractions of MF2 fibre, after the peak load, a very small drop in the load bearing capacity is followed by an appreciable value of residual strength up to crack width of 4 mm.

Figure 8b shows a comparison of different quantities of MF1 metallic fibre in the mix. Similar to MF2 fibre, when the quantity is increased from 20 to 40 kg/m³, the RFTS is greater. Unlike MF2 metallic fibre, after the peak load, RFTS is observed to drop gradually with the increase of CMOD, indicating a relatively brittle behaviour. The RFTS is seen to approach a value less than 1 MPa at crack width of 4 mm.

Figure 8c shows a comparison of C20MF1 and C20MF2 containing mono-fibre at 20 kg/m³ with C20HyF and C40HyF containing hybrid fibre 10 and 20 kg/m³ of each fibre respectively. In the case of mono-fibre concrete, it is observed that after the peak load at CMOD of 1 mm, the response of the two fibres in terms of RFTS is reversed: at smaller crack opening, the mix reinforced with MF1 fibre exhibits greater RFTS while at larger crack opening, RFTS of the mix containing only MF2 is high. In the case of the C20HyF hybrid mix, the residual strength values are found to be higher than those of C20MF2 and lower than those of C20MF1 at CMOD < 1 mm, while after 1 mm the reverse happens. As a result of different actions of the two fibres at different loading levels, the hybrid mix containing each fibre at 20 kg/m³ (40HyF) exhibits greater values of RFTS over a wide range of crack opening.

Figure 8d shows a comparison of C40MF1 and C40MF2 containing single fibre at 40 kg/m³ with C40HyF and C80HyF containing hybrid fibre at 20 kg/m³ and 40 kg/m³ of each fibre respectively. A similar trend as mentioned above for Figure 8c was observed. In the case of C40HyF hybrid mix, RFTS is observed to be higher than that of C40MF2 and lower than that exhibited by C40MF1 up to the crack opening of 1 mm. At 1-1.7 mm CMOD, the RFTS is lower than that of C40MF2. The hybrid mix containing each fibre at 40 kg/m³ (C80HyF) exhibits greater values of RFTS compared to all types of mix over a wide range of crack opening.

Flexural toughness (FT)

To mitigate the hazard for structures subjected to dynamic loads such as seismic, impact and blast, high-energy absorbing materials are needed [3]. Flexural toughness (or energy absorption capacity) is measured by the area under the load-deflection curve as shown in Figure 5. Flexural toughness also demonstrates the ductile behaviour of the material. The effect of fibre addition, fibre type and hybridisation of fibres on FT is illustrated in Figure 9 using FT values calculated as area under the load-deflection curve up to 4 mm deflection. Addition of metallic fibres is observed to increase FT of the brittle matrix appreciably. Moreover, the effectiveness of fibre increases with increase in fibre content; this is true for both types of metallic fibres. As far as the effect of fibre type is concerned, it is observed that at both fibre dosages (20 and 40 kg/m³), MF2 fibre is more effective than MF1 fibre. Among the hybrid concrete mixtures in series II, C80HyF mix exhibits the highest value of FT, which is significantly greater than that of the control concrete. In the case of C20HyF, the value is the same as for C20MF1 but less than that attained by C20MF2. For C40HyF the FT is found to be higher than that of C40MF1 but lower than that of C40MF2 although the difference is small in both cases.



Figure 9. FT values of all concrete mixtures

Synergetic effect

In FRC composites, one can consider that the contribution of various components are additive and this implies [10]:

$$F(C_f) = F(C) + \sum_{i=1}^{n} F(f_i)$$
(2)

where $F(C_f)$ is the mono-fibre concrete response; F(C) is the matrix response and $F(f_i)$ is the fibre contribution. Symbol *n* indicates the number of fibres present in the matrix. Similarly, for HyFRC, Eq.2 becomes

$$F(HyC_f) = F(C) + \sum_{i=1}^{n} F(af_i) + \sum_{j=1}^{m} F(cf_j)$$
(3)

where n and m indicate the number of amorphous metallic and carbon steel fibres respectively, symbol a is for amorphous metallic fibre and symbol c is for carbon steel fibre.

Simple arithmetic sum of responses from two single fibres in the reinforced concrete can be represented by:

$$\left\{F(C) + \sum_{i=1}^{n} F(af_i)\right\} + \left\{F(C) + \sum_{j=1}^{m} F(cf_j)\right\}$$

$$\tag{4}$$

To determine the synergetic effect between fibres, the sum of responses from single-fibre reinforcement are compared to the response from hybrid-fibre reinforcement. Note that while adding response from mono-fibre reinforcement (Eq. 4), the matrix contribution is added twice. For true determination of synergetic effect, one matrix effect should then be subtracted from the expression. Based on this consideration, for positive synergetic effect between the two fibres used in HyFRC the following equation should be satisfied:

$$F(HyC_f) > \left\langle \left(F(C) + \sum_{i=1}^{n} F(af_i) \right) + \left(F(C) + \sum_{j=1}^{m} F(cf_j) \right) - F(C) \right\rangle$$
(5)

In this study, the synergetic effect has been investigated in two formulations of HyFRC, i.e. C40HyF and C80HyF, in terms of MOR, RFTS and FT. For a hybrid concrete, a positive synergetic effect exists between two fibres if their combined response is greater than the arithmetic sum of responses from mono-fibre concretes each containing single fibre at the same volume fraction as in the hybrid combination.

In Figures 10-11, it is observed that no synergetic effect exists in terms of MOR and FT at fibre quantity of 40 kg/m³ (C40HyF). However, at 80 kg/m³ (C80HyF), a small positive synergetic effect for both properties exists between the fibres. A similar result can also be observed for RFTS as shown in Figure 12: synergetic effect does not exist at a total fibre quantity of 40 kg/m³ (C40HyF), but it does when both fibres are combined to the total quantity of 80 kg/m³ (C80HyF).

Another important fact about the synergetic effect is that if one fibre is efficient at microcracking level and the other fibre is at macro-cracking one, and when both fibres are present in HyFRC, resulting in improved response at both levels (micro- and macro-cracking), this can also be interpreted as a synergetic effect. In this case, it is not necessary that the response of the hybrid









Figure 12. Synergy assessment in terms of RFTS

composite in terms of one particular property must be greater than the sum of responses of the single-fibre-reinforced concretes. In this study, type-I fibre (MF1) is effective at micro-cracking level and type-II fibre (MF2) is effective at macro-cracking level. As a result, the hybrid composition exhibits a globally improved flexural behaviour at both cracking levels.

From all the above experimental results, it is clear that there is a marked difference in the responses of two metallic fibres used in this study. At low level of deflection or CMOD, the high-bonding non-slipping fibre (MF1) shows high efficiency whereas at high level of crack opening or deflection, the low-bonding slipping hook-ended fibre (MF2) exhibits better performance.

Since micro-cracking in a specimen subjected to flexure is initiated prior to the peak load (at 60% of the peak load) [16], high-bonding amorphous metallic fibre (MF1) stops the development of these micro cracks and as a result the peak resistance of the composite increases. With the increase of crack opening, the stress increases in the fibre and a stage comes when localised tensile stress in the part of fibre between the crack edges exceeds its tensile strength and the fibre breaks instead of pulling out from the matrix. During tests on specimens with MF1 fibre, after the peak load, the sound produced by the breaking of fibres could be heard, and this breaking was also visible when the fractured surface of the specimen was examined (Figure 13). Similar observations were also made by Pons et al. [10]. Due to the sudden breaking of fibres, a rapid drop in the load bearing capacity was observed and the RFTS approached a negligible value very quickly over a very short range of CMOD or deflection. Increase in peak load capacity and a high value of RFTS over a short plateau are two main factors which cause increase in the area under the load-deflection curve (i.e. flexural toughness or energy absorption capacity).



Figure 13. Fractured surface of specimen with MF1 fibre (left) and with MF2 fibre (right)

The slipping, low-bonding hook-ended fibre (MF2) at a dosage of 20 kg/m³ did not significantly increase the peak resistance although at 40 kg/m³, peak resistance was increased by 35.3% compared to control concrete. Being ductile and having adequate anchorage in the matrix

through hook ends, the fibre is effective in bridging macro-cracks. After the peak load, a drop in the load-carrying capacity was observed, but soon after, the fibre started acting and bridged the macro-cracks. Crack bridging by the fibre resulted in significant RFTS, which was observed to be attained over a long plateau. Finally, these individual fibres were pulled out from the concrete matrix instead of breakage as shown in Figure 13 and their hook ends were turned straight.

Since the two fibres were observed to act differently at two levels, viz. micro-cracking and macro-cracking levels, hybridisation of these fibres resulted in an improved flexural response at both loading stages in terms of strength and energy absorption capacity. For HyFRC at a total fibre quantity of 80 kg/m³ (40 kg/m³ of each fibre), positive synergetic effect between metallic fibres resulted in maximum flexural response compared to all other mixtures containing fibres in single or hybrid forms. Moreover, two combined actions, one of amorphous metal fibre to stop the micro-cracking mechanism and the other of carbon steel hooked-ended fibre to stop the propagation of macro-cracks, were another positive synergetic effect between the two metallic fibres.

However, it should also be mentioned here that by keeping the same fibre volume in a composite, mechanical performance of the FRC composite can also be improved by changing the constituents of the cement-based matrix. Maximum aggregate particle size (d_{max}) usually governs the length of the fibre, i.e. length of fibre (l_f) should be equal or greater than 3 times of d_{max} [17]. If d_{max} is reduced, fibre with shorter length can be selected, and for the same dosage the number of the shorter fibre will increase. Moreover, by reducing d_{max} , the matrix compactness will also improve. Increased number of fibres and improved compactness of the matrix can result in improved mechanical response of the FRC, which sometime leads to the development of ultra-high performance fibre-reinforced concrete composite [18].

Conclusions

On the basis of three-point bending tests performed on notched prismatic specimens constructed with mono- and hybrid-fibre-reinforced concretes containing two different fibres used in this study, the following conclusions can be drawn:

- Adhering amorphous stainless metallic fibre, due to its high-bonding with the matrix, is very effective in controlling the micro-cracking mechanism, which results in an improved behaviour in terms of smaller crack openings at peak resistance. On the other hand, high-modulus hook-ended carbon steel fibre is effective in controlling macro-cracks over a wide range and at high stress level. As a result, the toughness of the material is significantly increased. It should also be noted that these fibres also have very different properties in terms of fibre geometry and tensile strength. These variables are also effective on flexural performance of beam samples at different loading levels.
- The use of metallic fibres in hybrid form investigated in this study has resulted in improved behaviour of the composite regarding cracking control, strength and toughness. For such structural application as in water retaining structures, hybrid combination of these fibres could be promising.
- The response of the hybrid mixture containing both fibres at a total quantity of 80 kg/m³ (40 kg/m³ of each fibre) in terms of modulus of rupture, residual flexural tensile strength and

flexural toughness has shown that there exists a positive synergetic effect between the metallic fibres used in this study.

Future Work

In the ongoing research work, more fibre composites containing fibres in single and hybrid forms at the same dosage will be tested and results will be compared to further highlight the benefits of mixing metallic fibres used in this study. For example, mono-fibre composition containing fibres at the dosage of 80 kg/m³ will be tested in comparison with the hybrid composition containing both fibres at a total quantity of 80 kg/m³.

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Report

Phenological observation and population dynamics of six uncommon medicinal plants in the grasslands of Nilgiris, Western Ghats, India

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Abstract: Phenological observation and a population density study for six uncommon medicinal plant species were made in four grasslands in Nilgiri Biosphere Reserve, Western Ghats, viz. Thiashola, Korakundah, Ebbenadu and Wenlockdown, at monthly intervals from April 2007 to March 2008. The six plant species were Anaphalis elliptica DC. (Compositae), Ceropegia pusilla Wight & Arn. (Asclepiadaceae), Hedvotis articularis R. Br. ex G. Don (Rubiaceae), Heracleum rigens Walli. ex DC. (Umbelliferae), Leucas vestita Benth. (Lamiaceae) and Luzula campestris (L.) DC. (Juncaceae). Generally, all six species exhibited peak bud formation between February and May and bud break in June. Most of the leaves were produced in a single flush. Leaf expansion continued up to August in L. vestita. Flowering phenophase was observed from July to October, but in A. elliptica it extended to December. The active period of fruit formation occurred during August to December for all species except A. elliptica, which was during January and February. Seed maturation and seed dispersal happened during December - February for all the species except A. elliptica which happened during May-June. The study of population dynamics shows that there was a net decrease in the population of A. elliptica, L. vestita and L. campestris over a period of one year at Korakundah, Ebbenadu and Wenlockdown grasslands. C. pusilla, H. articularis and H. rigens maintained their populations at the same level in the respective grasslands without any major change during the study period.

Keywords: phenology, population dynamics, medicinal plants, grasslands, Nilgiris, Western Ghats

Introduction

Plants respond biologically to various parameters in the holocoenotic environment [1]. Besides this, many extrinsic factors, e.g. the time of phenophase and seed dispersal, are very important in distribution, survival and success in the establishment of a species in the community. Considering these facts, it is known that phenological studies are important for the conservation of genetic resources and forest management as well as for a better understanding of ecological capabilities of plant species and community-level interactions. Another important attribute which decides the establishment of a species is population size. This is regulated by an array of environmental factors. In this study we have observed the times of phenophase and determined the influence of environmental variables on the population density of six uncommon medicinal plants in four grasslands in Nilgiri Biosphere Reserve, Western Ghats. These are *Anaphalis elliptica, Ceropegia pusilla, Hedyotis articularis, Heracleum rigens, Leucas vestita* and *Luzula campestris*. Since no attempts have been made in the conservation of these six species in these grasslands, they have been selected for this study. The ecological and medicinal characters of these species are given in Table 1.

Species	Family	Habit	Ecological	Medicinal /
Anaphalis elliptica DC.	Compositae	Herb (annual)	Endemic	Antipyretic
Ceropegia pusilla Wight & Arn.	Asclepidaceae	Herb (annual)	Rare and threatened	Antidote for snake bite
Hedyotis articularis R. Br. ex G.Don	Rubiaceae	Shrub (perennial)	Endemic	Treatment of nervous disorders
Heracleum rigens Walli. ex DC.	Apiaceae	Herb (annual)	Endemic	Anticancer
Leucas vestita Benth.	Lamiaceae	Herb (annual)	Endemic	Treatment of rheumatism
Luzula campestris (L.) DC.	Juncaceae	Herb (annual)	Threatened	Agricultural indicator (indicates high possibility of agriculture practice by selecting any local crop)

Table 1. Ecological and medicinal attributes of the six studied species

Methods of Observation

Detailed phenological records of the six plant species were carried out from April 2007 to March 2008 at monthly intervals. During high activity periods, observations were made more frequently. Phenological observations of each species were made by marking 20 randomly selected individuals in one of the grasslands. Since all four grasslands are located at a more or less similar geographical position and elevation (11° 13' N and 76° 39' E, elevation between 2050-2200 m)

(Figure 1) and under uniform macroclimatic conditions (1560-mm annual rainfall and temperature between 5°C during January and 26°C during April : Table 2), the phenological observations were made at only one grassland where the species was present. The associated species in the plots were generally grasses with a few dicot herbs. When a phenophase was noticed in about 10% of individuals under observation, it was considered to be initiated and considered at a peak when it occurred in more than 80% of individuals. The phenograms were drawn according to phenophases which occurred in more than 80% of individuals following the methodology of Lodhiyal et al [3].

The study on population density and the results were expressed in a 100-m² scale. In the grasslands of the species occurrence, five 1-m² quadrats were made. In the case of adults, each plant with a height of 30 cm was considered as an individual. For the study of seedling population, seedling cohorts were marked in each of the quadrats in April 2007 and February 2008. Individuals arising from seeds were marked with dots of different colours. The survival of adults and seedlings were recorded at monthly intervals.

Results and Discussion

Phenological observations of bud formation, vegetative growth, flowering, fruiting, seed maturity and seed dispersal for each species are presented in Figure 2. Generally, for all species studied, bud formation occurred during February and May. For Anaphalis elliptica and Leucas vestita bud formation extended to June. It has been noted that more abundant and shallow roots in the upper soil layers contributed by herbs favour the sudden appearance of buds immediately after adequate rain in dense forests [4]. Bud aestivation started during June and most leaves were produced in a single flush. For Anaphalis elliptica, phenophase happened during September and October. Continued leaf expansion for Leucas vestita was until August. This may be explained due to the need of high temperature thresholds [5-6]. It has been observed that the rainy season was most favoured for the vegetative growth of all six species. Similar patterns on bud formation and vegetative growth have been observed for certain grassland and forest understorey species [7-9]. Flowering period after vegetative growth was mostly from July through October. For Anaphalis elliptica, this happened during November and December. The flowering response of plants is related to the high elevation and temperature factors and is species specific [10]. Generally, from August to December fruit formation occurred for all six species except Anaphalis elliptica, whose fruiting period was during January and February. In the months after fruiting, seed maturation happened for all six species. Seed dispersal was noted during December and February except for Anaphalis elliptica, which was in May and June. Seed maturation and seed dispersal generally happened with the onset of the cold, dry season (December-February) when growth cessation in plants normally happens in the area [11].



Figure 1. Location of study areas in Western Ghats

Year and month	Tempe (°(erature C)	Rainfall (mm)	No. of Rainy	Relative humidity		
	Max.	Max. Min.		days	(%)		
2007							
Apr	26.1	15.6	25	7	95		
May	25.3	17.6	68	6	94		
Jun	22.8	17.6	212	15	90		
Jul	21.5	16.8	210	14	95		
Aug	19.4	17.4	161	13	95		
Sep	21.8	16.6	86	15	94		
Oct	16.9	15.8	298	19	97		
Nov	16.6	15.6	240	13	96		
Dec	15.3	9.8	82	4	92		
2008							
Jan	14.6	5.3	0	0	85		
Feb	15.6	11.7	51	7	78		
Mar	16.8	13.1	85	11	75		
Apr	21.9	14.7	42	6	83		

 Table 2.
 Climate in the study area

Data Source: Government Meteorological Station, Ootacamund, the Nilgiris

Variation in population density of the six species is presented in Table 3. Anaphalis elliptica and Ceropegia pusilla, which were only present in Thiashola and Ebbenadu grasslands varied between 0.03 (in Ebbenadu during April 2008) and $0.21/m^2$ (in Thiashola during November 2007), and 0.04 (in both Ebbenadu and Thiashola during June and February) and 0.21/m² (in Thiashola during September, Hedyotis articularis, which was present in Thiashola, Korakundah and Ebbenadu 2007). grasslands, had population density variations between 0.02 (during May, 2007 in Korakundah and Ebbenadu) and 0.17/m² (during October, 2007 in Korakundah). The species density of Heracleum rigens ranged between 0.04 (during June, 2007 in Ebbenadu and Wenlockdown) and 0.19/m² (during October, 2007 in Ebbenadu). Leucas vestita also showed great variation in population size with between 0.03 and 0.21/m² in the grasslands of its occurrence. Luzula campestris, which was present only in Wenlockdown grassland, had its population sizes between 0.04 (in March, 2008) and $0.25/m^2$ (in October, 2007). The overall low population of all six species $(0.02-0.21/m^2)$ in the grasslands is due to the collective influence of several factors such as limited natural distribution and variation in microclimatic conditions [12]. Dry and cold conditions after seed dispersal during February and March can be a reason for the low population density for these species. In spite of severe habitat protection, illegal exploitation by local people and other herb gatherers for medicinal plants also have reduced their population sizes.

Species	2007 Apr	May	Iun	Iuly	Αιισ	Sen	Oct	Nov	Dec	2008 Jan	Feb	Mar
Anaphalis elliptica	$\hat{\mathcal{D}}$	$\hat{\mathcal{D}}$	$\tilde{\mathbf{v}}$	\Diamond		Ũ	Ũ	Q	Q	\bigcirc	Q	$\hat{\mathbf{D}}$
Ceropegia pusilla	\Diamond	Û	()	\bigcirc	Q	\bigcirc	$\hat{\mathbf{Q}}$	$\hat{\mathbf{Q}}$	\bigcirc	\bigcirc	\bigcirc	\Diamond
Hedyotis articularis	\Diamond	Ũ	()	\bigcirc	Q	Q	Q	Ŵ	$\hat{\mathbf{Q}}$	\bigcirc	\bigcirc	\Diamond
Heracleum rigens	\bigcirc	\Diamond	\diamond	$\langle \rangle$	\Diamond	\bigcirc	\bigcirc	\bigcirc	$\hat{\mathbf{Q}}$	\bigcirc	Σ	\bigcirc
Leucas vestita	\bigcirc	\bigcirc	\diamond	$\langle \rangle$	()	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\mathfrak{O}
Luzula campestris	Ũ	Û	\bigcirc	\bigcirc	\bigcirc	\bigcirc	$\hat{\mathbf{Q}}$	$\hat{\mathbf{Q}}$	\mathfrak{O}	\bigcirc	\Diamond	\Diamond



Phenophases

- 1. Budding
- 2. Vegetative growth (stem and leaves)
- 3. Flowering
- 4. Fruiting
- 5. Seed maturity

Figure 2. Phenograms for the six uncommon medicinal plant species studied

	Grassland													
Year		Thiashola		Korakundah			Ebbenadu					Wenlockdown		
and month	Anaphalis elliptica	Ceropegi a pusilla	Hedyotis articulari s	Heracleum rigens	Leucas vestita	Anaphalis elliptica	Ceropegi a pusilla	Hedyotis articulari s	Heracleu m rigens	Leucas vestita	Heracleum rigens	Leucas vestita	Luzula campestris	
2007														
Apr	-	-	0.04 ± 0.00	$0.04{\pm}0.00$	0.04 ± 0.00	0.04 ± 0.00	-	0.04±0.00	-	0.04±0.00	-	$0.04{\pm}0.00$	0.07 ± 0.00	
May	-	-	-	$0.02{\pm}0.00$	0.03±0.00	-	-	0.02 ± 0.00	-	0.03±0.00	-	0.03±0.00	0.05 ± 0.00	
Jun	0.08 ± 0.00	0.06±0.00	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06±0.00	0.04 ± 0.00	0.05±0.01	0.04 ± 0.00	0.05±0.01	0.04±0.00	0.05 ± 0.00	0.10±0.00	
Jul	0.11±0.01	0.12±0.00	0.09 ± 0.00	0.06±0.01	0.06 ± 0.00	0.09±0.01	0.05 ± 0.00	0.06±0.00	0.07 ± 0.00	0.06±0.01	0.06±0.01	0.08 ± 0.01	0.14±0.01	
Aug	0.15±0.00	0.18±0.00	0.10±0.00	0.11±0.02	0.12±0.01	0.15±0.01	0.08 ± 0.00	0.10±0.01	0.12±0.00	0.13±0.00	0.10±0.01	0.12±0.00	0.17±0.02	
Sep	0.17±0.00	0.21±0.03	0.12±0.00	0.13±0.00	0.17±0.02	0.12±0.01	0.12±0.01	0.13±0.01	0.16±0.02	0.18±0.02	0.15±0.00	$0.14{\pm}0.01$	0.21±0.01	
Oct	0.19±0.02	0.17±0.02	0.14±0.01	0.17±0.02	0.21±0.02	0.18±0.00	0.14±0.01	0.15±0.01	0.19±0.02	0.20±0.00	0.14±0.01	0.17±0.02	0.25±0.03	
Nov	0.21±0.00	0.14±0.01	0.12±0.01	0.13±0.01	0.14±0.01	0.17±0.02	0.12±0.00	0.12±0.02	0.14±0.01	0.14±0.02	0.17±0.02	0.16±0.01	0.19±0.01	
Dec	0.14±0.01	0.10±0.01	0.11±0.00	0.11±0.01	0.11±0.01	0.13±0.00	0.09±0.00	0.11±0.00	0.12±0.01	0.12±0.01	0.10±0.00	0.09±0.00	0.12±0.01	
2008														
Jan	0.11±0.00	0.08±0.00	0.09±0.01	0.08 ± 0.00	0.08 ± 0.00	0.11±0.01	0.07 ± 0.00	0.09±0.01	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	0.10±0.00	
Feb	0.09±0.00	0.04 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07±0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.08 ± 0.00	
Mar	0.05 ± 0.00	-	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.06±0.00	-	0.05±0.00	-	0.04 ± 0.00	0.05 ± 0.00	$0.04{\pm}0.00$	0.04 ± 0.00	
Apr	-	-	0.04±0.00	0.04±0.00	0.03±0.00	0.03±0.00	-	0.04±0.00	-	0.04±0.00	-	0.03±0.00	0.05±0.00	

Note: - indicates the absence of the species.

Recommendations

Based on the results of this study it is suggested that the seeds of all six species be collected and kept in cold storage until the next growing season. These seeds can be air sown in the grasslands to augment population levels. To confirm this concept, experiments on seed germination as influenced by cold storage must be conducted.

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Communication

Phenolic content and antioxidant properties of green chilli paste and its ingredients

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Abstract: Green chilli paste and its ingredients (chilli, red onion and garlic) from different stages of processing were analysed for total phenolic content and antioxidant properties, i.e. total antioxidant capacity, DPPH radical scavenging activity, and β -carotene bleaching activity. The effects of processing stage on total phenolic content and antioxidant properties of green chilli paste and its ingredients were discussed, along with the correlation between the total phenolic content and the antioxidant properties.

Keywords: green chilli paste, total phenolic content, antioxidant properties

Introduction

An antioxidant refers to any substance which, when present at low concentration compared to that of an oxidisable substrate, significantly delays or prevents oxidation of that substrate. Antioxidants are divided into two groups: natural enzymatic antioxidants and non-enzymatic ones. The natural enzymatic antioxidants, e.g. superoxide dismutases, catalases and enzymes, are located mostly in peroxisomes. Natural and synthetic non-enzymatic antioxidants consist of vitamin E and related antioxidants such as vitamin C, BHT, BHA, carotenoids, glutathione and derivatives, phenolic compounds, flavonoids and alkaloids [1-2]. Foods or food materials are an important source of antioxidant compounds for human consumption. Natural antioxidants present in the diet increase the resistance to oxidative damage. Fruits and vegetables are immensely valuable not only for their nutritional value but also for their potential health functionality against various degenerative diseases [3-4].

Green chilli paste (GCP) is a traditional food from the northern part of Thailand. In a large local market it is not unusual that about 500 kg/day of GCP are sold. GCP is made from chilli, red onion and garlic. Chilli (*Capsicum annuum* Linn.) is a rich source of phenolics and a good source of flavonoids, which of late have aroused great interest owing to their antioxidant activities. Red onion (*Allium ascalonicum* Linn.) and garlic (*Allium sativum* Linn.) are widely used vegetables in diets around the world. The antioxidant activity of *Allium* plants has mainly been attributed to a variety of sulphur-containing compounds and their precursors [5-7].

Some GCP manufacturers have been developing and improving the quality of GCP to delay spoiling. Sterilisation methods of GCP have been improved [8-9]. Normally the cooking process and storage are the main causes of the loss of nutritional value and desired physical characteristics in food and beverages [10-13]. However, thermal processing, which inactivates microorganisms and enzymes, is also the most common method for extending the shelf life of a food product [14].

In this study, the total phenolic content and antioxidant properties of GCP are investigated along with its ingredients, i.e. chilli, red onion and garlic. GCP at different stages of processing and both fresh and heat-processed ingredients are similarly examined.

Materials and Methods

Chemicals

Chemicals were purchased from the following: β-carotene, quercetin and Folin-Ciocalteu reagent from Sigma-Aldrich (USA); 1,1-diphenyl-2-picrylhydrazyl (DPPH) and linoleic acid from Fluka (Switzerland); ammonium molybdate and Tween 20 from AJAX (Australia); acetonitrile from Fluka (USA); gallic acid from Merck (USA); and BHT from BDH (UK). Reference compounds were of HPLC grade while the rest including other common chemicals were of analytical grade.

GCP and its ingredients

Unprocessed GCP was prepared by blending together roasted and peeled ingredients, i.e. chilli, red onion and garlic, and then the resulting mixture was salted. Degassed GCP was prepared by steaming and agitating unprocessed GCP contained in a bottle at 90-100°C for 5 minutes before tightly capping the bottle. Sterilised GCP was prepared by heating degassed GCP at 108°C for 30 minutes at a reduced pressure of 5 psi before storage. Each heat-processed ingredient was prepared in a similar manner as sterilised GCP except no salt was added in the process. All samples for analysis were freeze-dried and ground in a mortar, then kept under nitrogen at -4 °C before experiment.

All prepared GCP and its ingredients mentioned above were kindly supplied by Chiang Mai Vanusnun Co., Ltd., Chiang Mai, Thailand.

Sample extraction

A sample extract was obtained by methanol extraction [6]. A ground sample (1.5 g) was extracted with methanol (10 ml) by stirring for 1 hour at room temperature followed by sonicating for 20 minutes in an ultrasonic bath. The mixture was then centrifuged at 3000 rpm for 20 minutes, the supernatant decanted, and the above extraction was repeated with an additional 10 ml of methanol. The combined supernatant from the two extractions was used for analysis as described below.

Total phenolic content

The total phenolic content was measured spectrophotometrically following the procedure outlined by Siddhuraju [15]. The reaction mixture contained 50% Folin-Ciocalteu reagent (0.5 ml), 20% (w/v) sodium carbonate solution (2.5 ml), and gallic acid solution or sample extract (1.0 ml). The mixture was placed in the dark for 40 minutes and the absorbance was recorded at 750 nm against a blank with a spectrometer (Perkin-Elmer: Lamda 25 UV/VIS).

Preparation of the calibration curve for total phenolic content determination was carried out using gallic acid (2-10 μ g/ml). The total phenolic content was expressed based on gallic acid equivalent (GAE).

Determination of total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. The procedure set out by Banergee et al. [16] was followed. Standard gallic acid solution or the extract (1 ml) was combined with 3.0 ml of the reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the solution was measured at 725 nm.

Preparation of the calibration curve for total antioxidant capacity was carried out using gallic acid (2.5-10 μ g/ml). The total antioxidant capacity was expressed based on GAE.

DPPH radical scavenging activity

Free radical scavenging activity was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The extract (1 ml) was added to 3.0 ml of 0.004% methanolic solution of DPPH. After 30 minutes the absorbance (A) at 517 nm was measured. The per cent inhibition was calculated as $[1 - A_{extract} / A_{blank}] \times 100$. The relationship between per cent inhibition and sample concentration was plotted to determine the IC₅₀ value [16].

β -Carotene bleaching activity

The determination of the antioxidant activity as the ability to delay the bleaching of β -carotene in a water/linoleic acid emulsion was performed according to Nsimba et al [17]. To prepare the β carotene emulsion, 0.2 ml of β -carotene solution (0.2 mg/ml in chloroform) was transferred to a roundbottom flask containing linoleic acid (20 µl) and Tween 20 (200 µl). The mixture was evaporated at 40°C for 10 minutes to remove the solvent and distilled water (100 ml) was immediately added. The β carotene emulsion (5.0 ml) was transferred to a test tube containing the test sample (0.2 ml). The mixture was shaken and placed in a water bath at 50°C for 2 hours before its absorbance was measured at 470 nm.

The percent inhibition was calculated as $[1 - (A_0 - A_t)/(A_0^0 - A_0^t)] \times 100)$, where A_0 and A_0^0 are the absorbance values measured at initial time of the incubation for sample and control respectively, and A_t and A_0^t are the absorbance values of sample and control respectively at t minutes. The relation between per cent inhibition of β -carotene oxidation and sample concentration was plotted to determine the IC₅₀ value.

Results and Discussion

Total phenolic content

Thermal processing can cause both positive and negative changes in total phenolic content of GCP and its ingredients as shown in Table 1. Heat-processed chilli and garlic have slightly lower phenolic content than their fresh counterparts while heat-processed onion has an increase of total phenolic content. On the one hand, high temperature involved in the processing might have assisted in the decomposition of complex phenolic compounds thus releasing the free phenolics leading to an increase in total phenolic content [8, 18]. On the other hand, many other studies have shown that heating process has both negative and positive effects on total phenolic content of plant materials including fruits and vegetables, depending on the type of raw materials and the groups of compounds present [13-14, 18-21]. Li et al. [22] reported that heat treatment caused a reduction in total phenolic content during the processing of purple wheat bran. Zhang and Hamauzu [13] also reported that antioxidant components in broccoli are significantly lost during cooking.

From Table 1, the total phenolic content in degassed GCP, which has also been subjected to heat treatment, is not lowered compared to that in unprocessed GCP. Apparently, oxygen removal reduces the oxidation process and inactivates enzymatic reactions in GCP. Oms-Oliu et al. [12] have also found that a low oxygen level is ideal for maintaining vitamin C and phenolic content during storage.

As in the case of onion, the total phenolic content in sterilised GCP is significantly higher than untreated GCP, which is also probably due to the same reason, i.e. the release of free phenolic compounds from some more complex molecules upon sterilisation.

Sample		Total phenolic content (mg GAE/ mg DPM)	Total antioxidant capacity (mg GAE/ mg DPM)
Chilli	Fresh	3.42 <u>+</u> 0.08	14.91 <u>+</u> 0.75
	Heat-processed	2.69 <u>+</u> 0.02	14.22 <u>+</u> 0.60
Red onion	Fresh	10.59 <u>+</u> 0.34	10.98 <u>+</u> 0.60
	Heat-processed	12.82 <u>+</u> 0.46	11.44 <u>+</u> 0.30
Garlic	Fresh	2.00 <u>+</u> 0.13	6.65 <u>+</u> 0.89
	Heat-processed	1.82 <u>+</u> 0.07	7.14 <u>+</u> 0.36
Unprocessed GCP		5.93 <u>+</u> 0.26	18.2 <u>2+</u> 0.49
Degassed GCP		5.97 <u>+</u> 0.38	24.89 <u>+</u> 0.64
Sterilised GCP		7.88 <u>+</u> 0.38	18.78 <u>+</u> 0.55

Table 1. Total phenolic content and total antioxidant capacity of GCP and its ingradients

Note: $GAE = gallic acid equivalent; DPM = dry plant material; Results are reported with <math>\pm$ SD.
Antioxidant properties

The antioxidant properties were investigated using in vitro methods. The total antioxidant capacity is shown in Table 1. Fresh and heat-processed chilli apparently has a higher level of total antioxidant capacity than that of red onion or garlic. An earlier study also reported that chilli has a strong antioxidant activity [21]. Banerjee et al. [16] reported that the phenolics of green pepper (*Piper nigrum* L.) has higher DPPH radical scavenging capacity than the acetone extract of nutmeg mace (*Myristica fragrans*).

Heat processing is observed in this study to have pronounced effects on the three ingredients of GCP. In addition, removal of chilli skin prior to heat processing may result in the loss of some important compounds in chilli such as vitamin C, tocopherol, β -carotene and alkaloids, with consequent reduction of antioxidant activities of heat-processed chilli compared to the fresh one. However, they are enhanced in heat-processed red onion and garlic (Tables 1-2). In this case, it is most probable that heat processing has released such compounds as free aglycones or Maillard products that can reduce Mo(VI) to Mo(V) and also react as an electron donor or transfer a hydrogen atom to the DPPH radical, thus increasing the antioxidant properties.

From Tables 1-2, the antioxidant capacity and DPPH radical scavenging activity of both types of processed GCP apparently increase compared to unprocessed GCP. Earlier studies showed that sterilisation causes changes in the texture, colour and flavour of GCP [8, 23]. Sterilisation also produces a bitter taste in GCP. The main bitter compounds, identified as catechins, were shown to increase upon sterilisation [8]. This result supports the hypothesis that sterilisation releases some phenolic compounds and these products increase the antioxidant properties. Randhir et al. [18] found that thermal processing of sprouts and seedlings of wheat, buckwheat, corn and oat causes changes in their health-relevant functionality and suggested that these changes are due to modifications in the total phenolic content leading to a higher content with consequent increase in scavenging-linked antioxidant activity. However, the antioxidant capacity of sterilised GCP is seen to be lower than that of degassed GCP, which may be accounted for by the postulate that certain compounds with reducing property might have been adversely affected by the sterilisation process.

In attempting to correlate the total phenolic content with antioxidant properties, one can see that the correlation is rather poor. Among the three ingredients, fresh and heat-processed chilli is second in total phenolic content, yet it is strongest in both antioxidant properties. Red onion has the highest total phenolic content although it shows only medium levels in both antioxidant properties (Tables 1-2). In this regard, it is clear that the antioxidant properties may not only come from a phenolic group [6]; substances such as capsaicin and sulfur compounds may also be associated with the antioxidant properties of chilli and garlic [24]. Conversely, it is well known that not every phenolic substance is a good antioxidant. This may be evident from a result in Table 1, in which sterilised GCP, though highest in total phenolic content among the three GCPs, is not highest in total antioxidant capacity.

The β -carotene bleaching method is based on the loss of the yellow colour of β -carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β -carotene bleaching can be slowed down in the presence of an antioxidant. However, it can be seen in this experiment that the different processing steps did not seem to affect the antioxidant property of GCP as

determined by the β -carotene bleaching method. The IC₅₀ remains relatively constant as shown in Table 2. Conforti et al. [25] reported that a high level of phenolic content and an acyclic diterpene alcohol (phytol) in green pepper fruit are responsible for the inhibition of lipid peroxidation, although this effect takes place only at the ripening stage of the fruit. Furthermore, although the correlation between total phenolic content and antioxidant activity by the DPPH test was found for small green and red pepper extracts, no correlation was found for β -carotene bleaching test and bovine brain peroxidation assay.

S	ample	IC_{50} (mg/ml)			
		DPPH method	β-Carotene method		
Chilli	Fresh	2.12 <u>+</u> 0.02	-		
	Heat-processed	2.33 <u>+</u> 0.11	-		
Red onion	Fresh	4.00 <u>+</u> 0.34	-		
	Heat-processed	3.75 <u>+</u> 0.28	-		
Garlic	arlic Fresh		-		
	Heat-processed	17.63 <u>+</u> 0.93	-		
Unprocessed GCP		3.48 <u>+</u> 0.15	1.02 <u>+</u> 0.02		
Dega	ussed GCP	3.07 <u>+</u> 0.12	1.10 <u>+</u> 0.05		
Sterilised GCP		2.67 <u>+</u> 0.08	1.07 <u>+</u> 0.03		
Standard	BHT	8.31 <u>+</u> 0.18*	0.54 <u>+</u> 0.01*		
quercetin		0.31 <u>+</u> 0.01*	4.37 <u>+</u> 0.06*		

Table 2. DPPH radical scavenging and β -carotene bleaching activities of GCP and its ingredients

Note: - = Not investigated; * = in μ g/ml;

Results are reported with \pm SD.

Conclusions

The total phenolic content and antioxidant properties of green chilli paste and its ingredients are observed to be affected on being processed. The overall effect of processing, though not apparently extensive or outstanding, nevertheless seems to be somewhat beneficial to both of the green chilli paste products (i.e. degassed and sterilised) compared to the unprocessed product.

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Full Paper

Assessment of lubricating oil degradation in small motorcycle engine fueled with gasohol

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Abstract: Assessment of the degradation of lubricating oil was performed on the lubricants which had been used in a small motorcycle engine fueled with gasohol in comparison with the lubricants from gasoline-run engine. The lubricant properties examined in the assessment were lubricating capacity, viscosity and stability to oxidation. Lubricating capacity was evaluated by accelerated wear test on the Timken tester. Lubricating oils from gasohol-run engine appeared to produce about 10% greater wear than that made in oils from gasoline-run engine. There was no significant difference between the effect of gasohol and gasoline on the viscosity of the used lubricating oils. Moreover, no oxidation products in any used oil samples could be detected.

Keywords: engine wear, lubrication, gasohol, lubricating oil degradation

Introduction

Advanced lubricants have been formulated to reduce wear and friction of the tribological components of an engine by interposing a film of material between rubbing surfaces. Apart from lubrication, an engine oil also cleans and cools the engine, inhibits corrosion and improves sealing. The lubricant consists mainly of a base oil and chemical additives, which are blended according to its grade and specific duty. When adjusted optimally to its task, wear and maintenance requirements can be reduced leading to a greater saving and less problem with air pollution. During use, however, lubricating oil properties tend to degrade. Engine lubricant is degraded by heat, oxidation, and possible contamination from fuel and other materials. Any unburned fuel may leak from the engine combustion chamber into the lubricating oil sump, possibly via blow-by flow mechanism [1]. Contamination can degrade the performance of engine lubricants, thus affecting engine parts and properties. The extent of degradation depends on the severity of engine conditions and length of use. Used lubricating oil

contaminated by dirt, fuel, water, metals, products of combustion, and other materials is therefore replaced on a regular basis in all operating equipment. Degradation of lubricating oils after various degrees of their use can be observed and studied by a number of analytical methods such as Fourier transform infrared (FTIR) spectroscopy [2-5], atomic absorption spectroscopy [5-6], and measurement of change in viscosity and acidity [7-10]. The information obtained is often used to predict useful lubricant service life.

Ethanol is a renewable fuel derived from domestic feedstock. It is considered as an important alternative fuel and extender for engines. In Thailand, gasohol, a blended mixture of 10% ethanol and 90% gasoline, has been used extensively as transport fuel for the past decade. Relatively few studies on the degradation of tribological performance of a lubricant in small gasohol-fueled engines have been reported in the literature. The aim of this study is therefore to investigate the lubricating oil degradation in a small motorcycle engine fueled with gasohol for a prolonged period. This is done in comparison with oil from the same type of engine which is run on regular gasoline by measuring changes in the lubricant's properties, viz. lubricating capacity, viscosity and stability to oxidation.

Materials and Methods

Lubricants

Two different types of engine oil, i.e. mineral- and synthetic-based oils, whose properties are shown in Table 1, were used in this study. Both oils are commercially available from local distributors.

Small engines

Four motorcycles of similar built and age were used to run four test combinations of fuels and lubricants in parallel: (i) gasoline and mineral-based oil; (ii) gasoline and synthetic-based oil; (iii) gasohol and mineral-based oil; and (iv) gasohol and synthetic-based oil. Their engines were of a Honda, fourstroke of the *Dream* series. It is a lightweight, rugged, simple-to-maintain, high-performance engine. Its specifications are given in Table 2. Prior to the test, all engines were flushed to make sure that no other oil was present to contaminate the test oil. Then they were filled with the new lubricant. Road tests were performed on these vehicles with both mineral- and synthetic-based oils for up to 3000 km, a recommended oil-change mileage. This amounted to the test duration of 4-6 months. At the mileage of 1500 km and 3000 km, samples of the lubricating oil from each engine were collected from the crankcase sump with a syringe for later analysis (with replenishment of fresh oil at 1500 km). After completion of the test, the engine components were inspected visually to assess the degree of wear and tear.

Wear machine

All samples of fresh and used lubricating oils from the road test were subjected to bench wear test. The accelerated wear test was carried out using a Timken universal wear and friction testing machine. Schematic diagrams of the machine and testing arrangement of the specimen are shown in Figure 1. The machine comprises a motor-driven, rotating axle which provides a relative motion to the loaded specimen, fully immersed in the lubricant to be tested. A test specimen was used to imitate solid metallic contact and simulate the lubricity effect of the oil at the contact. The load on the test specimen can be adjusted. The bench test conditions used were: load of 33 N, rotating speed of 500 rpm, and

temperature of 25°C in accordance with ASTM standard [11]. The wear was generated under lubricated condition on the cylindrical steel specimen subjected to the rolling contact. The specimen was weighed using an electronic balance at a fixed time interval and wear scar area on the test specimen was monitored. Each test for each oil sample was done in triplicate and high repeatability (> 90%) was obtained.

Property	Mineral-based oil	Synthetic-based oil
Grade	SAE 20W-50	SAE 5W-40
Kinematic viscosity at 100°C (cSt)	18.5	14.2
Viscosity index	128	176
Cold cranking simulator at -25°C (cP)	7800	5410
Flash point (°C)	246	227
Pour point (°C)	-21	-45
Colour	red	yellow

Table 1. Specifications of engine lubricants

Engine	: Honda
Model	: Dream 125
Туре	: 4-stroke, OHC
Combustion	: Direct injection, naturally aspirated
Number of cylinder	:1
Bore	: 52.4 mm
Stroke	: 57.9 mm
Displacement	: 124.9 cc
Compression ratio	: 9.3:1
Ignition system	: CDI
Ignition timing	: 15° BTDC
Cooling system	: air cooled
Sump capacity	: 700 cc

Table 2. Motorcycle engine specifications

Analysis methods

A viscometer was used to measure the viscosity of the tested lubricants according to ASTM standard [12]. Analysis of the lubricants was also performed by FTIR spectroscopy [13]. The method is based on the fact that specific functional groups absorb in unique regions of the infrared spectrum, thus allowing the identification of contaminants and oxidation products. Spectra were acquired on a Bruker FTIR Tensor 27 model. The resolution for the spectral data was 4 nm and the number of scans was set at 200. The wear surfaces generated on the cylindrical specimens subjected to rolling contact under lubricating condition in the Timken tester were examined with an optical microscope. Apparent areas of wear scar were measured.



Figure 1. (a) Timken universal wear machine; (b) Testing arrangement of the specimen

Results and Discussion

The motorcycles completed the road test without any performance problem. After completion of the test, their engines were disassembled and the deposit formations on the cylinder head, piston crown, piston rings, inlet and exhaust valves were examined. Visual inspection of components showed little change compared to their initial conditions. The engine components did not show any sign of wear and the critical components were in good working conditions, although their surfaces were observed to be covered with light deposits. Carbon residuals were also found on the piston ring and ring grove. Exhaust ports and valve stems were coated with thin deposits but they could be easily removed. It should be noted that erratic operation from deposit accumulation was not encountered. The formation of carbon deposits did not seem to affect the overall performance of the engine.

The sliding contact between metal components of any mechanical system is always accompanied by wear, which results in the generation of minute particles of metal [1]. In this study, however, when accelerated wear tests were performed on the Timken tester, wear rate in terms of mass loss was found to be insignificant. Wear scars on the test specimens were observed to be smooth. Their dimensions and areas (shown schematically in Figure 2) are summarised in Table 3. It can be seen that the wear scar area increases with increasing distance travelled for all cases. Mineral-based oil gives larger scar areas than those produced in synthetic oil. For the same type of engine oil, the one from gasohol-run engine appears to produce about 10% larger scar areas than those produced in oil from gasoline-run engine.



Figure 2. Wear scar of test specimen and its measurement

Oil type	Fuel used	Distance travelled (km)	x (mm)	y (mm)	Area (mm ²)
Synthetic	Gasoline	0	3.73	6.57	76.95
		1500	4.69	6.58	96.90
		3000	4.93	8.09	125.23
	Gasohol	0	3.73	6.57	76.95
		1500	4.36	7.87	107.74
		3000	5.33	8.61	132.34
Mineral	Gasoline	0	4.69	7.41	95.16
		1500	4.75	7.58	110.26
		3000	4.97	8.22	128.28
	Gasohol	0	4.69	7.41	95.16
		1500	4.82	7.38	111.69
		3000	5.15	8.20	144.10

 Table 3.
 Average dimensions and areas of wear scar from Timken test

The lubricating property of an engine oil changes with running time due to effects of oxidation, thermal degradation, reaction with sliding surfaces, contamination by engine blow-by, and additive depletion [14]. In this investigation, the effects are shown in terms of kinematic viscosity and viscosity index of the lubricating oils (Figures 3-4). Oil viscosity has an effect on heat generation in bearings, gears, pistons and other engine components due to internal fluid friction. Formation of lubricating films and rate of oil consumption are also affected by viscosity. A proper level of viscosity is desired over a wide range of temperature. The viscosity index is used as a measure of response of an oil to temperature change. As seen from Figure 3, the viscosity of all oil samples decreases with operation time (measured as distance travelled), the viscosity changes being about 20% and 45% at 3000 km mileage for synthetic- and mineral-based oils respectively. This is contrary to the expectation that the viscosity is likely to increase due to loss of lighter fractions from evaporation or oxidation [15]. The corresponding changes in viscosity index (Figure 4) are smaller—about 10% and 20% for synthetic- and mineral-based oils respectively. Within the test conditions under study, however, there is no statistically significant

difference between the effect of gasoline and gasohol on viscosity. Incidentally, when simulated lubricant contamination was carried out by adding gasohol at merely 1% by volume to the lubricant, the viscosity was found to drop by more than 20% for both types of oils.



Figure 3. Change in kinematic viscosity of lubricating oils used in gasoline- and gasohol-fueled engines



Figure 4. Change in viscosity index of lubricating oils used in gasoline- and gasohol-fueled engines

As oil is exposed to oxygen in air at elevated temperature oxidation reactions may take place during operation, which leads to the formation of oxygenated species including carboxylic acids, thus increasing the acidity of the oil and contributing to corrosion. IR spectroscopy may be used to identify the presence of these oxidation products. This is characterised by changes in the O-H, C=O and C-O spectral regions at 3600-2500, 1900-1600 and 1500-900 cm⁻¹ respectively [2, 16]. Figure 5 shows the



Figure 5. FTIR spectra of fresh and used lubricating oils: (a) synthetic; (b) mineral

spectra of fresh and used oils at the end of the road test. Very small changes are found in these three spectral regions, suggesting negligible formation of oxidation compounds in any of the oil samples. Confirmation of this finding is possible by employing such methods as cyclic fast neutron activation analysis and nuclear magnetic resonance spectroscopy [17].

Conclusions

From this investigation, there seems to be no significant difference between the effect of gasohol and that of gasoline on the lubricating oil performance and degradation in a small motorcycle engine under normal engine operation.

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Short Review

Rheological study of chitosan and its blends: An overview

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Abstract: Chitosan, a modified natural carbohydrate polymer derived from carapaces of crabs and shrimps, has received a great deal of attention for its applications in diverse fields owing to its biodegradability, biocompatibility, non-toxicity and anti-bacterial property. The wide-ranging applications involve a broad spectrum of characterisation techniques and rheology represents one technique of growing importance in this field. This paper is an attempt to review the latest development in the rheology of chitosan, either on its own or associated with other materials, including the parameters that strongly influence its rheological behaviour such as concentration, pH and temperature.

Keywords: chitosan, chitosan blends, chitosan gels, cross-linked chitosan, rheology

Introduction

Chitosan, or $(1 \rightarrow 4)$ -2-amino-2–deoxy- β -D–glucan (Figure 1), is the deacetylated derivative of chitin [1-2], the most abundant natural polymer on earth after cellulose [3-5] and obtained from crustaceans [6-7] such as shrimps, squids and crabs. Chitosan is readily prepared from chitin [8-9].



Figure 1. Chemical structure of chitosan

Chitosan can be dissolved in aqueous solutions of organic acids such as formic acid and acetic acid at a pH below 6.2 due to protonation of the free amino groups present in its molecular structure [10]. Chitosan hardly dissolves in pure acetic acid. In general, the properties of chitosan solutions depend on several parameters including degree of deacetylation [11], pH [12-13], ionic strength [12], concentration [14], temperature [15], acid concentration [16], type of acid [17], and distribution of the acetyl groups along the chain [18-19]. For example, Matsumoto et al. [11] demonstrated that the solutions of chitosan with 62% and 96% degree of deacetylation (DD) are homogeneous while that with 76% DD is heterogeneous. Chen et al. [12] reported that the viscosity of chitosan solutions is significantly affected by ionic strength, pH and counter ions. Tsaih and Chen [13] revealed that viscosities of solutions of chitosan with the same molecular weight decrease with increasing pH, while viscosities of solutions of chitosan with the same pH increase with increasing molecular weight. A chitosan solution stored at 4°C is found to be relatively stable from a viscosity point of view [20].

Similar to most natural polymers, chitosan has an amphiphilic character which can influence its physical properties in solutions and solid states. This is attributed to the presence of the hydrophilic amino groups and the hydrophobic acetamido groups in its molecular structure. Unlike most polysaccharides, chitosan can carry strong positive charges because it possesses a great number of amino groups, thus endowing this polymer with many useful properties such as the capacity to form composite materials when blended with other polymers [21].

Chitosan shows great promise for a wide variety of uses. Unique properties such as biodegradability [22], biocompatibility [23-24], non-toxicity [25] and anti-bacterial activity [26] are the main driving forces pushing studies on new applications of this polymer. Chitosan has already been used in many fields including wastewater treatment, medicine, food and cosmetics [27-33]. Due to the importance of rheology in various fields of science and technology, this short review tries to present the recent (approximately from 1980 to the present) rheological studies involved with chitosan as well as its blends, although such studies are quite limited in the literature.

Rheological Properties

Rheology is defined as the science of the deformation and flow of matter [34-36]. It investigates the response of materials to an applied stress or strain [37-38]. Rheological properties describe flow characteristics and textural behaviour of substances. The success of a wide range of commercial products and industrial processes depends on meeting specific flow requirements. Rheological behaviour can be generally divided into two types [39]: elastic behaviour, where the material restores its original shape when the external force is removed, and viscous or plastic behaviour (such as in ideal Newtonian liquids), where deformation ceases and the material does not regain its original shape when the applied force is removed. The fluid flow behaviour is summarised in Scheme 1 and Figure 2.



Scheme 1. Fluid flow behaviour



Figure 2. The relationship between (a) apparent viscosity and shear rate for time-independent fluids and (b) apparent viscosity and time for time-dependent fluids

Rheology of Pure Chitosan Solutions and Gels

The rheological behaviour of chitosan depends on the concentration of its solution [40-48]. Kienzle-Sterzer et al. [42] found that the viscosity of concentrated chitosan solutions increases with increasing chitosan concentration and that a shear thinning behaviour is observed at the polymer concentrations above 0.50 g/dL. They also reported the independence and dependence of the zero shear viscosity on the ionic strength and pH of the media respectively.

Wang and Xu [43] reported that the non-Newtonian behaviour of chitosan solutions increases with increasing DD, which can be attributed to the chain-expanding structure and consequent increase in entanglement. Mucha [44] also reported an increase in the shear stress and viscosity of chitosan solutions with increasing chitosan concentration also due to the increase in entanglement among the macromolecular chains.

The rheology of two aqueous systems of chitosan–unmodified chitosan and hydrophobicallymodified chitosan–was studied by Nyström et al [46]. Their results revealed that the linear and nonlinear viscoelasticity are affected by several factors such as pH, temperature, amount of surfactant, and polymer concentration. The HM-chitosan was found to be more influenced by these factors. In addition, the shear thinning behaviour is more pronounced at higher shear rates for both systems. Hwang and Shin [47] noted that the shear rate dependence of viscosity is more remarkable at higher chitosan concentrations.

Martinez et al. [48] studied the influence of some parameters such as temperature, acid type and addition of salt, on the steady-shear rheology of concentrated chitosan solutions. Viscosity and normal stress were observed to decrease with increasing temperature and decreasing chitosan concentration. In addition, chitosan in hydrochloric acid solutions exhibits lower steady-shear viscosity and normal stress than in other acid solutions. Addition of salt was found to be the most effective parameter on the rheology of chitosan solutions, i.e. the presence of salt decreases viscosity due to the interaction between charged chains and small ions in solution.

Bodek [49] studied the rheological properties of microcrystalline chitosan hydrogels prepared by adding a methylcellulose hydrogel to aqueous chitosan dispersions. It was found that, depending on the kind and content of the pharmaceutical substances as well as the interactions between the polymer and the pharmaceutical substances, a pseudo-plastic system can be observed. Anchisi et al. [50] showed that rheological properties of chitosan dispersions are affected by the molecular weight and that all chitosan dispersions show a pseudo-plastic and shear thinning behaviour. It was also found that the presence of glycols leads to decrease in the apparent viscosity compared to that in the corresponding base dispersions.

The formation of chitosan hydrogels in an acetic acid-water-propanediol medium was reported by Montembaulta et al [51]. The gelation at different polymer concentrations, the degree of acetylation (DA) of chitosan, and the composition of the initial solvent were studied by rheometry. It was found that the optimal gelation conditions for cartilage regeneration application are: DA = 40%, ratio of water/alcohol (solvent) = 1:1, and polymer concentration = 1.5%.

The production of pH-induced monolithic hydrogels through uniform neutralisation of slightly acidic chitosan solutions with ammonia generated from enzymatic hydrolysis of urea was analysed via

rheology and reported by Chenite et al [52]. A decrease in gelation time with increase in temperature from 15 to 45°C was pronounced to be a result of the synergistic influence of increased reactant diffusion and increased urease activity. It was also observed that gelation is accelerated as the urea concentration increases up to a certain limit and then a slow decrease of the gelation kinetics was noticed. This study indicates the possibility of using the auto-gelling solutions of chitosan as injectable gels for tissue engineering and drug delivery.

Fernandez et al. [53] reported the rheology of chitosan gels with different (low and medium) molecular weights. Non-Newtonian flow independent of time was observed for the two gels, with pseudo-plastic behaviour for low-molecular-weight gel and plastic behaviour for medium-molecular-weight gel. Mironov et al. [54] reported a decrease in the dynamic viscosity of chitosan solutions in acetic acid during storage due to polymer degradation.

The effect of some parameters such as temperature, concentration, shearing time and storage time on the rheological properties (apparent viscosity and shear stress) of chitosan dissolved in acetic acid was reported by Elhefian et al [55]. The shear-thinning behaviour, which is more pronounced at a lower temperature, was observed for chitosan solutions at all temperatures and concentrations studied. When shearing time was studied, no significant changes in the dynamic viscosity and shear stress of chitosan solutions were observed. In addition, when the period of storage was extended to 3 months, an increase in viscosity was recorded at a constant shear rate, after which a drop in the viscosity was observed.

Rheology of Cross-Linked Chitosan Solutions

Argüelles-Monal et al. [56] reported an apparent yield stress at very low frequencies for chitosan in acetic acid solution and a cross-linking reaction between chitosan and glutaraldehyde, in which the chemical cross-linkage between the aldehydic groups of glutaraldehyde and the amino groups of chitosan was supposed to take place leading to the weak self-associated network of chitosan being gradually replaced by a permanent covalent network. A strong permanent gel was reported to form at higher cross-linking levels.

Moura et al. [57] reported the rheology of solutions of chitosan cross-linked with a natural cross-linker, genipin, at various concentrations. Stronger elastic gels of the cross-linked solutions were obtained under physiological conditions compared to those of the pure component. They also reported that the value of the gelation time obtained from the crossover of G'' (loss modulus) and G' (storage modulus) and that recorded by the Winter-Chambon criterion were in excellent agreement.

Rheology of Solutions and Gels of Chitosan Blends

The rheological characteristics of solutions of chitosan and polyoxyethylene of various molecular weights were reported by Nikolova et al [58]. Pseudo-plastic non-Newtonian behaviour was observed for each of the pure polymer solutions as well as those of their mixtures. It was assumed that the rheological behaviour of the chitosan/polyoxyethylene mixtures is determined by chitosan macromolecules. Khalid et al. [59] reported the rheological properties of a semi-interpenetrating

chitosan–polyethylene oxide network. Their results showed that the elastic properties increase with the semi-interpenetration due to the presence of the polyethylene oxide physical network.

Jiang et al. [60] investigated the phase transition behaviour of water within the chitosan/polyacrylate hydrogels with variation in temperature and frequency using oscillatory shear rheology. The results indicated that the water phase transition which occurs with a decrease in temperature has a significant influence on all the viscoelastic properties measured (shear storage modulus, shear loss modulus and shear loss tangent). The changes of the viscoelastic properties were found to be related to the mobility of water within the gels.

The preparation of thermogelling chitosan/glycerol-phosphate solutions by neutralising highly deacetylated chitosan solutions up to physicological pH (~ 7.2) with β -glycerol phosphate was reported by Chenite et al [61]. Rheological measurements demonstrated that the hydrogel formation by subsequent heating of these solutions is quickly obtained. The study also showed the pH sensitivity of the sol/gel transition temperature and the temperature dependence of the gelling time. Based upon the observations obtained, multiple interactions between chitosan, glycerol phosphate and water were suggested for the molecular mechanism of the gelation.

Salomé Machado et al. [62] reported the preparation and characterisation of collagen/chitosan blends by rheological studies. A decrease of storage modulus, viscous loss modulus and apparent viscosity (as a function of frequency) was observed with the addition of chitosan to collagen. It was also found that collagen/chitosan blends present a more fluid-like viscoelastic behaviour than solid-like one.

The rheological properties of kaolin/chitosan aqueous dispersions were characterised by Bezerril et al [63]. The kaolin/chitosan dispersions show a pseudo-plastic behaviour which increases at lower shear rate. The increase in pseudo-plasticity was related to a higher occurrence of particle-polymer-particle interactions stemming from the adsorption of chitosan macromolecules on the surface of kaolin particles. The rheological behaviour of these dispersions could not be described by a simple power law.

The rheological properties of chitosan/xanthan hydrogels were studied by Martı'nez-Ruvalcaba et al. [64]. Their results showed that chitosan/xanthan hydrogels behave like weak gels. An almost linear increase in the shear modulus was observed with frequency in the range between 0.1-65 s⁻¹. It was also found that other factors such as hydrogel concentration and nature of dispersion have a significant role in the final structure and the final properties of the hydrogels. The viscoelastic properties of chitosan/PVA hydrogel were investigated rheologically by Tang et al [65]. Their results indicated a good mechanical strength of the gel.

Madrigal-Carballo et al. [66] determined the rheological behaviour of lecithin/chitosan vesicles by means of shear stress against shear rate measurements. The results showed that chitosan can promote the transition of planar sheets into closed structures such as vesicles. It was also found that this system suggests a thixotropic behaviour. Chitosan solutions containing glycerol-2-phosphate were prepared and analysed by Kempe et al [67]. Their rheological properties were studied using oscillating rheology for characterising the micro-viscosity of the sol and gel systems. It was found that an amount of 6% glycerol-2-phosphate is necessary to induce gel formation and that neither the gelation process nor the chitosan/glycerol-2-phosphate proportion has an effect on the pH to a significant extent.

Wanchoo et al. [68] investigated the miscibility of chitosan blends with hydrophilic polymers: chitosan/polyvinyl alcohol, chitosan/polyvinylpyrrolidone and chitosan/polyethylene oxide. Rheological

data showed that rheograms of all the blends lie between those of pure components over the entire compositional range, indicating that the miscibility of the blends may occur.

The rheology of chitosan/magnesium aluminum silicate (MAS) composite was reported by Khunawattanakul et al [69]. The addition of MAS to chitosan dispersions leads to an increase in viscosity and a change in flow type of chitosan from Newtonian to pseudo-plastic flow with thixotropic properties. It was suggested that the change in the flow behaviour is due to the electrostatic interaction between chitosan and MAS since they carry a positive and a negative charge respectively. Based on these findings, it was concluded that the chitosan–MAS composite dispersions can be applied as suspending and gelling agents in pharmaceutical products.

The rheological study of chitosan/gelatin composite was investigated by Wang et al [70]. Their results showed that the formation of a complex between chitosan and gelatin is mainly through hydrogen bonds and that there is a close relation between the interactions of these two polymers in solution and the mechanical properties of the films formed.

The preparation of chitosan ferrogels was reported by Herandez et al [71]. The method used was the simultaneous co-precipitation of Fe ions in alkali media and chitosan. The reinforcement of the chitosan ferrogels is achieved in the presence of magnetite nanoparticles, as evidenced by the increase in the viscoelastic modulus measured.

Elhefian et al. [72] studied the rheological properties of solutions of chitosan/agar blends with chitosan as the major component at temperatures from 40 to 55°C. A Newtonian behaviour was demonstrated at all temperatures studied for the blend proportions of 100/0, 90/10, 80/20 and 70/30. However, a shear-thinning behaviour was observed for the 60/40 and 50/50 proportions, which could be attributed to the formation of a good interaction between chitosan and agar. All the blend solutions were found to obey the Arrhenius equation. No significant difference in the apparent viscosity of any blend solution was observed at all shearing times applied with exception of the 50/50 blend where a decrease in the viscosity was observed with increasing shearing time. Different behaviours were observed for the blend solutions when the period of storage was extended to three weeks.

Conclusions

Owing to the importance of rheology in several fields and products inclucing chitosan, this brief review has attempted to describe the recent studies on the rheology of solutions of chitosan including some of its cross-linked derivatives and a variety of its blends. It is anticipated that rheology will be an indispensable tool for chitosan research in the future.

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Full Paper

An adaptive optimisation scheme for controlling air flow process with satisfactory transient performance

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Abstract: A non-identifier-based adaptive PI controller is designed using a gradient approach to improve the performance of a control system when device aging and environmental factors degrade the efficiency of the process. The design approach is based on the model reference adaptive control technique. The controller drives the difference (error) between the process response and desired model output to zero asymptotically at a rate constrained by the desired characteristics of the model. The tuning rules are designed and justified for a non-linear process with dominant dynamics of second order. The advantage of this method for tracking and regulation compared to adaptive MIT control was validated in real time by conducting experiments on a laboratory air flow control system using the dSPACE interface in the SIMULINK software. The experimental results show that the process with adaptive PI controller has better dynamic performance and robustness than that with traditional adaptive MIT controller.

Keywords: non-linear system, non-identifier-based adaptive control, model reference adaptive control (MRAC), adaptive MIT (AMIT) control, adaptive PI (API) control

Introduction

Traditional non-adaptive controllers are generally "good enough" for most industrial process control applications. The ubiquitous proportional-integral derivative (PID) controller, or PID loop, is especially cheap and easy to implement. The simplicity of the PID controller also makes it fairly easy to understand and easy to diagnose [1]. A setpoint dependent or non-linear process can be particularly

difficult to control with a fixed parameter controller since it reacts differently to the efforts of the controller depending on the current value of the setpoint. To improve the control performance, Li et al. [2] have applied the traditional PID, fuzzy PID, neural network PID, pole assignment method, optimal control and adaptive control methods to control non-linear systems. Under some specified conditions, these strategies prove to be effective. However, the above-mentioned control methods are within the domain of model-based control, built upon the system's mathematical model [3-5].

As most physical systems behave in a non-linear fashion, there exists a strong incentive to develop non-linear controller design methods. The usual approach to control non-linear systems is to linearise about an operating point the non-linear dynamics and apply proven linear control design approaches. The linearised model is then verified and validated by exhaustive computer simulations with the linear controller over a variety of initial conditions and disturbances. Such an approach is practical for only a small range of operating conditions. Hence, to control non-linear systems, adaptive controllers are designed. An adaptive controller adapts not only its output, but also its underlying control strategy, providing adaptation mechanisms (adaptive laws) that adjust a controller for a controlled system (plant) with parametric, structural or environmental uncertainties, to achieve a desired system performance [6-7]. It can tune its own parameters or otherwise modify its own control law so as to accommodate fundamental changes in the behaviour of the process. Thus the adaptive controller can significantly improve the system behaviour [6, 8].

Although adaptive controllers improve responses of the non-linear systems and systems with variable parameters, they are not yet used very often. The obvious reason is their complexity [9]. Considering the limitation of the above-mentioned control strategies, a model reference adaptive control (MRAC) has been developed and implemented to control a non-linear system. The idea of the MRAC is based on forcing the plant to follow the reference model, i.e. the adaptive controller has to decrease the error vector between the reference model and plant to zero. This method of MRAC has been implemented in the feedback loop to improve the performance of processes by many researchers [e.g. 10-11]. In all this work the gradient method of adaptation technique based on the minimisation of a chosen loss function (J) is applied. The MIT rule is the original approach to MRAC. The name MIT is derived from the fact that the rule was first developed at the instrumentation laboratory at Massachusetts Institute of Technology (MIT). Since MIT rule is used here for the adaptation of the controller parameters, it can be called as Adaptive MIT (AMIT) controller. In the present work, an auto-tuning of the proportional integral (PI) controller using MRAC concept is designed and implemented for a non-linear air flow process using the dSPACE Real Time Interface (RTI) card DS1104 (DS1104 – Digital Signal Processor used in dSPACE card). The DS1104 board of dSPACE performs the real-time control application, which is designed by SIMULINK and transferred to the board through Real-Time Workshop. The qualitative and quantitative improvement in the performance of the proposed controller to the traditional adaptive MIT (AMIT) controller is examined and the behaviour of this scheme is analysed.

However, a limitation of this gradient method of adaptation technique is that it is unsuitable for a system that exhibits fast dynamics because the period between the consecutive parameter updates has to be sufficiently long to ensure that all the system dynamics have enough time to contribute, directly or indirectly, to the cost function. The rest of the paper is organised as the following: the non-linear air flow control system available in our lab is first described, followed by the section that deals with adaptive control algorithm. The real time implementation along with the results are presented in the last section before the concluding remarks.

Laboratory Air Flow Control System

The process considered in this paper is a simple laboratory air flow process. This process is non-linear but can show an acceptable range of linearisation. Air flow process can be modelled as a second-order process whose dynamics depends on operating conditions. The piping and instrumentation diagram (Figure 1) depicts the air flow process and its associated control system. The controlled variable (air flow rate) through the process line is measured by the electronic differential pressure flow transmitter (EDPFT). The sensor output is the feedback signal for closed-loop control via DS1104 dSPACE. The controller consists of the hardware of dSPACE DS1104 board and the software for the implementation of adaptive PI (API) control algorithm. The control algorithm runs in the DS1104 board and the real system data can be monitored by the control desk software. The connection scheme of air flow process is given in Figure 2a. Figure 2b provides the justification for treating the air flow process as a non-linear process.



Figure 1. Piping and instrumentation diagram of laboratory air flow control system

(V1 to V4 - Manifold valves; MV-1 to MV-3 - Manual control valves; PS - Power Supply; M1 and M2 - Manometer connections; mA - Milliammeter; DH - De humidifier; I/P – Current-to-pressure converter; AFR - Air filter regulator; PCV -Pneumatic control valve; PRG - Pressure gauge; G-2 - Galvanised pipe for cold air flow; EDPFT – Electronic differential pressure flow transmitter)



Figure 2(a). Connection scheme of air flow process (D/A - Digital to Analog Convertor; A/D - Analog to Digital Convertor; I/V - Current to Voltage Convertor; V/I - Voltage to Current Convertor; I/P - Current to Pressure Convertor; FCE - Final Control Element)



Adaptive Control Algorithm

Most advanced control techniques for designing control systems are based on a good understanding of the process and its environment. If quantitative knowledge of the process is not available then the situation is usually called a "black-box" problem. In many cases the operator may have some knowledge of the process but is not sure whether the knowledge is accurate or not. This is usually called a "grey-box" problem. If quantitative knowledge of the process is available, a "whitebox" model are to be dealt with.

Non-identifier-based adaptive control (NIAC)

A trade-off between the persistent excitation of signals for correct identification and steady system response for control performance exists. Non-identifier-based adaptive PI controller avoids this fundamental problem by not using any identification mechanism in the system [12]. The controller is defined to possess knowledge about the order and minimum relative degree of the process. The algorithm used in the controller updates its parameters based on the sole objective, viz. minimisation of the loss function.

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The structure of the control system with non-identifier-based adaptive PI controller is shown in Figure 3 [2]. This control system consists of a reference model, an adjustment mechanism and a controller. The reference model describes the desired input/output dynamics of the closed loop. The controller derives the control signal (U) so that the plant's closed-loop characteristics from the command signal U_C to the plant output (Y) is the same as the dynamics of the reference model. The convergence of the modelling error to zero for any given U_C is assured when Y exactly follows the output of the model (Y_M).



Figure 3. Block schematic diagram of the system with adaptive control $(U_{\rm C}$ - command signal; U - control signal; e - modelling error; Y - process output; $Y_{\rm M}$ - model output)

The modelling error *e* is given by equation (1):

$$e = Y - Y_M \tag{1}$$

The controller parameters are adjusted with the loss function $J(\theta)$:

$$J(\theta) = \frac{1}{2}e^2 \tag{2}$$

To minimise *J*, the parameters can be changed in the direction of negative gradient of *J*. The rate of change of controller parameters (θ) with respect to time is defined by equation (3) where the adaptation gain is defined by γ :

$$\frac{d\theta}{dt} = -\gamma \frac{dJ}{d\theta} = -\gamma e \frac{\partial e}{\partial \theta}$$
(3)

The following parameter adjustment mechanism, called MIT algorithm [6] and represented as in equation (4), is used to control the laboratory air flow control system:

$$\frac{d\theta}{dt} = -\gamma e \frac{\partial e}{\partial \theta} \tag{4}$$

Adaptive MIT (AMIT) algorithm

Although the adaptive control can actually deal with black-, grey- and white-box problems, it is more suitable for dealing with the grey-box one, since there is no need to apply a "no model" control method when a process is clear and it is not a good idea to attack a black-box problem without making the effort to understand the process. Based on a priori knowledge, the process is modelled as second order. The transfer function of the laboratory air flow process after linearisation can be represented by equation (5) as a function of Laplace transform (operator *s*, complex frequency variable) [13]:

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$$\frac{Y}{U} = \frac{K}{s^2 + a_1 s + a_2} \tag{5}$$

where *K*, a_1 and a_2 are positive and are the process parameters. The AMIT control law is given by $U = \theta_1 U_C - \theta_2 Y$ (6)

The closed-loop transfer function related to the output and input with the AMIT controller in the loop is given by equation (7) [14]:

$$\frac{Y}{U_C} = \frac{K\theta_1}{s^2 + a_1s + (a_2 + K\theta_2)}$$
(7)

$$Y = \frac{K\theta_1}{s^2 + a_1 s + (a_2 + K\theta_2)} U_C$$
(8)

where $U_{\rm C}$ is the command signal (reference input). The controller parameters (θ_1, θ_2) are updated by the adaptation mechanism such that the process output follows the model output (equation (9)):

$$\frac{Y_M}{U_C} = \frac{K_M}{s^2 + A_1 s + A_2}$$
(9)

where K_M , A_1 and A_2 are the reference model parameters. This model is introduced to match the structure of equation (8) and also has the same rise time and settling time as that of the reference model of adaptive PI controller [15]. The controller parameters are to be chosen as in equations (10) and (11) so that the input-output relations of the system and the model are the same. This is called perfect model following.

$$\theta_1 = \frac{K_M}{K} \tag{10}$$

$$\theta_2 = \frac{A_1 s + A_2 - a_1 s - a_2}{K} \tag{11}$$

To apply the AMIT controller, the sensitivity derivatives are obtained by calculating the partial derivatives of modelling error with respect to the controller parameters θ_1 and θ_2 . The process parameters K, a_1 and a_2 are not known. An approximation based on the observation : $s^2 + a_1s + (a_2 + K\theta_2) = s^2 + A_1s + A_2$ is applied for perfect model following. Then,

$$\frac{\partial e}{\partial \theta_1} = \left(\frac{K}{s^2 + A_1 s + A_2}\right) U_c \tag{12}$$

$$\frac{\partial e}{\partial \theta_2} = -Y \left(\frac{K}{s^2 + A_1 s + A_2} \right)$$
(13)

Based on equations (12) and (13) the following equations are obtained for updating the controller parameters θ_1 and θ_2 :

$$\theta_1 = -\frac{\gamma'}{s} e\left(\frac{1}{s^2 + A_1 s + A_2}\right) U_C \tag{14}$$

$$\theta_2 = \frac{\gamma'}{s} e\left(\frac{1}{s^2 + A_1 s + A_2}\right) Y \tag{15}$$

where $\gamma' = \gamma K$. By varying γ , the tracking speed and thus the controller parameter convergence rate are varied.

Structure of AMIT reference model

The numerical values for the second-order reference model for equation (9) considered in this work are given below:

$$\frac{Y_M}{U_C} = \frac{K_M}{s^2 + A_1 s + A_2} = \frac{\omega_n^2}{s^2 + 2\delta\omega_n + \omega_n^2} = \frac{2.069}{s^2 + 1.41556s + 2.069}$$

The reference model for AMIT control is chosen with a damping ratio (δ) of 0.7 and natural frequency (ω_n) of 1.4 to match with the dynamics of the reference model of adaption PI control.

Adaptive PI (API) control

It is common that most of the industrial and mechatronic control systems are based on PI and PID controllers [1-2]. Even a slight modification in the design of PI controller can lead to large improvement for the industries. PI controllers are simple and easy to implement; hence, one based on MRAC using a gradient approach is designed and implemented in this work. The PI algorithm used in the controller is given by equation (16):

$$U = K_{P}(U_{C} - Y) + \frac{K_{I}}{s}(U_{C} - Y)$$
(16)

where K_P and K_I are the proportional and integral gains of the controller. Based on a priori knowledge the process considered for control is represented by equation (5). The closed-loop transfer function with PI controller is given by

$$\frac{Y}{U_{c}} = \frac{KK_{P}s + KK_{I}}{s^{3} + a_{1}s^{2} + (a_{2} + KK_{P})s + KK_{I}}$$
(17)

The reference model to follow the dynamics, introduced to match the structure of equation (17), is given by equation (18):

$$\frac{Y_{M}}{U_{C}} = \frac{\alpha s + \beta}{s^{3} + A_{1}s^{2} + A_{2}s + A_{3}}$$
(18)

where α, β, A_1, A_2 and A_3 are the reference model parameters. For perfect model matching,

 s^3

$$+ a_1 s^2 + (a_2 + KK_P) s + KK_I$$

= $s^3 + A_1 s^2 + A_2 s + A_3$ (19)

Then, two approximate parameter-adaptation laws are derived by replacing θ in equation (4) with K_P and K_I . This results in equation (20) and (21) respectively:

$$K_{P} = -\frac{\gamma'}{s} e \left(\frac{s}{s^{3} + A_{1}s^{2} + A_{2}s + A_{3}} \right) (U_{C} - Y)$$
(20)

$$K_{I} = -\frac{\gamma'}{s} e \left(\frac{1}{s^{3} + A_{1}s^{2} + A_{2}s + A_{3}} \right) (U_{C} - Y)$$
(21)

where the original adaptation gain γ is replaced by γ' (= γK). Thus, the controller parameters are manipulated by the adaptation mechanism to match the response of the process with the dynamics of the reference model. The performances of the designed AMIT and API controllers are observed by implementing them on a non-linear process in real time.

Structure of the API reference model

Based on equation (18), the reference model for the adaptive PI controller is given below. It has a damping factor of 0.7 and a natural frequency of 1. The remaining time constant is selected around 0.3 so that the dynamics is not much affected.

$$\frac{Y_M}{U_C} = \frac{\alpha s + \beta}{s^3 + A_1 s^2 + A_2 s + A_3} = \frac{10s + 10}{s^3 + 11.4s^2 + 15s + 10}$$

Real-Time Experimentation

Experimentation work was carried out to demonstrate the tracking capability of the proposed API and AMIT controllers using dSPACE. This system has the advantage of high computing power and the possibility to download models realised in MATLAB/SIMULINK to the real-time hardware in an automated way. In Figure 4(a) the air flow control system available in our laboratory is displayed. The hardware set-up to control the chosen process using dSPACE is presented in Figure 4(b).



Figure 4(a). Laboratory air flow control system



Figure 4(b). Experimental set-up for real-time implementation of adaptive controllers

A step change in the feed flow rate of 150 litres per minute (lpm) was introduced (from 1025 to 1175 lpm) at 20 seconds and the responses of the process with API and AMIT controllers are presented in Figures 5(a) and 5(b) respectively. The adaptation gain (γ) was set as 5 and 10 for AMIT controller

and API controller. Figure 5(a) shows the tracking response of the process (*Y*) towards the model output (*Y*_M). The process output settled 9.63 seconds (γ =5) and 9.5 seconds (γ =10) after the application of servo disturbance. Figure 5(b) shows the tracking response of the process (*Y*) with AMIT controller for the same servo change. The process settled to the desired flow rate of 1175 lpm after 67 seconds of the applied input change with controller's adaptation gain of 10. With a decrease in the adaptation gain (γ =5), the process settled after 100 seconds for the same operating range. For the operating range of 1025 lpm the process settled with adaptation gain 10, but did not settle with adaptation gain 5. These responses reveal the need for proper selection of adaptation gain. The speed of response of the process with AMIT controller.



Various performance criteria of the system for the flow range of 1025 to 1175 lpm were observed and tabulated (Table 1). The speed of response of the process with API controller was 10 times more than that of the process with AMIT controller with the same adaptation gain (γ =5). The modelling error variation and the overshoot/undershoot of the process were 40% more with AMIT controller when the command signal changed from 1025 to 1175 lpm. In the decreasing range (1175-1025 lpm) there was no such significant change in the overshoot and undershoot.

Flow rate (1025 – 1175 lpm)	Adaptation gain	Increasing (1025-1	g flow rate 175 lpm)	Decreasing flow rate (1175-1025 lpm)		
		AMIT	API	AMIT	API	
$T_{S}(sec)$	5	100.00	9.6300	-	10.7000	
	10	67.370	9.5000	53.3800	10.2000	
os / us (lpm)	5	367.8200	235.4700	360.2500	337.5000	
	10	313.0000	232.4700	358.2500	337.0000	
$\Delta e (lpm)$	5	- 0.0700	-0.0300	0.0576	0.0300	
	10	-0.0650	-0.0295	0.0557	0.0295	
Δmv (volt)	5	0.0642	0.0505	0.0643	0.0527	
	10	0.0624	0.0500	0.0625	0.0524	
$\Delta \theta_1 / \Delta K_p$	5	0.0001	0.3652	0.0001	0.0001	
_•1' <i>p</i>	10	0.0001	0.3650	0.0001	0.0001	
$\Delta \theta_2 /$	5	0.0148	0.5308	0.0136	0.0136	
ΔK_{I}	10	0.0149	0.7310	0.0138	0.0134	

Table 1. Performance criteria comparison for flow range of 1025-1175 lpm

Note: Δmv - change in manipulated variable; T_s - settling time; os/us - overshoot/undershoot

The servo and regulatory response of the process in the flow range of 575-725 lpm with AMIT controller (adaptation gain 5 and 10) is shown in Figures 6(a) and 6(b) respectively. The load disturbance was provided by manipulating the position of the manual valve MV-1 (Figure 1). The reference input signal was a square wave with amplitude of 150 lpm. When the air flow rate was 575 lpm, the manual valve MV-1 was opened at 90 seconds to bypass more air, thus disturbing the process flow rate (Figures 6(a)). Once the disturbance was rejected the MV-1 was brought back to its original position. At 725 lpm the regulatory disturbance was applied by opening the manual valve MV-1 at 190 seconds (Figures 6(a)).



Figure 6(a). Servo and regulatory response of the process with AMIT controller (γ =5) ----- Response of the model ------ Response of the process



Figure 6(b). Servo and regulatory response of the process with AMIT controller (γ=10) ----- Response of the model ----- Response of the process

Initially the flow rate was at 725 lpm (Figure 6(a)). Then at 54 seconds the flow rate was changed to 575 lpm. The servo and regulatory response of the process presents improvement in tracking the set point and rejection of disturbance by increasing the adaptation gain from 5 to 10 (Figure (6(b)). For this decrease in flow rate the AMIT controller took action such that the adaptation of the controller parameter, θ 1, decreased and θ 2 increased (Figures 7(a) and 7(b)) to track the reference signal. To reject the regulatory disturbance, MRAC took action and the process was brought back to its nominal operating condition as shown in Figure 6(a) and 6(b).







Figure 7(b). Adaptation of AMIT controller parameters (γ =10)

The response of the controlled system with API controller for adaptation gain of 5 and 10 is presented in Figures 8(a) and 8(b) respectively. The process was disturbed suddenly by rotating the manual valve MV-1 half turn counterclockwise at 105 seconds when the flow rate was 725 lpm and at 160 seconds when the flow range was 575 lpm (Figure 8(a)).



Figure 8(a). Servo and regulatory response of the process with API controller (γ =5) ----- Response of the model ----- Response of the process



Figure 8(b). Servo and regulatory response of the process with API controller (γ=10) ----- Response of the model ----- Response of the process

The adaptation of the controller parameters for servo and regulatory changes are displayed in Figures 9(a) and 9(b). At the instant the servo changes or the regulatory disturbs the process, the adaptation of the controller parameters starts. After the flow rate settles to the desired value, the adaptation of the controller parameters vanishes and the controller operates with constant parameters. Tables 2 and 3 present the performance of the control systems with proposed AMIT and API controllers for the flow range of 575-725 lpm. Based on the quantitative data from Table 2 one can infer that the parameters such as settling time (T_s), change in manipulated variable (Δmv) and variation in controller parameters ($\Delta \theta_1 / \Delta K_p$, $\Delta \theta_2 / \Delta K_1$) are lower when adaptation gain is set to 10 compared to when it is set to 5 for servo and regulatory changes. Further, the controller parameter also converges at a faster rate with less manipulation in the controller output with γ =10.







Figure 9(b). Adaptation of API controller parameters (γ =10)

Flow rate	Parameter	Adaptation gain	Increasing flow rate (575-725 lpm)		Decreasing flow rate (575-725 lpm)	
			AMIT	API	AMIT	API
575-725	T_{s} (sec)	5	0.107	0.079	0.125	0.107
lpm		10	0.079	0.072	0.075	0.071
(both servo	Δmv (volt)	5	0.057	0.0510	0.054	0.0380
and		10	0.048	0.0060	0.052	0.0180
regulatory)	$\Delta \theta_{\rm l} / \Delta K_{\rm P}$	5	0.184	0.0090	0.135	0.0090
		10	0.167	0.0030	0.146	0.0250
	$\Delta \theta_2 / \Delta K_I$	5	0.140	0.0120	0.150	0.0020
		10	0.136	0.0530	0.175	0.0030

Table 2. Performance comparison of the control systems

Note: Ts - settling time; Amv - change in manipulated variable

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The performance comparison of the chosen process for load disturbance alone is presented in Table 3. For the load disturbance, the overshoot or undershoot (os/us) of the process is greater for AMIT controller and this is also the case for controller with adaptation gain 5. The speed of response of API controller is greater with minimal overshoot and undershoot. The load is applied by changing the position of the manual valve MV-1. It is applied and withdrawn for 575-lpm flow whereas for 725-lpm flow the MV-1 position is changed and is not brought back to its original position. The time integral performance criteria (integral square error (ISE), integral absolute error (IAE) and integral of time-weighted absolute error (ITAE) values) of the NIAC system under study are analysed in Table 4.

Flow rate	Parameter	Adaptation gain	Increas rate (575	sing flow 5-725 lpm)	Decreasing flow rate (575-725 lpm)		
			AMIT	API	AMIT	API	
575-725	T_{S} (sec)	5	179.600	120.7000	469.2000	447.600	
lpm		10	144.200	113.5000	370.8000	279.900	
-	os /	5	16.840	9.5800	13.12/13.28	12.91/11.4	
(only regulatory)	us (lpm)	10	16.600	7.0200	9.41/9.96	7.71/5.64	
regulatory)	Δmv (volt)	5	0.026	0.0260	0.031	0.0260	
		10	0.078	0.0060	0.018	0.0410	
	$\Delta \theta_1 / \Delta K_P$	5	0.038	0.0009	0.062	0.0002	
		10	0.049	0.0030	0.011/0.04	0.0040	
	$\Delta \theta_2 / \Delta K_I$	5	0.040	0.0010	0.060	0.0020	
		10	0.049	0.0010	0.040	0.0010	

Table 3. Performance comparison of the systems with report to load disturbance

Note: T_s - settling time; os/us - overshoot/undershoot; Δmv - change in manipulated variable

Flow rate Adaptatio		ISE		IAE		ITAE	
	gain	AMIT	API	AMIT	API	AMIT	API
Both servo and regulatory (575-725 lpm)	5	0.5368e ⁻³	0.1461e ⁻³	0.02317e ⁻¹	0.1209e ⁻²	9.1780	-0.4788
(<i>373-723</i> ipin)	10	0.7455e ⁻⁵	0.1023e ⁻⁵	0.8634e ⁻²	0.1012e ⁻²	-0.5421	-0.4007
Regulatory alone (575 lpm)	5	613.4000	18.3300	42.8100	24.7700	8990.0000	-3512.0000
	10	2048.0000	93.8900	45.2500	9.6900	9729.0000	-1541.0000
Regulatory alone (725 lpm)	5	40050.0000	25190.0000	200.1000	158.7000	352200.0000	25620.0000
	10	5774.0000	5052.0000	75.9900	71.0800	164200.0000	9498.00000

Table 4. Comparison of time integral performance criteria of the systems

The practical results presented reveal the dynamic character of the applied strategy. The comparative study indicates that the performance of the process with API controller is better than that with AMIT controller. The performance analysis based on the above-mentioned criteria for regulatory response alone also reveals that the API controller outperforms the AMIT controller.
Conclusions

The automatic tuning of PI controller has been investigated using MRAC concept and AMIT rule. Simple adaptation laws for the controller parameters are presented for a second-order process with third-order reference model. Furthermore, when the technique is applied to a non-linear laboratory air flow process, the overall system performance with adaptive PI is observed to have better tracking and disturbance rejection than that of the system with AMIT controller. From the plots, it is clear that the transient performance in terms of tracking error and control signal has been significantly improved by the API controller. Its adaptation gain variations are negligible when compared to the AMIT controller. Due to this, the adaptation of the controller parameters vanishes at a faster rate for API controller. The resulting performance could be improved by a better choice of the adaptation gain. Thus, the API controller supports the process to track the desired model response at a faster rate with less control effort.

A major setback in the AMIT rule is the speed of adaptation, and second, the AMIT rule does not guarantee the stability of the nominal system. The Lyapunov approach can be used to provide guaranteed nominal stability.

A further limitation of the approach is the assumption of the structure for the nominal system. In this paper a second-order model is used and may be simple for many applications. A more flexible nominal model could be used at the expense of more complicated adaptation laws.

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Review

Mathematical transforms and image compression: A review

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Abstract: It is well known that images, often used in a variety of computer and other scientific and engineering applications, are difficult to store and transmit due to their sizes. One possible solution to overcome this problem is to use an efficient digital image compression technique where an image is viewed as a matrix and then the operations are performed on the matrix. All the contemporary digital image compression systems use various mathematical transforms for compression. The compression performance is closely related to the performance by these mathematical transforms in terms of energy compaction and spatial frequency isolation by exploiting inter-pixel redundancies present in the image data. Through this paper, a comprehensive literature survey has been carried out and the pros and cons of various transform-based image compression models have also been discussed.

Keywords: image transforms, compression, entropy, coding gain, truncation error, quantisation error

Introduction

All practical-purpose images are a collection of some structured data generating some degree of correlation between neighbouring pixels. Correlation is closely related to redundancy which is known as inter-pixel redundancy. It requires a reversible transform to remove the inter-pixel redundancy by decorrelating the image in a more compact manner [1-2]. Thus any image having the correlated pixels

can be compressed using transform coding methods where the transform coefficients are highly decorrelated. An image transform can achieve a compression if the numbers of non-zero transform coefficients are smaller on average than the original pixels or data points. After quantisation of the transform coefficients lossy compression can be achieved [3]. An image transform aiming for compression should follow two properties : (a) inter-pixel redundancy minimisation; and (b) spatial frequency isolation.

In digital images the spatial frequencies are important as the low-frequency components correspond to important image features and the high-frequency ones to image details. High frequencies are a less important part of the images and can be quantised more heavily than low frequency coefficients to achieve low-bit rates. Also, the image transforms should be fast and simple giving a choice for linear transformations [3-9].

A linear transformation matrix [W], whose transpose $[W]^T$ will rotate the data matrix X to produce a diagonal covariance matrix for the transformed variable Y where $X = [x_1, x_2, x_3, ..., x_N]^T$ is a vector having N pixel or data points. Then,

$$Y = [W]^T X \tag{1}$$

Each column vector w_i of [W] is a basis vector of new space. So alternatively each element y_i of Y is calculated as

$$y_i = w_i^T X \tag{2}$$

For simple rotation with no scaling, the matrix [W] must be orthogonal, that is

$$[W]^{T}[W] = I = [W][W]^{T}$$
(3)

where *I* is the identity matrix. This means the column vectors of matrix [W] are mutually orthogonal and are of unit norm. From equation (3) it is clear that the inverse of an orthogonal matrix is simply its transpose :

$$[W]^{T} = [W]^{-1} \tag{4}$$

The inverse transformation is calculated as

$$X = [W]Y \tag{5}$$

The total energy after transformation is given as follows:

$$\begin{aligned} \left\|Y\right\|^{2} &= Y^{T}Y \\ &= \left(\left[W\right]^{T}X\right)^{T}\left(\left[W\right]^{T}X\right) \\ &= X^{T}\left[W\right]\left[W\right]^{T}X \\ &= \left\|X\right\|^{2} \end{aligned}$$
(6)

where $||X||^2$ is the norm of vector X, defined as follows:

$$\|X\| = \sqrt{X^T X} = \sqrt{\sum_{i=1}^{N} x_i^2}$$
(7)

Factors Affecting the Performance of Image Transforms Used for Compression

There are several factors such as entropy, coding gain, quantisation error, truncation error and block size which affect the compression performance of transform-based image compression systems [10].

Entropy

Entropy is a useful means of determining the performance of compression [7-8] and theoretically gives a lower bound on the average number of bits required for encoding without introducing error [10]. The probability of any real-value sample may be zero, causing discrete entropy to be undefined. To cure the problem of undefined discrete entropy, the differential entropy is used as generalised measure for the distribution of information. The differential entropy is given as follows [7]:

$$h(x) = -\int_{-\infty}^{\infty} p(s) \log[p(s)] ds$$
(8)

where h(x), s and p(s) are the entropy, samples of a sample space x and probability of samples respectively. For simple distribution such as Gaussian, Uniform and Laplacian, the differential entropy is given as follows:

$$h(x) = \frac{1}{2}\log(\sigma_x^2 + k)$$
(9)

where σ_x^2 is the variance of random variable and k is the constant which depends on the data or random-variable distribution.

From equations (8) and (9) it is evident that the image transformation should minimise the sum of differential entropy or the product of variances of the coefficients due to logarithmic terms [8]. The total energy is preserved after transformation due to orthonormality, hence the fixed sum of the coefficient variance [7, 10].

Coding gain

Coding gain is a measure of the compression efficiency of transformation and is given as follows [9-10]:

$$G_{w} = \frac{\frac{1}{N} \sum_{i=1}^{N} \sigma_{y_{i}}^{2}}{\left(\prod_{i=1}^{N} \sigma_{y_{i}}^{2}\right)^{1/N}}$$
(10)

where the numerator is the algebraic mean of variances which is transform-independent and the denominator is the geometric mean of variances and is transform-dependent. For any arbitrary signal or data, all the variances are almost equal giving a coding gain of 1. For a given energy signal, minimising the product of variances maximises the coding gain and minimises the lower bound on the number of bits required [10, 12].

Quantisation

Quantisation error also plays a very important role in the compression system and should be very low after transformation. Let \hat{Y} be a set of quantised coefficients for a block of data. The reconstructed data is then given as:

$$\hat{X} = [W]\hat{Y} \tag{11}$$

The square error for such block of data is given as follows:

$$e^{2} = \left\| \hat{X} - X \right\|^{2} = \left(\hat{X} - X \right)^{T} \left(\hat{X} - X \right)$$
$$= \left([W] \hat{Y} - [W] Y \right)^{T} \left([W] \hat{Y} - [W] Y \right)$$
$$= \left(\hat{Y} - Y \right)^{T} [W]^{T} [W] \left(\hat{Y} - Y \right)$$
$$= \left(\hat{Y} - Y \right)^{T} \left(\hat{Y} - Y \right)$$
$$\Rightarrow e^{2} = \left\| \hat{Y} - Y \right\|^{2}$$
(12)

From equation (12), it is clearly visible that for any linear orthogonal transformation having orthonormal vectors, the squared error on reconstruction is the same as that of the coefficients [10].

Truncation error

Another method of reducing the data is to remove some transformed coefficients completely leaving only M out of N coefficients. The truncation error is given in equation (13):

$$E(e^{2}) = E\left[\frac{1}{N}\sum_{i=1}^{N} (y_{i} - \hat{y}_{i})^{2}\right]$$

$$= \frac{1}{N}E\left[\sum_{i=1}^{M} (y_{i} - y_{i})^{2} + \sum_{i=M+1}^{N} (y_{i} - 0)^{2}\right]$$

$$= \frac{1}{N}E\left[\sum_{i=M+1}^{N} y_{i}^{2}\right]$$

$$\Rightarrow E(e^{2}) = \frac{1}{N}\sum_{i=M+1}^{N} \sigma_{i}^{2}$$
(13)

where E, y_i , \hat{y}_i and σ_i^2 are expected and original values of transformed coefficients, quantised transform coefficients and variances of the transformed coefficients respectively.

If the variances for the truncated coefficients are smaller and smaller, then the truncation error can be minimised [1, 4, 11].

Block size

The linear orthogonal transforms having orthonormal vectors are applied on some block of data to be transformed. The larger the block size is, the greater the decorrelation becomes, hence the greater coding gain [5, 7]. The number of arithmetic operations increases linearly as the block size increases, hence the complexity. Also, the block-based image transform reduces the inter-pixel redundancy among the pixels or data points within the block, leaving no assurance to remove the inter-block redundancy [9, 13].

Types of Image Transforms Used for Compression

Karhunen-Loeve transform and image compression

The Karhunen-Loeve transform (KLT) is a linearly reversible, orthogonal transformation which accomplishes the removal of redundancy by decorrelating the data block elements and is defined by Eigen values of covariance matrix [14]. Hotelling in 1933 [9] developed a method of principal components for removing the correlation from discrete random variable. A continuous version of Hotelling's transform was developed by Karhunen and Loeve in 1960's [15]. KLT is also known as Hotelling Transform or PCA (principal component analysis) [15-16]. The covariance matrix of an arbitrary data block is real and symmetric so the real Eigen values and corresponding Eigen vectors can be found easily. The diagonal covariance matrix $[C]_{y}$ of a transformed variable *Y* is given as:

$$[C]_{Y} = \begin{bmatrix} \lambda_{1} & 0 & . & 0 \\ 0 & \lambda_{2} & . & 0 \\ . & . & . & 0 \\ 0 & 0 & 0 & \lambda_{N} \end{bmatrix}$$
(14)

where λ_1 , λ_2 λ_N are variances of transformed data *Y*. The diagonal matrix can be calculated from the original covariance matrix $[C]_X$ as follows:

$$[C]_{Y} = E[YY^{T}]$$

$$= E\left[\left([W]^{T} X\right)\left([W]^{T} X\right)^{T}\right]$$

$$= E\left[[W]^{T} (XX^{T})[W]\right]$$

$$[C]_{Y} = \left[W^{T}\right][C]_{X}[W]$$
(15)

The column vector of W are found as

$$\left[C\right]_{\mathcal{X}} \mathcal{W}_{i}^{\mathsf{T}} = \lambda_{i} \mathcal{W}_{i} \tag{16}$$

where λ_i and w_i are Eigen value and Eigen vector pairs for i = 1, 2, 3, ..., N. The orthonormal Eigen vectors are found by using Gram-Schmidt orthonormalisation process [10]. KLT minimises the geometric mean of the variance of transform coefficients, thus providing largest coding gain [17]. The basis vectors of KLT are calculated from the original image pixels and are therefore data-dependent. In practical applications these vectors should also be included in the compressed bit streams, making this transform less ideal for practical applications of image compression [12, 18].

Discrete cosine transform and image compression

Discrete cosine transform (DCT) [19] is very important for compression. DCT is a discrete time version of Fourier-cosine series and can be computed with fast-Fourier-transform-like algorithms. Unlike discrete Fourier transform, DCT is a real value and provides a better approximation of a signal with fewer transform coefficients [20].

The DCT of a discrete signal X is given as

$$Y(f) = \sqrt{\frac{2}{N}} C_f \sum_{t=0}^{N-1} X(t) Cos\left[\frac{(2t+1)f\pi}{2N}\right]$$
(17.a)

$$C_f = \frac{1}{\sqrt{2}}$$
 : $f = 0$ (17.b)

= 1 : $f \neq 0, f = 1, 2, 3, \dots, N-1$

$$\Rightarrow Y = [Y(0), Y(1), \dots, Y(N-1)]$$
(17.c)

where t, f, N and Y(0) are time, frequency, number of points and DC coefficient respectively. $Y(1), \dots, to Y(N-1)$ are the AC coefficients and frequency increases as we go from $Y(1), \dots, to Y(N-1)$. The inverse DCT transform is given as Maejo Int. J. Sci. Technol. 2010, 4(02), 235-249

$$X(t) = \sqrt{\frac{2}{N}} \sum_{j=0}^{N-1} C_j Y(f) Cos\left[\frac{(2t+1)j\pi}{2N}\right]$$
(17.d)

where $t = 0, 1, 2, \dots, N-1$ and C_j is the j^{th} component in frequency domain for $j=0,1,\dots,N-1$, which is similar to C_f in time domain. The 2-dimensional discrete cosine transform (2D-DCT) and 2-dimensional inverse discrete cosine transform (2D-IDCT) for $M \times N$ matrix are given in equations (18.a) and (18.b) respectively:

$$Y(i,j) = \frac{2}{\sqrt{MN}} C_i C_j \sum_{i=0}^{N-1} \sum_{j=0}^{M-1} X(x,y) Cos\left[\frac{(2t+1)j\pi}{2M}\right] Cos\left[\frac{(2t+1)i\pi}{2N}\right]$$
(18.a)

where $0 \le i \le N-1$; $0 \le j \le M-1$ and Y(0,0) is DC coefficient and $Y(i, j) : (i \ne j) \ne 0$ are AC coefficients.

$$X(x,y) = \frac{2}{\sqrt{MN}} \sum_{i=0}^{N-1} \sum_{j=0}^{M-1} C_i C_j Y(i,j) Cos\left[\frac{(2y+1)j\pi}{2M}\right] Cos\left[\frac{(2x+1)i\pi}{2N}\right]$$
(18.b)

where $0 \le i \le N - 1$ and $0 \le j \le M - 1$.

The DCT has as good energy compaction as KLT [12]. The advantage of DCT over KLT is that the former uses a fixed basis which is independent of data or signal. Also, DCT is a block-based transform so performance and complexity is compromised with the block size [19-20].

Discrete sine transform and image compression

Discrete sine transform (DST) is a complementary transform of DCT. DCT is an approximation of KLT for large correlation coefficients whereas DST performs close to optimum KLT in terms of energy compaction for small correlation coefficients. DST is used as low-rate image and audio coding and in compression applications [21-22].

Discrete Walsh-Hadamard transform and image compression

The discrete Wlash-Hadamard transform (DWHT) is the simplest transform to be implemented for any application and is a rearrangement of discrete Hadamard transform matrix [23]. The amount of energy compaction efficiency of DWHT is poorer than that of DCT or KLT so it does not have a potential to be used for data compression [12, 23].

Discrete wavelet transform and image compression

All the linear orthogonal transformations, i.e. KLT, DST and DCT, are blocked transformations which remove the correlation among the pixels or data points inside the block. These transforms do not take care of correlation across the block boundaries [24]. The blocking artifacts are dominating at low bit rates. The blocking effect can be reduced by Lapped orthogonal transforms (LOT) but at the cost of increased computational complexity [25]. A wavelet transform does not require blocking of signal or data points before transformation, resulting in removal of blocking artifacts even at very low bit rates.

Also, wavelet-based subband coding is robust under decoding error and has a good compatibility with human visual system [26]. There are several ways to decompose a signal into various subbands using the wavelet transform, such as octave, adaptive and packet decompositions [27-29]. The octave decomposition is the most used decomposition technique, which non-uniformly splits the bands, rendering the lower frequency part narrower and narrower while leaving out any further decomposition of higher frequency coefficients. Figures 1-3 show a 1-level 2D-wavelet transform (DWT) [29]. Over the past few years many improvements of wavelet-based coding have been developed such as EZW, SPIHT, EBCOT, EPWIC, SFQ, CREW, SR, second generation wavelet coding, wavelet packet image coding, wavelet packet with VQ, and integer wavelet transform coding [30-40].



Figure 1. 1-Level 2D-wavelet decomposition: h(x) and h(x) are horizontal low pass and high pass filter functions whereas h(y) and $\tilde{h}(y)$ are vertical low pass and high pass filter functions respectively; $f_L(x, y)$ and $f_H(x, y)$ are horizontal low pass and high pass wavelet coefficients respectively; approximation, horizontal, vertical and diagonal details are respectively represented by $f_{LL}(x, y)$, $f_{LH}(x, y)$, $f_{HL}(x, y)$ and $f_{HH}(x, y)$.



Figure 2. 1-Level 2D-wavelet recomposition: h(x) and h(x) are horizontal low pass and high pass filter functions whereas h(y) and $\tilde{h}(y)$ are vertical low pass and high pass filter functions respectively; $f_L(x, y)$ and $f_H(x, y)$ are horizontal low pass and high pass wavelet coefficients respectively; approximation, horizontal, vertical and diagonal details are respectively represented by $f_{LL}(x, y)$, $f_{LH}(x, y)$, $f_{HL}(x, y)$ and $f_{HH}(x, y)$.

LILL LIHL	IIIH IIHH	LH
HL		HH

Figure 3. Spectral decomposition and ordering of wavelet coefficients: *L* and *H* are the low pass and high pass wavelet transformed coefficients respectively.

Fractional discrete transform and image compression

In 1929 Wiener [41] introduced a concept of fractional transforms, which led to the development of fractional Fourier transform (FrFT) first developed in 1980 [42]. Almeida [43] explored the time-frequency localisation property and provided a possible application of FrFT in image compression. In the case of fractional transform, one extra free parameter is also there besides time and frequency. In 2000 Gerek and Erden proposed a discrete fractional cosine transform by taking an advantage of the relation between DCT and DFT [44], which was similar to the method of finding DFrFT by Ozaktas et al. in 1996 [45]. In 2005 Singh and Saxena [46] explored the possible application of DFrCT and DFrFT in image compression. The compression performance of fractional transforms depends on the value of free parameter. However, any direct relation between free parameter and compression performance has not been reported. Hence, it is impractical to optimise the free parameter, which results in a recursive and a very slow process for image compression.

Directional discrete transform and image compression

All the transforms as discussed above are 2D transforms implemented by using 1D separable architectures and are not suitable to preserve the image features with arbitrary orientation that is neither vertical nor horizontal [47]. In these cases, they result in large-magnitude high-frequency coefficients. At low bit rates, the quantisation noise from these coefficients is clearly visible, in particular causing annoying Gibbs artifacts at image edges with arbitrary directions. Some work on wavelet and subband transform to incorporate directional information into transforms has been reported. The lifting structure developed by Sweldens provides a good way to incorporate directional information into the wavelet transform [47-49]. Zeng and Fu [50] are the first authors to propose how to incorporate directional information into DCT. Their directional DCT is motivated by SA-DCT (shape-adaptive DCT). Hao et al. [51] proposed a lifting-based directional DCT-like transform for image coding and used it for image compression. The main problem with directional transforms is the selection of optimum direction.

Singular-value decomposition and image compression

Image transform is a very important part of image compression. The optimum transform coder which minimises the mean square distortion of the reproduced data for a given bit rate is the KLT [9]. Other transforms investigated for image or picture compression include DCT, piecewise Fourier Transform, slant transform, linear transform with block quantisation and Hadamard transform [53-57]. Though the energy compaction efficiency of the KLT is very suitable for compression, it is not used in real applications due to its computational complexity [53, 59-60]. Singular-value decomposition-based transformation has an optimal energy-compaction property making it the most appropriate for compression in spite of computation complexity [61]. In the case of singular-value decomposition (SVD) the singular values are image-dependent and must therefore be coded with the associated singular vectors as side information [62]. The optimal energy compaction property was exploited and utilised by McGoldrick et al. [62] and Yang and Lu [63]. McGoldrick et al. calculated singular values as well as singular vectors and the latter were coded by variable-rate vector quantiser. JPEG image coder based on DCT was superior to SVD-based method. Yang and Lu also used SVD in conjunction with vector quantisation giving a superior method by reducing the computational complexity to that of DCTbased method. However, with the application of fast DCT algorithm, this was not a preferred technique [64]. Waldemar and Ramstad [65-66] proposed hybrid KLT-SVD image compression using transform adaptation technique exploiting the local variation of images. This hybrid method was better than KLTbased methods in terms of energy compaction but could not be sustained due to a large number of vectors to be coded. In 2000 Chen [67] used rank approximation method for SVD-based lossy image compression. In rank approximation for SVD-based image compression an image of size $N \times N$ was transformed by SVD to obtain matrices U_{NxN} , S_{NxN} and V_{NxN} , where S is a diagonal $N \times N$ matrix whose number of non-zero diagonal elements determines the rank "k" of the original matrix where $k \leq N$. In this method a smaller rank is used to approximate the original image. The total storage space required to restore the original approximated image is 2Nk + k, where $k \le N$. In order to achieve the goal of compression, used rank should be as follows:

$$k \le \frac{N \times N}{\left(1 + 2N\right)} \tag{19}$$

So by rank approximation method there is a restriction on reconstructed image quality for compressed image. Arnold and McInnes in 2000 [68] reported block-based adaptive rank approximation method similar to most of the popular image compression methods, to exploit the uneven complexity and correlation of image. The work reported by them was based on singular-value distribution of different subblocks in which higher ranks were used for complex subbands. Also, for the same storage space, smaller block sizes of subblocks produced better results [68-69]. Arnold and McInnes further reduced rank of the blocks by rank-one update, in which the respective mean was subtracted from all the elements of the blocks and then SVD and adaptive rank approximation was used. Dapena and Ahalt [69] and Wongsawat et al. [70] reported hybrid DCT-SVD and modified hybrid DCT-SVD image coding algorithms in 2002 and 2004 respectively. Both methods were based on an adaptive selection of block transforms to be used on the basis of complexity and correlation of different blocks. For high correlation, SVD was used while for the rest, DWT was used. In 2003 a hybrid DWT-

SVD-based image coding, which is also a block-based method, was reported by Ochoa and Rao [71-72], who used a criterion of threshold standard deviation for all blocks of Y component to determine whether DWT or SVD has to be used for any particular block. If standard deviation is high, rank-one update is used for that block, otherwise DWT method is used. Ochoa and Rao further extended this method for colour image compression also [73-74]. In 2007 Ranade et al. [75] proposed a modified SVD image compression based on SSVD (shuffled SVD). In this work the block-based shuffling operator was used to get subblocks. The performance of SSVD was shown to be better than SVD in terms of space for the same quality but involved more complex operations. Also, the performance was not even near to DCT-based coding systems. Aase et al. [76] gave a critique on SVD-based image compression and pointed out the major drawback of using lossless SVD transform for image compression. According to them, the singular vectors along with the singular values are stored for lossless reconstruction, which requires 2(1+1/N) times more space for $N \times N$ image.

Conclusions

On the basis of the above discussion it can be concluded that any image transform applied for image compression will have minimum entropy, maximum coding gain, minimum quantisation error, minimum truncation error, and moderate block size. Although the KLT shows highest energy compaction, it is a very complex transform and usually takes unfeasible time delay during the transformation. DCT shows as good performance as KLT though the advantage of DCT over KLT is that the former employs fixed basis which is independent of data or signal. Also, DCT is a block-based transform so performance and complexity is compromised with the block size. Another advantage of DCT is its blocking effect for low bit rate applications. DST is also a block-based transform and can be used only for the image or data which have very small correlation. DWHT is very simple to implement but has a very poor performance in terms of energy compaction efficiency. The compaction efficiency of DWT is not very good compared to that of DCT but it can provide a satisfactory performance for the entire range of bit rates. The blocking effect as shown in DCT is removed in the case of DWT as it is a global transform and not the block-based transform. The compression performance of fractional transforms depends on the value of free parameter and it is impractical to optimise the free parameter due to a recursive and very slow process, which is not favourable for compression. Directional discrete transforms are used at low bit rates when the quantisation noise from the transform coefficients is clearly visible, in particular causing Gibbs artifacts at the image edges with arbitrary directions. The optimisation of direction makes it unsuitable for compression. SVD transform has an optimum energy compaction property but needs the requirement of more storage space for lossless compression and has a high level of complexity if it is used globally.

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Full Paper

Effects of thermochemical pretreatment on the biodegradability of sludge from a biological wastewater treatment system

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Abstract: The effects of thermochemical pretreatment on the sludge biodegradability were examined. Two types of tests were conducted: aerobic biodegradation and denitrification using thermochemically pretreated sludge as carbon source. In the aerobic biodegradation tests, the biodegradation efficiency for the sludge pretreated at 60, 70, 80 and 90°C (pH 11) was 1.4-2.2 times higher than that for the untreated sludge. The biodegradation efficiency for the supernatant was also about 1.9 times higher than that for the particulates. The biodegradation enhancement for the thermochemically pretreated sludge was demonstrated in denitrification tests. The supernatant showed its potential as a carbon source for the denitrification process.

Keywords: wastewater treatment, sludge, thermochemical pretreatment, biodegradability, denitrification

Introduction

Sludge disintegration has been commonly practiced as a pretreatment for sludge digestion [1]. Pretreatment destroys cell walls leading to the solubilisation of extracellular and intracellular materials into the aqueous phase [2]. With pretreatment, not only hydrolysis is accelerated by the increase in dissolved components but improvement of biodegradability, sludge dewatering and reduction of pathogens and foaming can also be achieved as well [3]. There have been several kinds of pretreatment

method studied so far, which are either physical, chemical, mechanical or biological in nature, or a combination of any two of these methods [1].

Recently, ozone treatment of sludge followed by recirculation of the treated sludge into the bioreactor was reported to effectively reduce sludge production [4-5]. However, through ozonation at a high ozone dose, e.g. above 1.0 g O₃/g of mixed-liquor suspended solids, a large portion of sludge was mineralised [6-7]-in other words, some organic compounds or released soluble chemical oxygen demand (SCOD) were lost, probably due to complete oxidation to CO₂ since ozone has a strong oxidative power [8-10]. Although this phenomenon is good for the reduction of the sludge, it should be noted that the supernatant from the ozonated sludge also contains some nitrogen [6]. The carbon/nitrogen (C/N) ratio in the supernatant is consequently low and not sufficient for denitrification of released nitrogen in the supernatant [4, 10-12]. External organic compounds such as methanol, ethanol and acetic acid are usually added to the denitrification tank if the wastewater does not contain sufficient reducing power [13]. The use of these external electron donors imposes a financial burden [14]. A thermochemical method for sludge pretreatment may provide a solution for the problem. Organic compounds have not been lost during the pretreatment because the thermochemical process does not oxidise any materials but only promotes hydrolysis and helps split complex polymers into smaller molecules [15-16]. The thermochemical process is simple and cost-effective [1-2], with sodium hydroxide being found to be the most efficient alkali for inducing hydrolysis and cell lysis [17].

Themochemical method has in fact been studied elsewhere [1-2, 17-19]. However, there is still a lack of quantitative information about the enhancement of biodegradability of sludge pretreated thermochemically. Therefore, in the present study, the effects of thermochemical pretreatment on the biodegradability of sludge are evaluated. Two types of tests were first conducted to quantify the enhancement of biodegradability of the thermochemically pretreated sludge: aerobic biodegradation and denitrification. The aerobic biodegradation tests were carried out with the pretreated sludge, particulate and supernatant. The denitrification experiment using the supernatant as a carbon source was also examined.

Materials and Methods

Thermochemical pretreatment of sludge

The sludge used for the experimental work was obtained from the aerobic basin of a bench-scale anoxic-aerobic membrane bioreactor located in a wastewater treatment plant in Sungkyunkwan University, Suwon, Korea. The sludge was passed through a standard sieve (0.5 mm) to remove coarse debris.

In order to study the hydrolysis process of the sludge, a series of experiments were performed at different temperatures (60, 70, 80 and 90°C) while pH and experimental time were fixed at 11 and 7 hours respectively. All determinations were carried out in duplicate. The sludge sample (1.0 L) was put in a 2.0-L glass beaker. A stock solution of sodium hydroxide (>98%, Samchun Pure Chemical Co., Korea) was added to increase the pH of the sample to 11. The beaker was put in a water bath (Model KMC-1205WP, Vision Scientific Co., Korea) to maintain the desired reaction temperature for 7 hours. The sludge in the beaker was kept in suspension by a slow-speed mixer (Model GT-S20, 0-600 rpm,

Green Tech Co., Korea). In order to minimise evaporation during the pretreatment, the beaker was covered with aluminum foil. Chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD) and mixed-liquor suspended solids (MLSS) were measured every hour.

The sludge solubilisation efficiency after thermochemical pretreatment was evaluated through SCOD release efficiency and MLSS reduction efficiency as follows:

SCOD release efficiency =
$$\frac{SCOD}{COD} \times 100$$
 (%)

where SCOD (mg/L) was measured after thermochemical pretreatment and COD (mg/L) was measured before thermochemical pretreatment.

MLSS reduction efficiency =
$$\frac{MLSS}{MLSS_o} \times 100$$
 (%)

where MLSS (mg/L) was measured after thermochemical pretreatment and MLSS_o (mg/L) was mixed liquor suspended solids measured before thermochemical pretreatment.

Aerobic biodegradation experiment

After the thermochemical pretreatment, a part of the pretreated sludge was centrifuged at 5000 rpm for 10 minutes in a centrifuge (Model MF550, Hanil Science Industrial Co., Korea) to afford the supernatant and particulates. The aerobic biodegradation experiments were conducted in a series of 500-mL bioreactors (Comput-OX respirometer, Model AV4R, N-CON Systems Co., USA). The designed sample volume for testing in a bioreactor is 300 mL [20]. Therefore, three sets of testing samples were prepared for each bioreactor: (i) 150 mL of sludge (untreated sludge taken directly from the aerobic basin) and 150 mL of each of the differently pretreated sludge (60, 70, 80 and 90°C at pH 11 for 3 hours); (ii) 150 mL of untreated sludge and 150 mL of supernatant; and (iii) 150 mL of untreated sludge and 150 mL of particulates. Besides, a control test was prepared in another bioreactor which only consisted of 150 mL of untreated sludge and 150 mL of distilled water. Allylthiourea (>98%, Acros Organics, USA) was added to each bioreactor at 1.4 mg/L to inhibit nitrification process which can utilise some oxygen. As respiration takes place, oxygen is depleted and carbon dioxide is released at the same rate [20]. Potassium hydroxide pellets (>85%, Showa Chemical Co., Japan) were used to trap the generated CO₂ as potassium carbonate. As a result, the pressure in the reactor dropped. Oxygen consumption was determined by measuring the change in headspace pressure created by oxygen consumption. The Comput-OX system detected the drop in pressure and delivered pure oxygen to the reactor in measured increments and also recorded the volume of oxygen and the time it was delivered. The oxygen utilisation (in mg/L) was computed automatically by the Comput-OX program and presented both in numeric and graphic displays for each test. Also, COD of the sludge in each bioreactor was measured before and after the aerobic biodegradation tests.

Batch denitrification experiment

The biodegradability of the supernatant (obtained by centrifuging the pretreated sludge as described in the above section) was also evaluated by batch denitrification experiment. A 250-mL flask containing 35 mg NO₃⁻N per L (prepared from a stock solution of potassium nitrate (>99%, Yakuri Pure Chemical Co., Japan)) and 50 mL of sludge (taken from the anoxic basin) or the supernatant

(diluted to C/N ratio of 5, 10 or 15) was sealed and incubated at 30°C in an incubator (Model DH.WTH0115, Daihan Scientific Co., Korea) with slow agitation (50 rpm, using a magnetic stirrer). Aliquot samples were periodically taken from the flask and analysed for nitrate (NO₃⁻-N).

Analysis methods

The proper standard methods for the examination of water and wastewater by APHA [21] were used for all the monitoring analyses in this study.

Mixed-liquor suspended solids (MLSS) and mixed-liquor volatile suspended solids (MLVSS) were measured by using the methods 2540D and 2540E respectively. Sample for the measurement of MLSS and MLVSS was filtered through a 0.45-µm glass microfibre filter (GD/X, Whatman, UK). The solids retained by filter were dried in an oven (Memmert UFP600, GmbH, Germany) at 105°C for 2 hours for the MLSS measurement, and then ignited in a furnace (DH.WFH12.27, Daihan Co., Korea) at 550°C for 20 minutes for the MLVSS measurement.

COD was determined by the closed reflux, colorimetric method (Method 8000). Sample was added into the COD vial and heated in a COD heater (Model DRB200, Hach Co., USA) at 150°C for 2 hours, then cooled to room temperature and its absorbance measured at 620 nm by a spectrophotometer (DR/2500, Hach Co., USA) to afford COD (in mg/L). SCOD was similarly determined except the sample was first centrifuged at 5000 rpm for 10 minutes and then filtered through a 0.45-µm glass microfibre filter.

Total nitrogen (TN) was analysed by the persulphate digestion method (Method 10071). Sample was added into the TN vial. A Total Nitrogen Reagent Powder Pillow (Hach Co., USA) was also added to the TN vial, which was then heated at 105°C for 30 minutes (using COD heater, Model DRB200, Hach Co., USA). After the sample was cooled to room temperature, it was measured at 410 nm to obtain TN (in mg/L).

Nitrate (NO₃⁻-N) was measured by the cadmium reduction method (Method 8039). Sample was filtered through a 0.45- μ m glass microfibre filter and added to the NO₃⁻-N vial. NitraVer 5 Nitrate Reagent Powder Pillow (Hach Co. USA.) was then added to the vial. A 5-minute reaction period was required. Then the sample was analysed spectrometrically at 500 nm to afford NO₃⁻ -N (in mg/L).

The pH value of each sample was measured using a pH meter (Horiba Navi, Model F-54, Japan).

Results and Discussion

Sludge pretreatment and solubilisation efficiency

The initial MLSS and MLVSS were measured and found to be 7500 and 5540 mg/L respectively. The ratio of MLVSS/MLSS is about 74%. The solubilisation efficiency of sludge after thermochemical pretreatment can be inferred through two aspects, i.e. SCOD release efficiency and MLSS reduction efficiency. The experimental data related to SCOD release and MLSS reduction during thermochemical pretreatment process are given in Figures 1-2.



Figure 1. SCOD release efficiency during thermochemical pretreatment



Figure 2. MLSS reduction efficiency during thermochemical pretreatment

An increase in SCOD release efficiency and decrease in MLSS were used to evaluate the hydrolysis process. For all thermochemical pretreatment conditions, a rapid increase in SCOD release was observed during the first hour (Figure 1). Then a decreasing rate of SCOD release was observed until a maximum value was reached. Rocher et al. [18] reported that the cells were completely deactivated by a temperature above 60°C. Their study also showed that alkaline treatment (by using NaOH) combined with thermal treatment was an efficient process to induce cell lysis. With intermediate conditions of pH 10 and 60°C, the released dissolved organic carbon was higher than or equal to that obtained by thermal treatment at 100°C and neutral pH or by alkaline treatment at pH 12 and 30°C [19]. In this study, with intensive hydrolysis conditions (pH 11 and T \geq 80°C), the COD release efficiency increased significantly during the first 3 hours of the pretreatment process, in which about 75-80% of COD release efficiency was achieved.

The MLSS reduction efficiency is presented in Figure 2. The MLSS reduction efficiency during the first 3 hours was about 60-75% of the final values, obtained after 7 hours of pretreatment.

Increasing the temperature from 60 to 90°C after the third hour of operation at high pH value (pH 11) decreased MLSS concentration significantly from 7500 mg/L down to about 6750 and 5600 mg/L (at the 3rd hour), corresponding to about 10 and 25% of sludge reduction respectively.

Aerobic biodegradation tests

The biodegradability enhancement of sludge after thermochemical pretreatment is demonstrated in the aerobic biodegradation experiment. Figure 3 shows the oxygen consumption by the thermochemically pretreated sludge in the aerobic bioreactor. Two phases of biodegradation—the fast and slow degradation—were observed. The duration of the fast degradation phase increased with temperature. Although it is obvious that biodegradability of sludge was enhanced by thermochemical pretreatment, accurate quantification of the biodegradability of the pretreated sludge is not easy because the initial sludge added in the bioreactor itself consumed oxygen.



Figure 3. Oxygen consumption during aerobic biodegradation tests for the thermochemically pretreated sludge

As previously mentioned, during the aerobic biodegradation tests the oxygen utilised by nitrification was negligible since allylthiourea was added in the bioreactor to inhibit nitrification process [20]. Considering the volume ratio of the pretreated sludge and the untreated sludge (1:1), the biodegradation efficiency can be regarded as an average value of that for the thermochemically pretreated sludge and the untreated sludge. The biodegradation efficiency for the pretreated sludge is estimated based on the COD before and after aerobic biodegradation tests (after 150 hours) as presented in Figure 4. From the figure, the biodegradation efficiency for the differently pretreated sludge (60, 70, 80 and 90°C at pH 11 for 3 hours) is 41.4, 44.3, 53.1 and 62.4% respectively, compared to 28.7% for the untreated sludge. Thus, the biodegradation efficiency for the thermochemically pretreated sludge was 1.4-2.2 times higher than that for the untreated sludge. In fact, most of the biodegradation of the thermochemically pretreated sludge occurred within 80 hours while that of the untreated sludge was steady after more than 90 hours (Figure 3). One of the interesting observations is the suppression of biodegradation rate at relatively high temperature (> 80°C).



Figure 4. Biodegradation efficiency of thermochemically pretreated sludge prepared by different Conditions

The biodegradation efficiency for the particulates and supernatant obtained from sludge pretreated at 80°C (pH 11) for 3 hours was also determined by the aerobic biodegradation tests, in comparison with the pretreated and the untreated sludge (control). The oxygen consumption during the aerobic biodegradation for these cases is shown in Figure 5. It can be seen that the biodegradation of the supernatant was predominant during the first 40 hours. Also, the biodegradability of the particulates was better than that of the untreated sludge. Most of the biodegradation of the particulates occurred within 100 hours.



Figure 5. Oxygen consumption during aerobic biodegradation of sludge portions

Figure 6 presents the estimated biodegradation efficiency for the sludge portions. From the figure, the biodegradation efficiency for the supernatant was found to be 84.4% at 150 hours. In contrast, the biodegradation efficiency for the untreated sludge was found to be 28.7%. Thus, the supernatant was much easier to biodegrade, with biodegradation efficiency being enhanced approximately 55.7% more than that for the untreated sludge. The biodegradation efficiency for the

particulates was found to be 44.2% after 150 hours of aerobic biodegradation, showing an enhancement of 15.5% more than that for the untreated sludge. From the obtained results it is evident that the biodegradation efficiency for the supernatant was about 1.9 times higher than that for the particulates.



Figure 6. Biodegradation efficiency of sludge portions

Batch denitrification tests

Figure 7 shows the changes in nitrate concentration during denitrification tests using the supernatant (obtained by centrifuging the sludge pretreated at 80°C, pH 11, for 3 h) as carbon source at C/N ratios of 5, 10 and 15. Although the rate of denitrification is affected by the amount of carbon supply, it is known that the supernatant could be utilised as a carbon source for denitrification [19]. Figure 8 shows another denitrification experimental result obtained by using the supernatant pretreated at different temperatures at a fixed C/N ratio of 10.



Figure 7. Result of denitrification tests using supernatant at different C/N ratios



Figure 8. Result of denitrification tests using supernatant pretreated at different temperatures (at fixed C/N ratio of 10)

The result clearly indicates that the supernatant was available for biodegradation as a good carbon source regardless of the different thermal pretreatments. The average specific denitrification rate was found to be about 1.85 g NO₃⁻-N/g MLVSS/h (grams of nitrate per gram of mixed-liquor volatile suspended solids per hour). In Table 1, the feasibility of the solubilised organics after thermochemical pretreatment as carbon source is compared with that of other carbon sources commonly used for denitrification. It should be noted that the supernatant from the thermochemically pretreated sludge also contained some nitrogen. At 80°C and pH 11 for 3 hours, however, the C/N ratio of the supernatant became larger than 15.6, indicating that the recycling of the supernatant to the influent wastewater stream of the wastewater treatment plant can increase the overall C/N ratio.

Carbon source	Specific denitrification rate (g NO ₃ ⁻ -N/g MLVSS/h)		Reference
	Range	Average	
Methanol	2.88-21.6	10.32	[13]
Wastewater	0.72-2.64	1.68	[22]
Supernatant*	1.16-2.18	1.85	This study

Table 1. Comparison of specific denitrification rates by various carbon sources

*obtained from thermochemically pretreated sludge centrifuged at 5,000 rpm for 10 minutes.

Conclusions

The effects of thermochemical pretreatment on the sludge biodegradability were determined by aerobic biodegradation tests. Thermochemically pretreated sludge at 60, 70, 80 and 90°C (pH 11) showed 1.4-2.2 times greater biodegradation compared to the untreated sludge. The biodegradation of the thermochemically pretreated sludge mostly occurred within 80 hours while that of the untreated sludge was steady and required more than 90 hours. Thus, the biodegradability of sludge was greatly enhanced by thermochemical pretreatment. The biodegradation of the supernatant was about 1.9 times

greater than that of the particulates. The biodegradability of the particulates was also somewhat better than that of the untreated sludge.

The biodegradation enhancement of thermochemically pretreated sludge was confirmed by the denitrification tests. The supernatant obtained from the thermochemically pretreated sludge showed its potential as a carbon source for denitrification with an average specific denitrification rate of 1.85 mg NO_3 -N/g MLVSS/h. As a result, the supernatant demonstrated its capability as a carbon source for denitrification.

The sludge was also reduced significantly (up to 25%) after 3 hours of pretreatment. Thus, combining the thermochemical method with the biological wastewater treatment could be a good solution for the overall sludge reduction. Further study will be necessary to compare this pretreatment technique with others such as mechanical and biological pretreatments in terms of biodegradability enhancement.

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Short Communication

Effects of temperature, shearing time and rate of shear on the viscosity of chitosan/agar-blend solutions

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Abstract: In this study, a number of aqueous solutions of chitosan/agar blends (with chitosan as the major component) were prepared. The effects of temperature, shear rate and shearing time on the rheological property, i.e. the apparent viscosity, were investigated for the blend solutions. Results show that a non-Newtonian behaviour was pronounced at temperatures from 20° C to 50° C for all solutions and that a decrease in viscosity with increasing temperature was also observed. It was also found that all the blend solutions obeyed the Arrhenius equation to different degrees. In addition, shearing time independence was found to occur at a low shear rate only for pure chitosan solution. However, the shearing time independence slowly shifted to occur at higher shear rate with increasing agar content.

Keywords: rheology, chitosan, chitosan/agar mixture, viscosity of chitosan blend

Introduction

Chitosan and agar are natural polymers that are much used in food, cosmetic, pharmaceutical and some other industries [1-5]. Chitosan is a polysaccharide formed by alkaline deacetylation of chitin, the second most abundant naturally occurring polysaccharide from crab and shrimp shells [6]. Agar, on the other hand, is a hydrocolloid extracted from seaweed and mainly consists of agarose and agaropectin [7]. One unique property of agar is that the gelation occurs at a temperature far below the gel's melting temperature, which leads to the formation of a rigid gel. These polymers are non-toxic, biodegradable, biocompatible and easily available.

Extensive studies on both chitosan and agar have been done in order to provide useful data for their applications. However, the work on both of them together, especially with respect to rheological properties, is very limited and thus, this forms the basis for studying them in the form of blends.

Earlier, we reported on the rheology of chitosan/agar-blend solutions at different proportions [8]. The study was conducted at temperatures ranging between 40-55°C with shearing times of 15, 30, 45, 60 and 75 seconds. However, the temperature range studied was small due to the gel formation of some blends below 40°C. The objective of this work is to study the effects of temperature, shearing time and rate of shear on the viscosity of chitosan/agar-blend solutions using smaller amounts of agar and a wider range of temperature for a better understanding of the flow behaviour of these blends.

Materials and Methods

Materials

Shrimp-source chitosan was purchased from a local company with a deacetylation percentage of 88.1% (defined by UV method [9]). This chitosan was acid soluble, white-coloured and flaky. Agar, powdery and light beige in colour, was purchased from Sigma (average molecular weight = 1.3×10^4 g mol⁻¹). Acetic acid was purchased from Merck (Darmstadt, Germany). Ultra-pure water (Maxima Ultra Pure Water, Elga-Prima Corp., UK) with a resistivity greater than 18 MΩ/cm was used to prepare all solutions. All chemicals were used without further purification and freshly prepared solutions were used in all experiments.

Preparation of chitosan/agar-blend solutions

Chitosan, oven-dried to constant weight (5 g), was dispersed in 0.1 M acetic acid (500 mL) followed by stirring and heating at about 60° C overnight to form a 10 g L⁻¹ chitosan solution, which was then filtered to remove dust and other traces of impurities. Air bubbles were eliminated by keeping the solution at room temperature for 2 hours. An agar solution was similarly prepared following the above procedure except 0.1 M acetic acid was replaced by preheated ultra-pure water. The solution was then stirred and kept at 80°C for 3 hours to ensure complete dissolution of agar and a very clear solution.

The chitosan/agar blends were prepared by adding the aqueous agar solution drop by drop to the chitosan solution, which was kept stirring on a magnetic stirrer at about 90° C, and then the mixture was stirred at a moderate speed for a certain time. The chitosan/agar-blend solutions thus prepared, with chitosan as the major component, had the blend ratio of 100/0 (pure chitosan), 90/10, 80/20, 70/30, 60/40 and 50/50.

Methods

The molecular weight of chitosan was about 5.5×10^5 g mol⁻¹ determined by gel permeation chromatography [10].

The viscosity of all solutions was determined by using a Brookfield digital viscometer, model DV-II + Pro, with an attached UL adapter. The temperature was maintained by using a circulating water bath. The viscosity was determined in a 16-mL of sample from 20°C to 50°C and shearing times were 20, 40, 60, 80 and 100 seconds. The shear rate was measured automatically by the instrument once set. The apparent viscosity values were reported in centipoise (cP) unit.

It is worth mentioning that it is necessary to use freshly prepared mixtures as any delay can make it difficult to measure the viscosity due to the formation of gel, especially for the mixtures containing higher than 20% agar.

Results and Discussion

Figure 1 shows the relation between viscosity and shear rate at different temperatures for solutions of pure chitosan and its blends with agar. Generally, two regions of flow profiles—low-shear and high-shear regions—can be identified. In low-shear region, the shear-rate-dependent viscosity decreased dramatically suggesting shear thinning behaviour (non-Newtonian behaviour). However, the shear-rate-dependent viscosity was found to be nearly independent of shear rate at high shear rates. Non-Newtonian materials are of significance in the pharmaceutical area since they are used to produce liquid and solid dispersions [11]. In addition, lower shear thinning and higher viscosity was observed when the concentration of agar was increased. Furthermore, the pure chitosan solution showed increasing shear thinning with increase in temperature. The viscosity range for this solution was 7-20 cP for shear rate ranging between 2-15 s⁻¹. As the proportion of agar increased, the solution became more viscous for each shear rate value, but showed the same trend with respect to temperature. The viscosity increased further with increase in amount of agar and tailed off at a slightly higher viscosity at the highest shear rate. As expected the last blend (50/50) with the highest agar content showed the highest viscosity values. The reason for this is most likely due to the gelling capacity of the agar. It is thought that improving the viscosity of the solution may improve the strength of the resulting thin film.

According to the Arrhenius equation, the viscosity values (η) obtained at a constant shear rate can be correlated with temperature:

$$\eta = A \cdot e^{-Ea/RT}$$

where A is a constant related to molecular motion, E_a is the activation energy for viscous flow at a constant shear rate, R is the gas constant and T is the absolute temperature in K.

Figure 2 presents the Arrhenius plots of the natural log of viscosity versus the inverse of temperature for various ratios of chitosan/agar-blend solutions. This graph shows a nearly linear relationship for all solutions. The values of the apparent activation energy (E_a), which were calculated from the slope, are shown in Table 1. Knowing the activation energy is important in deducing the sensitivity of a process towards temperature [12]; the higher the activation energy, the more sensitive the process is to temperature. The activation energy is also useful for signifying the minimum amount of energy required for the reactants to start a reaction [13]. The lower the activation energy, the faster the reaction will proceed. The lower values of E_a may indicate a good compatibility of the two polymers.



Figure 1. The influence of shear rate and temperature on the rheological curves of pure chitosan solution and its blends with agar at different proportions



Figure 2. The relationship between apparent viscosity and temperature of chitosan/agar-blend solutions at a constant shear rate (6.15 s^{-1})

Chitosan/Agar	Viscous flow activation	
	energy, E_a (kJ mol ⁻¹)	
100/0	15.31	
90/10	10.52	
80/20	16.51	
70/30	15.12	
60/40	15.21	
50/50	15.40	

Table 1. The relationship between viscous flow activation energy and blend concentration

Figure 3 presents the effects of shear rate and shearing time on the rheological curves of pure chitosan solution and its blends with agar at room temperature. For pure chitosan solution at low shear rate, viscosity was independent of shearing time but becoming more dependent at higher shear rate. With added agar the opposite seemed to occur. At 10% agar, the viscosity became more dependent of shearing time at low shear rate and slightly less dependent of shearing time at higher shear rate. For the 80/20 blend, viscosity dependence on shearing time was almost the same for all shear rate values. For the 70/30 blend, clear independence of shearing time was observed at high shear rate values, and this is more pronounced with the 60/40 and 50/50 blends. In pure chitosan solution, shearing time independence occurred at low shear rate. The reason is most likely due to the absence of gelling property, which was present in the blend solutions, especially those with 30, 40 and 50% agar.



Figure 3. The influence of shear rate and shearing time on the rheological curves of pure chitosan and its blend solutions with agar at different proportions at room temperature

Conclusions

This study has shown that the viscosity of chitosan/agar-blend solutions increases significantly as the agar ratio is increased and that the temperature effect is the same for all blends, i.e. the overall viscosity decreases as the temperature increases. Also, a shear thinning behaviour was observed for all blend solutions at all temperatures studied. In addition, the relationship between the apparent viscosity and temperature can be described by the Arrhenius equation. Furthermore, shearing time independence occurs at low shear rate only for pure chitosan solution; for the blends, this phenomenon gradually shifts to higher shear rate as the agar content increases.

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Full Paper

Ecological status of the lowland deciduous forest in Chang Kian Valley, Chiang Mai, northern Thailand

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Abstract: An ecological assessment of lowland deciduous dipterocarp-oak, seasonal, hardwood forest on the base of the east side of Doi Sutep-Pui, Chiang Mai province, was conducted. Seven selected sites representing the most intact condition to the worst one were surveyed. Plant species diversity and abundance declined as fire damage increased. Details on the number of species, their habits, size classes of trees, pioneer vs climax species, woody seedlings, coppices, and herbaceous ground flora are presented. Species lists for all these categories and their abundance are also included. Profile diagrams and photographs of some sites are shown. The conservation value of lowland forests has been largely neglected and now many places require reforestation. The degree of degradation is serious and will continue to deteriorate unless effective protective and remedial action is done.

Keywords: deciduous dipterocarp-oak, seasonal, hardwood forest (DOF) ; forest fire ; successional ecology ; Chang Kian, Chiang Mai

Introduction

During the hot-dry season with the highest temperatures (often over 40°C) between March-April, Chiang Mai basin was covered with dense, noxious smog which was up to 700 m thick in 2007 and well over 1300 m in 2010 [1] The cause was due to massive fires in the mountainous areas of the north including the adjacent Doi Sutep-Pui National Park, combined with traffic and construction pollution within the city. Nothing effective had been done to prevent other pollution episodes as occurred in 2007 and the toxic conditions were repeated to a greater extent in 2010. Extensive fires have become a risk factor for the environment, health and tourism [1].

Burning of agricultural fields and forested areas is a traditional annual event which has become increasingly more destructive and widespread in the northern provinces of Thailand. In upland areas (above 1000 m), primitive agricultural practices involving slash and burn methods are conducted by hill-tribe communities during the dry season to remove agricultural wastes and also to expand fields [2]. This has ruined much of the original forests and has prevented natural recovery of vegetation,
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since fallow periods are reduced from many to mostly only a few years [3-5]. Biodiversity continuously decreases as the amounts of pesticides and chemical fertilisers increase and enter the environment. An overall increase in soil erosion and water pollution along with a decrease in water availability and quality, especially during the dry months of November to May, has resulted. Water shortages during this dry period and flooding during the rainy season are recent problems which Chiang Mai now experiences. All this, combined with deliberate burning of lowland vegetation and disturbance of adjacent soil, has resulted in the loss of forest cover and increases in environmental problems [6].

Research Site

Northern Thailand is situated in the monsoonal climatic region extending from Manipur State (NE India) throughout northern Burma, northern and eastern Thailand, central and southern Laos, Cambodia, to central and southern Vietnam. There is a distinct dry season from November to May and a hot period from March to May. The rainy season starts in May and ends in November. Lowland areas, i.e. below 850-m elevation, have deciduous forests while above this the forests become evergreen. There is a definite correlation between elevation and the amount of rainfall and temperature range. Lowland areas have less rainfall with Chiang Mai (350 m) receiving an annual average of about 1100 mm and at Puping Village on Doi Sutep (1375 m) it is 2095 mm [7]. Lowland temperatures frequently exceed 40°C in April-May while it is always considerably cooler at over 1000-m elevation.

Chiang Mai was established over 700 years ago with the forest of Doi Sutep (1601 m) and Doi Pui (1685 m) providing people with sustainable water supplies, timber, useful plants and wildlife. This situation was radically disrupted when lowland teak-dominated forests started to be cut in the late 1800's. By the mid-1900's all teak trees had been cut while other commercially valuable trees were being rapidly removed. About this time the first Hmong villages were established on the mountain. The upland forests were largely destroyed for agricultural expansion and the establishment of various "development" projects. Presently there are over 5500 hill-tribe people, mostly Hmong, living on the mountain [8].

Doi Sutep-Pui National Park was established on 14 April 1981 and since that time the amount of forest cover has steadily decreased along with increases in erosion and water turbidity. Wildlife has also been severely reduced to the extent that no large mammals or primates are present. The lowland vegetation on Doi Sutep-Pui, i.e. below *c*. 850 m, is deciduous dipterocarp-oak, seasonal hardwood forest (dof) which remains in various stages of degradation including that in the Chang Kian Valley, which is on the lower slopes on the east side of the national park [7]. Control of exploitation of natural resources on the mountain and attempts to properly reforest destroyed areas or control water pollution in settled places seem to have been unsuccessful, and although a fire control unit exists in the national park, fires still destroy several hundred hectares of vegetation each year.

Vegetation and Fire Threat

Lowland deciduous forests include deciduous, seasonal, hardwood + bamboo forest (bb/df) and deciduous dipterocarp-oak, seasonal, hardwood forest (dof)—the latter often incorrectly being referred to as "savanna" forest [9-10]. Areas of bb/df in Thailand began to be commercially exploited for valuable timber trees, especially teak (*Tectona grandis* L. f., Verbenaceae) and other hardwood trees, from about 1870 [11]. Presently there remains only one area in Thailand in Mae Yom National Park, Phrae province, which has natural teak populations [12-13]. This area has also been illegally logged for decades and repeatedly threatened with a dam construction project. The reservoir of this dam would inundate a teak-dominated area up to an elevation of 260 m. This would destroy much of the remaining teak habitat in the country [13]. Natural teak in Doi Sutep-Pui National Park is rare and is only known from a few individuals below Pra Taht Temple at 850 m on the east side of the mountain.

Destroyed bb/df is succeeded by dof, not by bb/df [7, 12]. This is enhanced by fire which not only prevents bb/df vegetation to develop, but also destroys organics in the soil. With consequent degradation of soil quality and erosion, only species from dof are able to establish and grow. Dof is a

kind of fire-climax or edaphic-climax facies which has also been considered as fire-dependent secondary growth [14]. Research by Stott on fire in dof in Thailand [9-10] indicates that, depending on the amount of dry ground litter present, the average ground temperature during fire can reach 388°C. At 0.5-1 m high, the average is 169°C with an amazing 700-900°C in thicker vegetation. At 5-cm depth the temperature never exceeded 75°C. A typical fire moves at a rate of 1.6 cm/second. The cremating effect of fire on vegetation, soil organics and underground biodiversity along with erosion, human encroachment and lack of any conservation or protection have maintained dof forest in the region for centuries. Destruction of dof will result in perpetual deciduous secondary scrub or tertiary grassland vegetation [14]. The districts of Mae Awn and Sarapee, both in Chiang Mai province, and adjacent Lampoon area are good examples of this deplorable situation.

A detailed study by Putnam [15] during February-March 2009 in On Nuea subdistrict, c. 45 km SE of Chiang Mai University, revealed the causes and effects of burning in lowland areas. Fires that occurred in that area can be classed into 5 categories: 1) forest fire, 2) agricultural burining, 3) roadside burning, 4) open area fire and 5) domestic burning. Forest fires are deliberately set mainly for the supposedly increased forest product yields, especially the earth star mushroom (Astreaus hygrometricus (Pers.) Morgan, or "Hed Thob" or "Hed Phor" in Thai), which has seasonal availability and a high market price. In order to collect such products local farmers use fire either to clear the forest floor to make it easier to find the mushroom or because the fire is believed to stimulate the growth of this mushroom. "Cheap and fast" is a crucial explanation for the intentional use of fire to clear agricultural fields, overgrown roadsides and open areas. Cattle herders also burn areas to stimulate the growth of Imperata grass which is able to quickly produce new leaves during the hot-dry season. New leaves produced on burnt areas have a higher nutrient value, which is perfect for cattle grazing. Domestic burning involves the burning of both natural and refuse wastes from households. Various local administrative officers correctly admit that all fire incidents are man-made. Fires produce large amounts of smoke which often stagnates in the community area causing eye irritation and respiratory ailments. Large areas of degraded forest are destroyed by fire each year. The situation in On Nuea, as on Doi Sutep-Pui, has come to a point where fire contributes to the dry season haze, which has been a serious threat for both human health and environmental quality of Chiang Mai for years.

Objectives

The aim of this study is to assess the present condition of a forest as well as determine how the vegetation should be conserved and replanted. The lowland dof in Chang Kian Valley has been regularly burned and exploited for centuries. This has resulted in a very degraded facies. To get a proper understanding of the actual condition of the lowland deciduous dipterocarp-oak forest in Chang Kian Valley, this ecological survey is initiated. The actual condition of the dof there seems to vary. So in order to determine the range of difference, an intense ecological-taxonomic study of several different dof areas is required. The specific effects of fire on plant diversity, forest structure and seed recruitment also need to be determined to gauge the overall condition of the dof in the valley.

Methodology

Vegetation sampling

The survey was conducted during October-December 2008. This was just after the rainy season when the vegetation was most luxurious. Due to time and budget constraints, the survey team selected seven areas as initial sites (Table 1) which could be used to rapidly assess the entire valley. Each survey plot was 50x5 m where all woody species (including seedlings) were identified and measured for height and canopy width. DBH (diameter at breast height) of a tree was measured according to European/Canadian standard at 1.3 m [16], whereas all other woody plants (seedlings, saplings, treelets, shrubs, woody climbers and coppices) were measured at ground level. A distinction was made between seedlings and coppices since the ratio of these two growth forms is a good indicator of the destructive effects of fire. Seedlings are cremated by fire while many saplings and treelets are burned

and able to regrow at the base. Growth deformities and fire damage to larger woody individuals were also noted. For the ground flora, i.e. all plants up to 1 m tall, a 10-m diameter circular plot was made 2 m from the corner of each plot. Photographs of some sites are shown in Appendix 1.

Site	Elevation (metres)	Description
1. Pah Laht Temple	607	The most intact forest which has been protected from major disturbance for more than 50 years* (Photo 8)
2. Chang Kian Stream	474	Above the boy scout camp near Chang Kian Village and close to Chang Kian Stream, severely degraded and frequently burned by mushroom collectors (Photos 4 & 8)
3. Mae Yuak Noi 1	455	Near Nong Haw meditation centre, a regenerating forest which has uniform tree regrowth after being cleared 25 years ago (Photos 2, 3 & 5)
4. Mae Yuak Noi 2	490	Near site 3, but with more grass cover (Photos 6 & 7)
5. Huay Dtueng Tao 1	439	Above Huay Dteung Tao Lake, a very exposed, frequently burned, very eroded ridge (Photo 1)
6. Huay Dtueng Tao 2	453	Near site 5 and similar to it, but with more trees (Photo 1)
7. Huay Dtueng Tao 3	411	Gully below site 6 with less frequent fire than site 5, almost closed canopy

Table 1.	Survey plot	locations and	descriptions
		10	

* Since a similar site could not be found in Chang Kian Valley, we selected Pah Laht Temple to represent the best dof conditions. This place is located in an adjacent valley, c. 2 km south of site 2.

To study the ground flora, i.e. all plants up to 1 m tall, a 10-m diameter circular plot was made 2 m from each corner of the plot. A diagram of the survey plot is shown in Figure 1.



Figure 1. Diagram of the survey plot

Information from the circular plots include listing of all species, individual abundance and total ground cover vs. bare soil. Abundance was estimated using a modified Braun-Blanquet cover class scale [17] where: x = sparse, few individuals; 1 = common, small % coverage; 2 = covering 5 % of the area; 3 = covering 25-50 %; 4 = covering 50-75 %; and 5 = covering more than 75 %.

Ecological parameters

To properly assess the overall health of forested areas, several parameters were used. These factors include: species diversity and their abundances, canopy height and tree density, understory and ground flora densities, seedlings and coppices, soil erosion, and soil quality. These parameters will be explained in relation to the condition of the plots.

Results and Discussion

Forest structure diagrams

The vegetation data from the sampling plots including the botanical name, number of species and maximum height were used to construct the forest diagram for vegetation layer analysis of each plot. Figures 2-8 show the forest profile diagrams at the 7 sampling sites. The details for each species in the diagram is shown in Tables 2-8.



Figure 2. Forest profile diagram of site 1 (Pah Laht Temple)

Table 2. Species found in site 1 (Pah Laht Temple)
(Abbreviations for habit: L = treelet, S = shrub, T = tree, WC = woody climber)

N.	Determined	E	TT-1.14	M
NO.	Botanical name	Family	Habit	
1		т ·	WG	height (m)
1	Acacia megaladena Desv. var. megaladena	Leguminosae,	WC	0.24
		Mimosoideae	wa	1.0.0
2	Amphineurion (Aganosma) marginata (Roxb.)	Apocynaceae	WC	4.00
	D.J. Midd.			
3	Anneslea fragrans Wall.	Theaceae	Т	5.50
4	Antidesma acidum Retz.	Euphorbiaceae	Т	3.50
5	Aporosa octandra (BH ex D. Don) Vick. var.	Euphorbiaceae	Т	3.00
	octandra			
6	Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	Т	5.00
7	<i>Breynia glauca</i> Craib	Euphorbiaceae	S	4.00
8	Bridelia stipularis (L.) Bl.	Euphorbiaceae	WC	1.80
9	Buchanania lanzan Spreng.	Anacardiaceae	Т	12.00
10	Calycopteris floribunda (Roxb.) Lmk.	Combretaceae	WC	0.65
11	Canarium subulatum Guill.	Burseraceae	Т	2.50
12	Cansjera rheedii J. F. Gmel.	Opilliaceae	WC	15.00
13	Celastrus paniculatus Willd.	Celastraceae	WC	3.00
14	Cissus hastata Mig.	Vitaceae	WC	5.00
15	Cissus repanda Vahl	Vitaceae	WC	0.41
16	<i>Clausena excavata</i> Burm.f. var. <i>excavata</i>	Rutaceae	L	1.20
17	Craibiodendron stellatum (Pierre) W.W.Sm.	Ericaceae	Т	5.50
18	Dalbergia cultrata Grah. ex Bth.	Leguminosae,	Т	7.00
	5	Papilionoideae		
19	Desmos dumosus (Roxb.) Saff. var. glabrior	Annonaceae	WC	1.23
	Craib			
20	Dimocarpus longan Lour. ssp. longan var.	Sapindaceae	Т	0.70
	longan	_		
21	Diospyros ehretioides Wall. ex G.Don	Ebenaceae	Т	3.50
22	Dipterocarpus obtusifolius Teijsm. ex Miq. var.	Dipterocarpaceae	Т	20.00
	obtusifolius			
23	Dipterocarpus tuberculatus Roxb. var.	Dipterocarpaceae	Т	1.40
	tuberculatus			
24	Elaeocarpus floribundus Bl. var. floribundus	Elaeocarpaceae	Т	0.79
25	Erythroxylum cuneatum (Miq.) Kurz	Erythroxylaceae	S	1.40
26	Eugenia albiflora Duth. ex Kurz	Myrtaceae	Т	3.00
27	Eugenia grata Wight var. grata	Myrtaceae	Т	10.00
28	Ficus hirta Vahl	Moraceae	Т	1.23
29	Flacourtia indica (Burm.f.) Merr.	Flacourtiaceae	Т	0.24
30	Gardenia sootepensis Hutch.	Rubiaceae	Т	2.50
31	Goniothalamus griffithii Hk.f. & Thoms.	Annonaceae	L	3.50
32	Irvingia malayana Oliv. ex A. Benn.	Irvingiaceae	Т	0.64
33	<i>Ixora cibdela</i> Craib	Rubiaceae	L	1.14
34	Lannea coromandelica (Houtt.) Merr.	Anarcardiceae	Т	3.00
35	Leea indica (Burm.f.) Merr.	Leeaceae	L	0.69

Table 2. (Continued)

No.	Botanical name	Family	Habit	Max.
		-		height (m)
36	Lithocarpus polystachyus (Wall. ex A. DC.)	Fagaceae	Т	12.00
	Rehd.			
37	Memecylon scutellatum (Lour.) Hk. & Arn.	Melastomaceae	L	2.50
38	Memecylon umbellatum Burm.f.	Melastomaceae	L	6.00
39	Millettia extensa Bth. ex Baker	Leguminosae,	WC	0.74
		Papilionoideae		
40	Mitragyna rotundifolia (Roxb.) O.K.	Rubiaceae	Т	5.00
41	Myxopyrum smilacifolium (Wall.) Bl. var.	Oleaceae	WC	0.34
	smilacifolium			
42	Ochna integerrima (Lour.) Merr.	Ochnaceae	Т	2.30
43	Pavetta tomentosa Roxb. ex Sm. var. tomentosa	Rubiaceae	L	0.75
44	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Т	1.18
45	Prismatomeris tetrandra (Roxb.) K. Sch. ssp.	Rubiaceae	L	1.50
	tetrandra			
46	Quercus kerrii Craib	Fagaceae	Т	8.00
47	Rothmannia sootepensis (Craib) Brem.	Rubiaceae	Т	5.00
48	Schima wallichii (DC.) Korth.	Theaceae	Т	0.09
49	Scleropyrum pentandrum (Denn.) Mabb.	Santalaceae	Т	7.00
50	Shorea obtusa Wall. ex Bl.	Dipterocarpaceae	Т	16.00
51	Shorea siamensis Miq. var. siamensis	Dipterocarpaceae	Т	12.00
52	Spatholobus parviflorus (Roxb.) O.K.	Leguminosae,	WC	6.00
		Papilionoideae		
53	Stereospermum colais (BH. ex Dillw.) Mabb.	Bignoniaceae	Т	0.40
54	Symplocos racemosa Roxb.	Symplocaceae	L	0.36
55	Tarennoidea wallichii (Hk.f.) Tirv. & Sastre	Rubiaceae	Т	0.59
56	Terminalia alata Hey. ex Roth	Combretaceae	Т	3.00
57	Tetrastigma leucostaphyllum (Denn.) Mabb.	Vitaceae	WC	23.00
58	Tristaniopsis burmanica (Griff.) Wils. & Wat.	Myrtaceae	L, T	1.40
	var. rufescens (Hance) Parn. & Lug.			
59	Vernonia parishii Hk.f.	Compositae	L	1.10
60	Vitex limoniifolia Wall. ex Kurz	Verbenaceae	Т	3.50
61	Vitex peduncularis Wall. ex Schauer	Verbenaceae	Т	5.00



Figure 3. Forest profile diagram of site 2 (Chang Kian Stream)

Table 3. Species foun in site 2 (Chang Kian Stream)
(Abbreviations for habit: L = treelet, S = shrub, T = tree, WC = woody climber)

No	Potenical nama	Eamily	Uabit	Mov
INO.	Dotanical name	Failiny	пари	Iviax.
				height (m)
1	Amphineurion (Aganosma) marginata (Roxb.)	Apocynaceae	WC	2.00
	D.J. Midd.			
2	Anneslea fragrans Wall.	Theaceae	Т	1.60
3	Antidesma acidum Retz.	Euphorbiaceae	Т	0.17
4	Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	Т	0.83
5	Breynia glauca Craib	Euphorbiaceae	S	1.15
6	Bridelia stipularis (L.) Bl.	Euphorbiaceae	WC	0.84
7	Buchanania lanzan Spreng.	Anacardiaceae	Т	9.00
8	Calycopteris floribunda (Roxb.) Lmk.	Combretaceae	WC	0.37
9	Canarium subulatum Guill.	Burseraceae	Т	1.70
10	Catunaregum spathulifolia Tirv.	Rubiaceae	L	1.48
11	Catunaregum tomentosa (Bl. ex DC.) Tirv.	Rubiaceae	L	0.69
12	Celastrus paniculatus Willd.	Celastraceae	WC	0.45
13	Clerodendrum serratum (L.) Moon var.	Verbenaceae	L	0.62
	wallichii Cl.			

Table 3. (Continued)

NT		F 1	TT 1 '4	M
NO.	Botanical name	Family	Habit	Max.
				height (m)
14	Craibiodendron stellatum (Pierre) W.W.Sm.	Ericaceae	T	5.50
15	Dalbergia cultrata Grah. ex Bth.	Leguminosae,	Т	2.50
		Papilionoideae		
16	Desmodium oblongum Bth.	Leguminosae,	L	2.50
		Papilionoideae		
17	Dillenia parviflora Griff. var. kerrii (Craib)	Dilleniaceae	Т	1.10
	Hoogl.		_	
18	Diospyros ehretioides Wall. ex G.Don	Ebenaceae	T	1.25
19	Dipterocarpus obtusifolius Teijsm. ex Miq.	Dipterocarpaceae	Т	15.00
•	var. obtusifolius	D		- 0.0
20	Dipterocarpus tuberculatus Roxb. var.	Dipterocarpaceae	T	7.00
	tuberculatus		G	0.00
21	Ellipeiopsis cherrevensis (Pierre ex Finet &	Annonaceae	S	0.89
	Gagnep.) R.E.Fr.			1.00
22	<i>Embelia tsjeriamcottam</i> (Roem. & Schult.) A.	Myrsinaceae	WC	1.20
	DC. var. tsjeriamcottam		G	0.57
23	Erythroxylum cuneatum (Mıq.) Kurz	Erythroxylaceae	S	0.57
24	Eugenia cumini (L.) Druce	Myrtaceae	L	8.00
25	Gardenia obtusifolia Roxb. ex Kurz	Rubiaceae		0.90
26	<i>Glochidion eriocarpum</i> Champ.	Euphorbiaceae	Т	0.68
27	<i>Grewia abutilifolia</i> Vent. <i>ex</i> Juss.	Tiliaceae	L	1.00
28	<i>Grewia lacei</i> Drum. & Craib	Tiliaceae	L	1.60
29	Hibiscus glanduliferus Craib	Malvaceae	S	1.14
30	Indigofera cassioides Rottl. ex DC.	Leguminosae,	S	2.00
		Papilionoideae		
31	<i>Ixora cibdela</i> Craib	Rubiaceae	L	0.32
32	Leea indica (Burm.f.) Merr.	Leeaceae	L	1.00
33	Linostoma persimile Craib	Thymelaeaceae	S	4.00
34	Lithocarpus polystachyus (Wall. ex A.DC.)	Fagaceae	Т	18.00
	Rehd.			
35	Lophopetalum wallichii Kurz	Celastraceae	Т	1.70
36	Mitragyna hirsuta Hav.	Rubiaceae	Т	3.00
38	Pavetta fruticosa Craib	Rubiaceae	S	0.38
39	Phoenix loureiri Kunth var. loureiri	Palmae	L	1.05
40	Premna nana Coll. & Hemsl.	Verbenaceae	S	1.00
41	Quercus kerrii Craib	Fagaceae	Т	7.00
42	Shorea obtusa Wall. ex Bl.	Dipterocarpaceae	Т	12.00
43	Shorea siamensis Miq. var. siamensis	Dipterocarpaceae	Т	1.12
44	Spatholobus parviflorus (Roxb.) O.K.	Leguminosae,	WC	4.00
		Papilionoideae		
45	Stereospermum colais (BH. ex Dillw.) Mabb.	Bignoniaceae	Т	0.20
46	Stereospermum neuranthum Kurz	Bignoniaceae	Т	0.40
47	Terminalia alata Heyne ex Roth	Combretaceae	Т	1.70
48	Tristaniopsis burmanica (Griff.) Wils. & Wat.	Myrtaceae	Т	2.00
	var. rufescens (Hance) Parn. & Lug.			
49	Vitex peduncularis Wall. ex Schauer	Verbenaceae	Т	0.90
50	Wendlandia tinctoria (Roxb.) DC. ssp.	Rubiaceae	Т	2.50
	floribunda (Craib) Cow.			



Figure 4. Forest profile diagram of site 3 (Mae Yuak Noi 1)

Table 4. Species found in site 3 (Mae Yuak Noi 1)
(Abbreviations for habit: L = treelet, S = shrub, T = tree, WC = woody climber)

No.	Botanical name	Family	Habit	Max.
				height (m)
1	Amphineurion (Aganosma) marginata (Roxb.) D.J.	Apocynaceae	WC	0.56
	Midd.			
2	Anneslea fragrans Wall.	Theaceae	Т	8.00
3	Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	Т	7.00
4	Bridelia stipularis (L.) Bl.	Euphorbiaceae	WC	0.19
5	Buchanania glabra Wall. ex Hk.f.	Anacardiaceae	Т	0.70
6	Buchanania lanzan Spreng.	Anacardiaceae	Т	5.00
7	Canarium subulatum Guill.	Burseraceae	Т	2.20
8	<i>Cansjera rheedii</i> J. F. Gmel.	Opilliaceae	WC	1.00
9	Catunaregum spathulifolia Tirv.	Rubiaceae	L	1.50
10	Craibiodendron stellatum (Pierre) W.W.Sm.	Ericaceae	Т	6.00
11	Dalbergia cultrata Grah. ex Bth.	Leguminosae,	Т	5.00
		Papilionoideae		
12	Dalbergia oliveri Gamb. ex Prain	Leguminosae,	Т	0.60
		Papilionoideae		

Table 4. (Continued)

No.	Botanical name	Family	Habit	Max.
				height (m)
13	Dillenia parviflora Griff. var. kerrii (Craib) Hoogl.	Dilleniaceae	Т	2.00
14	Dipterocarpus obtusifolius Teijsm. ex Miq. var.	Dipterocarpaceae	Т	12.00
	obtusifolius			
15	Dipterocarpus tuberculatus Roxb. var.	Dipterocarpaceae	Т	12.00
	tuberculatus			
16	Ellipeiopsis cherrevensis (Pierre ex Finet &	Annonaceae	S	1.18
	Gagnep.) R.E.Fr.			
17	Erythroxylum cuneatum (Miq.) Kurz	Erythroxylaceae	S	0.67
18	<i>Flacourtia indica</i> (Burm.f.) Merr.	Flacourtiaceae	Т	0.25
19	<i>Gardenia obtusifolia</i> Roxb. <i>ex</i> Kurz	Rubiaceae	L	0.48
20	Gluta usitata (Wall.) Hou	Anacardiaceae	Т	0.73
21	Grewia abutilifolia Vent. ex Juss.	Tiliaceae	L	5.00
22	Grewia lacei Drum. & Craib	Tiliaceae	L	0.60
23	Hibiscus glanduliferus Craib	Malvaceae	S	0.60
24	Irvingia malayana Oliv. ex A. Benn.	Irvingiaceae	Т	2.20
25	<i>Ixora cibdela</i> Craib	Rubiaceae	L	0.83
26	Lagerstroemia cochinchinensis Pierre var.	Lythraceae	Т	2.50
	ovalifolia Furt. & Mont.			
27	Leea indica (Burm.f.) Merr.	Leeaceae	Т	0.90
28	Lithocarpus polystachyus (A. DC.) Rehd.	Fagaceae	Т	0.90
29	Lophopetalum wallichii Kurz	Celastraceae	Т	0.11
30	Mammea siamensis (Mig.) T. And.	Guttifereae	Т	0.80
31	Memecvlon scutellatum (Lour.) Hk. & Arn.	Melastomaceae	L	0.13
32	Mitragyna hirsuta Hay.	Rubiaceae	Т	1.60
33	Ochna integerrima (Lour.) Merr.	Ochnaceae	Т	0.79
34	Pavetta fruticosa Craib	Rubiaceae	S	0.60
35	Phoenix loureiri Kunth var loureiri	Palmaceae	Ľ	0.45
36	Phyllanthus emblica L	Euphorbiaceae	T	0.55
37	Premna nana Coll & Hemsl	Verbenaceae	S	1 10
38	Quercus kerrii Craib	Fagaceae	T	0.80
39	Shorea obtusa Wall ex Bl	Dipterocarpaceae	T	1.60
40	Shorea sigmensis Mig. var. sigmensis	Dipterocarpaceae	T	11.00
40	Spatholobus parviflorus (Roxh) O K	Leguminosae	WC	20.00
41	Spainolobus par vijiorus (Roxo.) O.K.	Papilionoideae	we	20.00
42	Stereospermum colais (B-H er Dillw) Mabb	Bignoniaceae	Т	3.00
12	Stevelyos mur-vomica I	Loganiaceae	T	0.27
43	Supplocos racemosa Boxh	Symplocaceae	I	1 90
44	Terminalia alata Houne or Poth	Combretaceae		1.90
43	Terminalia chebula Potz vor chebula	Combretaceae	T	0.75
40	Tristanionsis hurmanica (Criff) Wile & Wat yer	Murtaceae		0.75
4/	rufascans (Hance) Parn & Lug	wrynaceae	1	0.93
10	Viter limoniifolia Woll or Virg	Varbanasaa	т	8.00
48	Vitex natural aris Wall or Schemer	Verbenaceae		0.00
49	Wondlandia tinotonia (Dorb.) DC and dorite a d	verbenaceae Dubiogogia		1.30
- 50	(Craib) Cow	Rublaceae	1	0.80
1		1	1	1



Figure 5. Forest profile diagram of site 4 (Mae Yuak Noi 2)

Table 5. Species found in site 4 (Mae Yuak Noi 2)
(Abbreviations for habit: L = treelet, S = shrub, T = tree, WC = woody climber)

No.	Botanical name	Family	Habit	Max.
				height (m)
1	Albizia odoratissima (L.f.) Bth.	Leguminosae,	Т	0.34
		Mimosoideae		
2	Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	Т	4.50
3	Buchanania glabra Wall. ex Hk.f.	Anacardiaceae	Т	2.00
4	Buchanania lanzan Spreng.	Anacardiaceae	Т	5.00
5	Canarium subulatum Guill.	Burseraceae	Т	1.40
6	Catunaregum spathulifolia Tirv.	Rubiaceae	L	1.05
7	Craibiodendron stellatum (Pierre) W.W.Sm.	Ericaceae	Т	1.30
8	Dillenia parviflora Griff. var. kerrii (Craib)	Dilleniaceae	Т	0.75
	Hoogl.			
9	Diospyros ehretioides Wall. ex G.Don	Ebenaceae	Т	0.40
10	Dipterocarpus obtusifolius Teijsm. ex Miq.	Dipterocarpaceae	Т	10.00
	var. obtusifolius			
11	Dipterocarpus tuberculatus Roxb. var.	Dipterocarpaceae	Т	6.00
	tuberculatus			
12	Ellipeiopsis cherrevensis (Pierre ex Finet &	Annonaceae	S	0.14
	Gagnep.) R.E.Fr.			

Table	5.	(Continu	ed)
		(

No.	Botanical name	Family	Habit	Max.
				height (m)
13	Eugenia cumini (L.) Druce	Myrtaceae	Т	12.00
14	Garcinia cowa Roxb. ex DC.	Guttifereae	Т	0.12
15	Gardenia obtusifolia Roxb. ex Kurz	Rubiaceae	L	2.00
16	Gluta usitata (Wall.) Hou	Anacardiaceae	Т	0.75
17	Leea indica (Burm.f.) Merr.	Leeaceae	L	0.40
18	Lophopetalum wallichii Kurz	Celastraceae	Т	0.90
19	Memecylon scutellatum (Lour.) Hk. & Arn.	Melastomaceae	L	0.80
20	Ochna integerrima (Lour.) Merr.	Ochnaceae	Т	0.70
21	Parinari anamense Hance	Rosaceae	Т	0.20
22	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Т	0.85
23	Premna nana Coll. & Hemsl.	Verbenaceae	S	0.07
24	Quercus kerrii Craib	Fagaceae	Т	9.00
25	Shorea obtusa Wall. ex Bl.	Dipterocarpaceae	Т	10.00
26	Shorea siamensis Miq. var. siamensis	Dipterocarpaceae	Т	2.50
27	Spatholobus parviflorus (Roxb.) O.K.	Leguminosae,	WC	5.00
		Papilionoideae		
28	Stereospermum neuranthum Kurz	Bignoniaceae	Т	0.30
29	<i>Strychnos nux-vomica</i> L.	Loganiaceae	L	3.00
30	Symplocos racemosa Roxb.	Symplocaceae	Т	1.60
31	Terminalia alata Heyne ex Roth	Combretaceae	Т	0.95
32	Terminalia chebula Retz. var. chebula	Combretaceae	Т	0.95
33	Tristaniopsis burmanica (Griff.) Wils. & Wat.	Myrtaceae	Т	3.50
	var. rufescens (Hance) Parn. & Lug.			
34	Vitex limoniifolia Wall. ex Kurz	Verbenaceae	Т	0.60



Figure 6. Forest profile diagram of site 5 (Huay Dtueng Tao 1)

Table 6. Species found in site 5 (Huay Dtueng Tao 1)(Abbreviations for habit: L = treelet, S = shrub, T = tree, WC = woody climber)

No.	Botanical name	Family	Habit	Max.
				height (m)
1	Amphineurion (Aganosma) marginata	Apocynaceae	WC	0.08
	(Roxb.) D.J. Midd.			
2	Aporosa villosa (Lindl.) Baill	Euphorbiaceae	Т	2.50
3	Blinkworthia lycioides Choisy	Convulvulaceae	S	0.71
4	Bridelia stipularis (L.) Bl.	Euphorbiaceae	WC	0.54
5	Buchanania glabra Wall. ex Hk.f.	Anacardiaceae	Т	7.00
6	Buchanania lanzan Spreng.	Anacardiaceae	Т	6.00
7	Canarium subulatum Guill.	Burseraceae	Т	0.64
8	Catunaregum spathulifolia Tirv.	Rubiaceae	L	0.05
9	Celastrus paniculatus Willd.	Celastraceae	WC	0.73
10	Cratoxylon formosum (Jack) Dyer ssp.	Guttifereae	Т	0.20
	pruniflorum (Kurz) Gog.			
11	Dalbergia cultrata Grah. ex Bth.	Leguminosae,	Т	0.18
		Papilionoideae		
12	Dillenia parviflora Griff. var. kerrii (Craib)	Dilleniaceae	Т	0.22
	Hoogl.			

Table 6. (Continued)

No.	Botanical name	Family	Habit	Max.
				height (m)
13	Dipterocarpus obtusifolius Teijsm. ex Miq.	Dipterocarpaceae	Т	0.08
	var. obtusifolius			
14	Dipterocarpus tuberculatus Roxb. var.	Dipterocarpaceae	Т	12.00
	tuberculatus			
15	Eugenia cumini (L.) Druce	Myrtaceae	Т	7.00
16	Gardenia obtusifolia Roxb. ex Kurz	Rubiaceae	L	1.60
17	Glochidion eriocarpum Champ.	Euphorbiaceae	L	0.36
18	Grewia abutilifolia Vent. ex Juss.	Tiliaceae	S	0.90
19	Hibiscus glanduliferus Craib	Malvaceae	L	0.40
20	Meyna velutina Roby.	Rubiaceae	L	0.72
21	Ochna integerrima (Lour.) Merr.	Ochnaceae	Т	0.53
22	Pavetta fruticosa Craib	Rubiaceae	S	0.09
23	Phoenix loureiri Kunth var. loureiri	Palmae	L	0.69
24	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Т	0.75
25	Quercus kerrii Craib	Fagaceae	Т	0.07
26	Shorea obtusa Wall. ex Bl.	Dipterocarpaceae	Т	8.00
27	Symplocos racemosa Roxb.	Symplocaceae	L	1.05
28	Terminalia alata Heyne ex Roth	Combretaceae	Т	1.30
29	Tristaniopsis burmanica (Griff.) Wils. &	Myrtaceae	Т	1.50
	Wat. var. rufescens (Hance) Parn. & Lug.			
30	Vitex peduncularis Wall. ex Schauer	Verbenaceae	Т	0.23
31	Wendlandia tinctoria (Roxb.) DC. ssp.	Rubiaceae	Т	0.90
	floribunda (Craib) Cow.			



Figure 7. Forest profile diagram of site 6 (Huay Dtueng Tao 2)

Table 7. Species found in site 6 (Huay Dtueng Tao 2)(Abbreviations for habit: L = treelet, S = shrub, T = tree, WC = woody climber)

No.	Botanical name	Family	Habit	Max.
				height (m)
1	Acacia megaladena Desv. var. megaladena	Leguminosae,	WC	0.10
		Mimosoideae		
2	Albizia odoratissima (L.f.) Bth.	Leguminosae,	Т	0.80
		Mimosoideae		
3	Amphineurion (Aganosma) marginata (Roxb.) D.J.	Apocynaceae	WC	1.60
	Midd.			
4	Anneslea fragrans Wall.	Theaceae	Т	0.65
5	Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	Т	8.00
6	Blinkworthia lycioides Choisy	Convulvulaceae	S	0.87
7	Buchanania glabra Wall. ex Hk.f.	Anacardiaceae	Т	0.22
8	Buchanania lanzan Spreng.	Anacardiaceae	Т	1.00
9	Calycopteris floribunda (Roxb.) Lmk.	Combretaceae	WC	0.60
10	Canarium subulatum Guill.	Burseraceae	Т	0.22

Table 7. (Continued)

No.	Botanical name	Family	Habit	Max.		
				height (m)		
11	Catunaregum spathulifolia Tirv.	Rubiaceae	L	1.10		
12	Celastrus paniculatus Willd.	Celastraceae	WC	0.05		
13	Cratoxylon formosum (Jack) Dyer ssp.	Guttifereae	Т	1.80		
	pruniflorum (Kurz) Gog.					
14	Desmodium oblongum Bth.	Leguminosae,	L	2.00		
		Papilionoideae				
15	Dillenia parviflora Griff. var. kerrii (Craib) Hoogl.	Dilleniaceae	Т	3.00		
16	Diospyros ehretioides Wall. ex G. Don	Ebenaceae	Т	0.60		
17	Dipterocarpus obtusifolius Teijsm. ex Miq. var.	Dipterocarpaceae	Т	12.00		
	obtusifolius					
18	Dipterocarpus tuberculatus Roxb. var.	Dipterocarpaceae	Т	15.00		
	tuberculatus					
19	Erythroxylon cuneatum (Miq.) Kurz	Erythroxylaceae	S	0.90		
20	Eugenia cumini (L.) Druce	Myrtaceae	Т	1.20		
21	Gardenia obtusifolia Roxb. ex Kurz	Rubiaceae	L	0.80		
22	Glochidion eriocarpum Champ.	Euphorbiaceae	L	0.52		
23	Hibiscus glanduliferus Craib	Malvaceae	L	0.90		
24	<i>Ixora cibdela</i> Craib	ra cibdela Craib Rubiaceae				
25	Lagerstroemia macrocarpa Kurz var. macrocarpa	Т	0.91			
26	Leea indica (Burm.f.) Merr. Leeaceae		Т	0.62		
27	Memecylon scutellatum (Lour) Hk & Arn Melastomaceae		L	0.80		
28	Millettia extensa Bth ex Baker Legumi		WC	1 60		
		Papilionoideae		1100		
29	Mitragyna hirsuta Hay.	Rubiaceae	Т	8.00		
30	Ochna integerrima (Lour.) Merr.	Ochnaceae	Т	0.30		
31	Phoenix loureiri Kunth var loureiri	Arecaceae	L	2 00		
32	Phyllanthus emblica L.	Euphorbiaceae	T	0.27		
33	Pterocarpus macrocarpus Kurz	Leguminosae.	T	1.00		
		Papilionoideae				
34	<i>Ouercus kerrii</i> Craib	Fagaceae	Т	8.00		
35	Shorea obtusa Wall. ex Bl.	Dipterocarpaceae	Т	7.00		
36	Shorea siamensis Mig. var. siamensis	Dipterocarpaceae	Т	1.00		
37	Spatholobus parviflorus (Roxb.) O.K.	Leguminosae.	WC	1.92		
		Papilionoideae				
38	<i>Strvchnos nux-vomica</i> L.	Loganiaceae	Т	0.50		
39	Symplocos racemosa Roxb.	Symplocaceae	L	0.63		
40	<i>Terminalia alata</i> Hevne <i>ex</i> Roth	Combretaceae	Т	17.00		
41	Terminalia chebula Retz, var. chebula	Combretaceae	Т	8.00		
42	Tristaniopsis burmanica (Griff) Wils & Wat var	Myrtaceae	T	7.50		
-	rufescens (Hance) Parn. & Lug.	,	-			
43	Ventilago denticulata Willd.	Rhamnaceae	WC	0.05		
44	Vitex peduncularis Wall ex Schauer	Verbenaceae	Т	1.10		
45	Walsura trichostemon Mig	Meliaceae	T	0.90		
46	Wendlandia tinctoria (Roxh) DC ssp floribunda	Rubiaceae	T	4.00		
	(Craib) Cow.		-			



Figure 8. Forest profile diagram of site 7 (Huay Dtueng Tao 3)

Table 8. Species found in site 7 (Huay Dtueng Tao 3) (Abbreviations for habit: B = bamboo, L = treelet, S = shrub, T = tree, WC = woody climber)

No.	Botanical name	Family	Habit	Max.
				height (m)
1	Albizia odoratissima (L.f.) Bth.	Leguminosae,	Т	0.80
		Mimosoideae		
2	Amphineurion (Aganosma) marginata (Roxb.) D.J.	Apocynaceae	WC	0.62
	Midd.			
3	Anneslea fragrans Wall.	Theaceae	Т	0.50
4	Antidesma acidum Retz.	Euphorbiaceae	Т	6.00
5	Aporosa octandra (BH ex D.Don) Vick. var.	Euphorbiaceae	Т	0.90
	octandra			
6	Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	Т	0.53
7	Bridelia stipularis (L.) Bl.	Euphorbiaceae	WC	0.72
8	Buchanania glabra Wall. ex Hk.f.	Anacardiaceae	Т	20.00
9	Canarium subulatum Guill.	Burseraceae	Т	1.20

Table 8. (Continued)

r				
No.	Botanical name	Family	Habit	Max.
				height (m)
10	Cansjera rheedii J. F. Gmel.	Opilliaceae	WC	0.90
11	Catunaregum spathulifolia Tirv.	Rubiaceae	L	0.82
12	Celastrus paniculatus Willd.	Celastraceae	WC	0.50
13	Cissus repens Lmk.	Vitaceae	WC	4.00
14	Clausena excavata Burm.f. var. excavata	Rutaceae	L	0.50
15	Clerodendrum serratum (L.) Moon var. wallichii	Verbenaceae	L	0.48
16			T	1.10
16	Colona flagrocarpa (CI.) Craib			1.10
1/	(Kurz) Gog.	Guttifereae	1	1.60
18	Dalbergia cana Grah. ex Kurz var. cana	Leguminosae,	Т	0.65
		Papilionoideae		
19	Dalbergia cultrata Grah. ex Bth.	Leguminosae,	Т	22.00
		Papilionoideae		
20	Dendrocalamus nudus Pilg.	Gramineae,	В	8.00
		Bambusoideae		
21	Dillenia parviflora Griff. var. kerrii (Craib) Hoogl.	Dilleniaceae	Т	0.38
22	Dimocarpus longan Lour. ssp. longan var. longan	Sapindaceae	Т	0.75
23	Diospyros ehretioides Wall. ex G.Don	Ebenaceae	Т	0.61
24	Dipterocarpus obtusifolius Teijsm. ex Miq. var.	Dipterocarpaceae	Т	0.90
	obtusifolius	tusifolius		
25	Embelia tsjeriamcottam (Roem. & Schult.) A. DC.	Myrsinaceae	WC	0.80
	var. tsjeriamcottam			
26	Erythroxylum cuneatum (Miq.) Kurz	Erythroxylaceae	S	0.95
27	Eugenia albiflora Duth. ex Kurz	Myrtaceae	Т	0.20
28	Gardenia sootepensis Hutch.	Rubiaceae	Т	0.76
29	Garuga pinnata Roxb.	Burseraceae	Т	0.20
30	Gluta usitata (Wall.) Hou	Anacardiaceae	Т	0.45
31	Grewia abutilifolia Vent. ex Juss.	Tiliaceae	S	1.20
32	Grewia eriocarpa Juss.	Tiliaceae	Т	0.86
33	Grewia lacei Drum. & Craib	Tiliaceae	L	1.00
34	Helicteres elongata Wall. ex Bojer	Sterculiaceae	S	0.17
35	Hibiscus glanduliferus Craib	Malvaceae	L	0.45
36	Holarrhena pubescens Wall. ex G.Don	Apocynaceae	Т	0.95
37	Irvingia malayana Oliv. ex A. Benn.	Irvingiaceae	Т	0.50
38	Ixora cibdela Craib	Rubiaceae	L	0.62
39	Jasminum scandens (Retz.) Vahl	Oleaceae	WC	0.22
40	Lagerstroemia cochinchinensis Pierre var.	Lythraceae	Т	5.00
	ovalifolia Furt. & Mont.	J		
41	Leea indica (Burm.f.) Merr.	Leeaceae	L	0.90
42	Millettia extensa Bth. ex Baker	Leguminosae.	WC	12.00
		Papilionoideae	_	
43	Morinda tomentosa Heyne ex Roth	Rubiaceae	Т	22.00
44	Ochna integerrima (Lour.) Merr.	Ochnaceae	Т	0.53
45	Oroxylum indicum (L.) Kurz	Bignoniaceae	Т	1.00
46	Pavetta fruticosa Craib	Rubiaceae	S	0.20

Table 8. (Continued)

No.	Botanical name	Family	Habit	Max.
		5		height (m)
47	Polyalthia cerasoides (Roxb.) Bth. ex Bedd.	Annonaceae	Т	1.20
48	Pterocarpus macrocarpus Kurz	Leguminosae,	Т	22.00
		Papilionoideae		
49	Quercus kerrii Craib	Fagaceae	Т	1.50
50	Rothmannia sootepensis (Craib) Brem.	Rubiaceae	Т	3.50
51	Schleichera oleosa (Lour.) Oken	Sapindaceae	Т	18.00
52	Shorea obtusa Wall. ex Bl.	Dipterocarpaceae	Т	20.00
53	Shorea siamensis Miq. var. siamensis	Dipterocarpaceae	Т	0.30
54	Spatholobus parviflorus (Roxb.) O.K	Leguminosae,	WC	15.00
		Papilionoideae		
55	Sterculia balanghas L.	Sterculiaceae	Т	0.80
56	Stereospermum neuranthum Kurz	Bignoniaceae	Т	0.96
57	Strychnos nux-vomica L.	Loganiaceae	Т	1.13
58	Symplocos racemosa Roxb.	Symplocaceae	L	4.00
59	Terminalia alata Heyne ex Roth	Combretaceae	Т	15.00
60	Terminalia chebula Retz. var. chebula	Combretaceae	Т	12.00
61	Terminalia mucronata Craib & Hutch.	Combretaceae	Т	2.00
62	Vernonia parishii Hk.f.	Compositae	L	0.22
63	Vitex canescens Kurz	Verbenaceae	Т	0.50
64	Vitex peduncularis Wall. ex Schauer	Verbenaceae	Т	1.20
65	Wendlandia tinctoria (Roxb.) DC. ssp. floribunda	Rubiaceae	Т	2.50
	(Craib) Cow.			
66	<i>Xylia xylocarpa</i> (Roxb.) Taub. var. <i>kerrii</i> (Craib &	Leguminosae,	Т	6.00
	Hutch.) Niels.	Mimosoideae		
67	Ziziphus oenopolia (L.) Mill. var. oenopolia	Rhamnaceae	WC	0.24

Forest profile diagrams make a clear illustration of the forest structure as they show the amount of species, their maximum sizes and vegetation layers which are different at each site. The number of species indicates the species richness and diversity. The canopy profile and layers can indicate the history of the area related to human disturbances such as logging and fire. Multi-vegetation layers indicate the stages of vegetation recovery with different size classes.

Site 1 at Pah Laht Temple had less disturbance than the other sites. The profile diagram shows that the size class 1-5 m was the main vegetation layer that densely covered the site. Above 5 m, tree size was randomly distributed, but enough to create cooler, shady conditions and an almost closed canopy. Some tall tree species were the deciduous *Dipterocarpus obtusifolius* var. *obtusifolius*, *Shorea obtusa*, *S. siamensis* var. *siamensis* and the evergreen *Lithocarpus polystachyus*. The highest number of individuals were found at this site. Other large species were *Buchanania lanzan* and *Eugenia grata* var. *grata*. The woody climbers at this site had the highest number of species, most individuals and largest sizes when compared with the other sites. This site also had a small seasonal stream with vegetation characteristic of mixed evergreen + deciduous (mxf) forest.

Site 2, in deciduous dipterocarp-oak forest (dof), had *Dipterocarpus obtusifolius* var. *obtusifolius*, *Lithocarpus polystachyus* and *Shorea obtusa* as the dominant tall and most abundant trees. This was the first time that the effects of fire were clearly seen. Most of the trees had burned stems and some damaged parts caused by fire which had resulted in coppiced regeneration. Two vegetation layers in this site were mainly coppiced species (*Glochidion eriocarpum* and *Buchanania lanzan*) and regenerating stumps (*Aporosa villosa*, *Tristaniopsis burmanica* var. *rufescens* and *Wendlandia tinctoria* spp. *floribunda*). This site is clearly a very degraded version of site 1.

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Sites 5, 6 and 7 are located above Huay Dteung Tao Lake and represent an extremely degraded dof forest. Only one tree was more than 10 m high and less than 10 individuals were higher than 5 m. Small and randomly distributed seedlings of *Buchanania lanzan*, *Cratoxylon formosum* spp. *pruniflorum* and *Gardenia obtusifolia* were sometimes found beneath the dominant grasses and sedges. Site 5 was the most degraded site that had suffered from repeated fires and had the fewest trees over 5 m tall.

Site 6 was in a slightly better condition than site 5. The largest tree at this site was *Terminalia alata*. From the profile diagram (Figure 7) the vegetation layer at 5-10 m mostly had *Aporosa villosa*, *Mitragyna hirsuta*, *Quercus kerrii*, *Shorea obtusa*, *Terminalia chebula* var. *chebula* and *Tristaniopsis burmanica* var. *rufescens*. Woody species less than 5 m tall were dominated by *Cratoxylon formosum* spp. *pruniflorum* and *Tristaniopsis burmanica* var. *rufescens*, which are indicators of frequently burnt areas. The herbaceous ground flora, especially two grasses, *Arundinella setosa* var. *setosa* and *Apluda mutica*, uniformly covered the site.

Site 7 was situated on the same hill as sites 5 and 6, but in a gully and was less disturbed by fire. The plot was also partially covered by dense bamboo growth, *Dendrocalamus nudus*, and had a small stream. The site had a partial mxf facies and had the highest species richness and abundance of vegetation. The canopy was almost closed with 4 layers (Figure 8). *Buchanania glabra, Dalbergia cultrata, Morinda tomentosa, Pterocarpus macrocarpus* and *Shorea obtusa* all exceeded 20 m. The subcanopy trees more than 10 m tall consisted of 3 species of *Terminalia* and the >1-5 m size class comprised *Lagerstroemia cochinchinensis* var. *ovalifolia, Quercus kerrii*, the 3 species of *Terminalia*, and *Xylia xylocarpa* var. *kerrii* (Table 8). More than 40 species of seedlings covered the plot.

Sites 3 and 4 were similar to sites 5 and 6 and had two indistinct tree layers. The tallest trees and most abundant coppices were Dipterocarpaceae, viz. *Dipterocarpus obtusifolius* var. *obtusifolius*, *D. tuberculatus* var. *tuberculatus*, *Shorea obtusa* and *S. siamensis* var. *siamensis* (Figures 4-5). Although the mean tree height at site 3 was more than at site 4, site 3 had a much denser tree layer up to 1 m tall. More than half of site 4 was covered by perennial grasses, which indicated that this site had had more fire damage than site 3.

Species composition and abundance

Sites 1 and 7 had the most woody species (61 and 67 respectively) while sites 4 and 5 had the least (34 and 31 respectively) (Figure 9). The other sites ranged between 46-50 species. The number of woody individuals at each site is depicted (Figure 10). Site 7 had the most with 1169 individuals, followed, surprisingly, by site 2 (952) and site 1 (876). Site 5 was lowest with 319 individuals.



Number of species

Figure 9. Number of woody species at each survey site



Figure 10. Number of individuals of woody species at each survey site

Species richness and diversity

Species richness is the total number of woody plant species in each site. Species diversity is calculated using both Shannon's and Simpson's diversity indices and MVSP 3.1, a multivariate statistical package program [18]. Shannon's index takes the number of species (species richness) and the relative abundance of the species (evenness) into account, while Simpson's reciprocal index measures the probability that two individuals randomly selected from a sample will belong to the same species. The details of these species diversity indices are shown in Appendix 2. Species richness and diversity at each site are shown in Table 9.

Species richness Shannon's index Simpson's reciprocal index Site 1 20.06 61 3.38 2 50 2.83 10.97 3 50 3.06 13.62 4 2.44 34 6.83 5 31 2.58 9.14 6 46 2.45 5.30 7 67 3.37 20.00

Table 9. Species richness and species diversity indices of each site

The highest species diversity values are at site 1 for both diversity indices. Shannon's index value at site 1 is slightly higher than at site 7. Although the number of species (species richness) in site 1 was less than that in site 7, the abundance of each species (evenness) in site 1 was to some extent higher than that in site 7. For Shannon's index, the more equal the values of species abundance are, the higher the evenness of the community is (sites 1, 7 and 3). In contrast, if one or few species are dominating, the evenness of the community is low (sites 2, 5, 4, and 6). The higher evenness means the higher value of Shannon's index. High evenness indicates a lack of dominant species, which reflects the good health of the sample plots, since the dominance of one or few species in an ecosystem often results from negative human impacts [19].

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Simpson's reciprocal index is the measurement of dominant species in the community, which could arise from disturbance. The higher the value, the greater is the diversity or the degree to which dominance is shared by many species. A lower value represents the possibility that two individuals randomly selected from a sample will belong to the same species. For example, site 2 and site 3 showed equal richness in species but site 2 was covered with coppiced regeneration species more than at site 3, which had less damage from fire. Simpson's reciprocal index shows that the influence by the dominant species is more than the actual diversity of degraded areas such as site 5, despite having the lowest species richness. Simpson's reciprocal index of site 5 is higher than those of sites 4 and 6, which were dominated by fire-tolerant, deciduous tree species.

Species categories and size class

All woody plants were classified into 6 size classes, viz. 1) 0-1 m, 2) >1-5 m, 3) >5-10 m, 4) >10-15 m, 5) >15-20 m and 6) >20 m, to show the composition of forest vegetation cover that consists of trees, shrubs and woody climbers, which are stratified from seedlings, saplings and mature plants growing to the top of the canopy. Woody plants in each size class at each survey site are shown in Table 10. Trees, shrubs and woody climbers are classified in different size classes as mentioned above for site comparison (Figures 11-13).

Site	Number of woody plants (number of species) for each size class								
	$\leq 1 m$	>1-5 m	>5-10 m	>10-15 m	>15-20 m	>20 m			
1	625 (51)	211 (42)	25 (14)	12 (5)	2 (2)	-			
2	773 (43)	168 (31)	10 (9)	6 (2)	1 (1)	-			
3	568 (47)	108 (21)	27 (7)	12 (5)	-	-			
4	591 (32)	55 (17)	11 (4)	1(1)	-	-			
5	278 (29)	31 (9)	9 (5)	1(1)	-	-			
6	735 (41)	44 (17)	12 (8)	2 (2)	1(1)	-			
7	1043 (63)	103 (23)	9 (2)	5 (3)	6 (5)	3 (3)			

Table 10. Number of woody plants in each size class at each survey site

Table 10 shows that woody plants ≤ 1 m dominated all survey sites. The number of species and the number of individuals decreased on going up the size class. It is important to note that the number of woody plants more than 1 m tall was highest at site 1 and lowest at site 5. The number of individuals over 1 m tall in site 1 (251 individuals) was approximately double that in the next highest place—site 7, which had 126 plants. No woody plants higher than 15 m were found in sites 3, 4, and 5. Those which exceeded 20 m were only found in site 7 (Figure 11). Shrubs were most abundant in site 3 and taller shrubs (>1-5 m) were absent from sites 4 and 5 (Figure 12). Woody climbers were abundant in sites 1 and 7 (Figure 13). Site 1 showed many size classes of woody climbers although small-sized ones dominated the site, some large ones being found on trees that exceeded 15 m tall.



Figure 11. Number of trees at each site shown in different size class charts: A) ≤ 1 m, B) >1-5 m, C) >5-10 m, D) >10-15 m, E) >15-20 m and F) > 20 m



Figure 12. Number of shrubs at each site according to size class



Number of woody climbers

Figure 13. Number of woody climbers at each site according to size class

Seedlings

Young woody plants below the canopy are very important for forest stability and represent the new generation of forest species. Woody seedling species from the circular plots are shown in Table 13, which compares the species composition among sites.

No.	Botanical name	Habit				Site			
			1	2	3	4	5	6	7
1	Acacia megaladena Desv. var. megaladena	WC							\checkmark
2	Albizia odoratissima (L.f.) Bth.	Т				~			√
3	Amphineurion (Aganosma) marginata (Roxb.) D.J. Midd.	WC	√		~				~
4	Anneslea fragrans Wall.	Т				\checkmark			\checkmark
5	Antidesma acidum Retz.	Т	\checkmark						\checkmark
6	<i>Aporosa octandra</i> (BH. <i>ex</i> D. Don) Vick. var. <i>octandra</i>	Т	✓						~
7	Aporosa villosa (Lindl.) Baill.	Т			\checkmark	\checkmark			\checkmark
8	Blinkworthia lycioides Choisy	S				\checkmark	\checkmark		\checkmark
9	Breynia glauca Craib	L,S	\checkmark		~	✓			
10	Bridelia glauca Bl.	L,S			~				
11	Bridelia stipularis (L.) Bl.	WC	\checkmark		✓				
12	<i>Buchanania glabra</i> Wall. <i>ex</i> Hk.f.	Т			~	~	✓		
13	Buchanania lanzan Spreng.	Т	\checkmark	✓		\checkmark	\checkmark	\checkmark	
14	Canarium subulatum Guill.	Т	\checkmark			\checkmark			\checkmark
15	Cansjera rheedii J. F. Gmel.	WC	\checkmark		\checkmark				
16	Catunaregum spathulifolia Tirv.	L	\checkmark		\checkmark	✓			\checkmark
17	Celastrus paniculatus Willd.	WC							\checkmark
18	Cissus hastata Miq.	WC	\checkmark						
19	<i>Cissus repanda</i> Vahl	WC							\checkmark
20	<i>Clausena excavata</i> Burm.f. var. <i>excavata</i>	L							~
21	<i>Clerodendrum paniculatum</i> L.	L	\checkmark						
22	<i>Clerodedrum serratum</i> (L.) Moon var. <i>wallichii</i> Cl.	L		~					~
23	Colona flagrocarpa (Cl.) Craib	Т							\checkmark
24	Craibiodendron stellatum (Pierre) W.W.Sm.	Т			~	~			
25	Cratoxylon formosum (Jack) Dyer ssp. pruniflorum (Kurz) Gog.	Т				~	~	\checkmark	~

Table 13. (Continued)

No.	Botanical name	Habit				Site			
			1	2	3	4	5	6	7
26	Dalbergia cultrata Grah. ex Bth.	Т	✓		✓	✓			✓
27	<i>Dalbergia oliveri</i> Gamb. <i>ex</i> Prain	Т			✓				
28	Dalbergia velutina Bth.	WC			~				
29	Desmodium oblongum Bth.	L	\checkmark						
30	Dillenia parviflora Griff. var. kerrii (Craib) Hoogl.	Т				~			~
31	<i>Diospyros ehretioides</i> Wall. <i>ex</i> G.Don	Т	√						~
32	<i>Dipterocarpus obtusifolius</i> Teijsm. <i>ex</i> Miq. var. <i>obtusifolius</i>	Т	√	~	~	~		~	~
33	Dipterocarpus tuberculatus Roxb. var. tuberculatus	Т	✓		~				~
34	<i>Ellipeiopsis cherrevensis</i> (Pierre <i>ex</i> Finet & Gagnep.) R.E.Fr.	S			~	~			
35	<i>Erythroxylum cuneatum</i> (Miq.) Kurz	S	\checkmark	✓	✓	✓			✓
36	<i>Eugenia albiflora</i> Duth. ex Kurz	Т							~
37	Eugenia cumini (L.) Druce	Т				~			
38	Eugenia grata Wight var. grata	Т	\checkmark						
39	Flemingia sootepensis Craib	L	~						
40	Garcinia cowa Roxb. ex DC.	Т	\checkmark		~				~
41	<i>Gardenia obtusifolia</i> Roxb. <i>ex</i> Kurz	L			~	~			
42	Gardenia sootepensis Hutch.	Т	\checkmark						
43	Glochidion eriocarpum Champ.	L,T					✓		~
44	Glochidion rubrum Bl.	L,T	\checkmark	✓					
45	Gluta usitata (Wall.) Hou	Т			✓	✓			✓
46	<i>Grewia abutilifolia</i> Vent. <i>ex</i> Juss.	S							~
47	Grewia eriocarpa Juss.	Т						✓	✓
48	Grewia lacei Drum. & Craib	L			✓				
49	Hibiscus glanduliferus Craib	L	\checkmark	\checkmark	\checkmark			\checkmark	\checkmark
50	<i>Hymenodictyon orixense</i> (Roxb.) Mabb.	Т							~
51	<i>Irvingia malayana</i> Oliv. <i>ex</i> A. Benn.	Т			~				~
52	<i>Ixora cibdela</i> Craib	L	\checkmark	\checkmark	\checkmark				✓
53	<i>Lagerstroemia cochinchinensis</i> Pierre var. <i>ovalifolia</i> Furt. & Mont.	Т							~
54	Leea indica (Burm.f.) Merr.	L	\checkmark	✓	✓	✓		✓	✓
55	Lophopetalum wallichii Kurz	Т			\checkmark	\checkmark			

Table 13. (Continued)

No	Botanical name	Habit				Site			
INU.	Botanicai nanc	maun	1	2	3	4	5	6	7
56	<i>Mammea siamensis</i> (Miq.) T. And.	Т	✓						
57	<i>Memecylon scutellatum</i> (Lour.) Hk. & Arn.	Т				~			
58	Millettia extensa Bth. ex Baker	WC	\checkmark						\checkmark
59	Mitragyna hirsuta Hav.	Т			\checkmark				
60	Ochna integerrima (Lour.) Merr.	L			✓	✓			\checkmark
61	Oroxylum indicum (L.) Kurz	L,T							✓
62	Pavetta fruticosa Craib	S			✓				
63	Pavetta tomentosa Roxb. ex Sm. var. tomentosa	L	✓						
64	Phoenix loureiri Kunth var. loureiri	L	√	~	~	~			~
65	<i>Phyllanthus emblica</i> L.	Т			✓				
66	Pterocarpus macrocarpus Kurz	Т				✓			\checkmark
67	Pueraria stricta Kurz	S						✓	
68	<i>Quercus kerrii</i> Craib	Т	\checkmark		✓	✓			~
69	<i>Rothmannia sootepensis</i> (Craib) Brem.	Т	\checkmark						
70	Schima wallichii (DC.) Korth.	Т	\checkmark						
71	Shorea obtusa Wall. ex Bl.	Т	\checkmark	✓	✓	✓			\checkmark
72	Shorea siamensis Miq. var. siamensis	Т			~	~			~
73	Spatholobus parviflorus (Roxb.) O.K.	WC	✓	~	~	~			~
74	<i>Stereospermum colais</i> (BH. <i>ex</i> Dillw.) Mabb.	Т	✓						
75	Stereospermum neuranthum Kurz	Т		~					✓
76	Strychnos nux-vomica L.	Т			✓	✓			\checkmark
77	Symplocos racemosa Roxb.	L					✓		
78	<i>Tarennoidea wallichii</i> (Hk.f.) Tirv. & Sastre	Т	✓						
79	Terminalia alata Heyne ex Roth	Т				✓			\checkmark
80	<i>Terminalia chebula</i> Retz. var. <i>chebula</i>	Т							~
81	<i>Tetrastigma leucostaphylum</i> (Denn.) Mabb.	WC	\checkmark						
82	<i>Tristaniopsis burmanica</i> (Griff.) Wils. & Wat. var. <i>rufescens</i> (Hance) Parn. & Lug.	L,T			~	~	~		~
83	Vernonia parishii Hk. f.	L	\checkmark						
84	<i>Vitex limoniifolia</i> Wall. <i>ex</i> Kurz	Т	\checkmark		✓	✓			

Table 13. (Continued)

No.	Botanical name	Habit	Site						
			1	2	3	4	5	6	7
85	Vitex peduncularis Wall. ex	Т							✓
	Schauer								
86	Wendlandia tinctoria (Roxb.) DC.	Т				\checkmark			~
	ssp. floribunda (Craib) Cow.								
87	<i>Xylia xylocarpa</i> (Roxb.) Taub.	Т							\checkmark
	var. kerrii (Craib & Hutch.) Niels.								
	Total Number of Species		39	12	37	34	7	7	51

Note: Abbreviations for habit: L =treelet, S =shrub, T =tree, WC =woody climber

The circular sampling plots had the most woody plant seedlings in site 7 (51 species), which was similar to the species composition in the 50x5-m survey plots. The fewest seedlings were found in sites 5 and 6 (7 species). Tree seedlings of Dipterocapaceae, *Dipterocarpus obtusifolius* Teijsm. *ex* Miq. var. *obtusifolius* and *Shorea obtusa* Wall. *ex* Bl., were found in 6 sites and 5 sites respectively. Other tree seedlings found in more than 4 sites were *Buchanania lanzan* Spreng., *Hibiscus glanduliferus* Craib, *Leea indica* (Burm.f.) Merr. and *Phoenix loureiri* Kunth var. *loureiri*. Common shrub and woody climber seedlings found in the circular plots were *Erythroxylum cuneatum* (Miq.) Kurz and *Spatholobus parviflorus* (Roxb.) O.K., except for sites 5 and 6.

Herbaceous ground flora

The proportion of herbaceous ground vegetation cover and open area, and dominant ground vegetation (any species which were not tree seedlings, covering more than 1% of the plot area) from the 4 circular plots in each site are listed in Table 14. It is notable that all ground flora circles had few individuals of most species and only a few species, mostly perennial, deciduous Gramineae (grasses) and Cyperaceae (sedges). These two families provide much of the fuel for fires (Photo 6). Dominant species were different between sites with more fire and sites with less fire. For example, *Arundinella setosa* Trin. var. *setosa* dominated sites 2, 3, 4 and 5. These sites suffered from more of fire while *Cyrtococcum accrescens* (Trin.) Stapf (Site 1) and *Eupatorium odoratum* L. (Site 7) were herbaceous cover dominating areas with less fire.

Site	Circular plot	Cover	Open	Dominant species	
Site 1				Cyrtococcum accrescens (Trin.) Stapf (Gramineae),	
	1	65%	35%	Globba schomburgkii Hk.f. var. schomburgkii (Zingiberaceae)	
	2	40%	60%		
	3	50%	50%		
	4	40%	60%		
Average		48.75%	51.25%		
Site 2				Inula indica L. (Compositae),	
	1	95%	5%	Scleria levis Retz. (Cyperaceae),	
	2	70%	30%	Arundinella setosa Trin. var. setosa, Apluda mutica L.,	
	3	85%	15%	Mnesithea striata (Steud.) Kon. & Sos. (all Gramineae),	
	4	80%	20%	Globba reflexa Craib (Zingiberaceae)	
Average		82.50%	17.50%		
Site 3				Arundinella setosa Trin. var. setosa, Eulalia siamensis Bor,	
	1	60%	40%	Hyparrhenia rufa (Nees) Stapf var. siamensis Clay.,	
	2	60%	40%	Imperata cylindrica (L.) P. Beauv. var. major (Nees) C.E.Hubb.	
	3	40%	60%	ex Hubb. & Vaugh., Polytoca digitata (L.f.) Druce (all Gramineae)	
	4	40%	60%		
Average		50%	50%		

Table 14. Proportion of the cover of herbaceous ground flora and open area, and dominant ground vegetation species from the 4 circular plots at each site

Site	Circular plot	Cover	Open	Dominant species
Site 4				Scleria teresstris (L.) Fass. (Cyperaceae),
	1	80%	20%	Arundinella setosa Trin. var. setosa and Polytoca digitata (L.f.) Druce
	2	60%	40%	(both Gramineae)
	3	50%	50%	
	4	50%	50%	
Average		60%	40%	
Site 5				Fimbristylis straminea Turr., Rhynchospora rubra (Lour.) Mak.,
	1	15%	85%	Scleria levis Retz. (all Cyperaceae),
	2	65%	35%	Arundinella setosa Trin. var. setosa, Apluda mutica L.,
	3	60%	40%	Themeda triandra Forssk. (all Gramineae),
	4	90%	10%	Dunbaria bella Prain (Leguminosae, Papilionoideae)
Average		57.50 %	43.50%	
Site 6				Fimbristylis dichotoma (L.) Vahl ssp. dichotoma,
	1	15%	85%	Fimbristylis straminea Turr., Scleria kerrii Turr. (all Cyperaceae),
	2	99%	1%	Arundinella setosa Trin. var. setosa, Apluda mutica L.,
	3	99%	<1%	Pennisetum polystachyon (L.) Schult. (all Gramineae),
	4	80%	20%	Pueraria stricta Kurz (Leguminosae, Papilionoideae)
Average		73.25%	27.75%	
Site 7				Eupatorium odoratum L. (Compositae),
	1	60%	40%	Scleria kerrii Turr.(Cyperaceae),
	2	40%	60%	Imperata cylindrica (L.) P. Beauv. var. major (Nees) C.E.Hubb. ex Hubb.
	3	65%	35%	& Vaugh. (Gramineae)
	4	70%	30%	
Average		58.75%	42.25%	

Fire

Fire has a disastrous effect on forest growth since it can kill young plants. Figures 14-16 show how damaging fire can be on vegetation. Site 1, which had been protected from fire more effectively than all the other sites, had the most woody species without fire damage (21 species), while sites 4 and 3 had the least (4 and 5 species respectively) (Figure 14). The ravages of fire are convincingly shown in Figures 15-16 where site 1 had the least number of individuals damaged by fire (176 or 20% of the total number of individuals) while site 5 had the fewest number of individuals (319) and highest percentage of damage (88.7%). The fire-damaged woody species and their fire-damaged percentage at each site are shown in Table 15. The species for each site are arranged in order of the number of fire-damaged individuals.

The percentage of damage of the woody plants caused by fire was lowest in site 1 where the most damage was to *Lithocarpus polystachyus* (Wall. *ex* A.DC.) Rehd. (63.16%). The damage of other species in site 1 ranged between 10.77-30%. In the more degraded sites, the highest percentages of damage of woody plants ranged between 84-100%, e.g. *Aporosa villosa* (Lindl.) Baill., *Dipterocarpus tuberculatus* Roxb var. *tuberculatus*, *Gluta usitata* (Wall.) Hou, *Tristaniopsis burmanica* (Griff.) Wils. & Wat. var. *rufescens* (Hance) Parn.& Lug. and *Shorea obtusa* Wall. *ex* Bl. High percentages of fire damage for many species (e.g. in site 7) revealed that extensive and frequent fire in the past had resulted in the coppicing of these species after fire.



Number of species

Figure 14. Number of woody species and number of species damaged by fire at each survey site



Figure 15. Number of individuals of woody species and the number of individuals damaged by fire at each survey site



Figure 16. Percentage of fire-damaged species and fire-damaged individuals at each survey site

% Fire damage

Table 15.	List of fire-damaged species at each site and number and percentage of fire-
	damaged individuals

Site	Species	Total number	Fire-damaged number	% Fire damage
1				
	1. <i>Lithocarpus polystachyus</i> (Wall. <i>ex</i> A.DC.) Rehd.	114	72	63.16
	2. Quercus kerrii Craib	36	10	27.78
	3. <i>Dipterocarpus obtusifolius</i> Teijsm. <i>ex</i> Miq. var. <i>obtusifolius</i>	66	8	12.12
	4. <i>Rothmannia sootepensis</i> (Craib) Brem.	65	7	10.77
	5. Gardenia sootepensis Hutch.	24	6	25.00
	6. Vernonia parishii Hk. f.	24	5	20.83
	7. Craibiodendron stellatum (Pierre) W.W.Sm.	28	5	17.86
	8. Buchanania lanzan Spreng.	29	5	17.24
	9. Breynia glauca Craib	31	4	12.90
	10. Shorea obtusa Wall. ex Bl.	10	3	30.00
2		150	10.4	
	1. Dipterocarpus tuberculatus Roxb. var. tuberculatus	150	124	82.67
	2. Dipterocarpus obtusifolius Teijsm. ex Miq. var. obtusifolius	151	64	42.38
	3. Shorea obtusa Wall. ex Bl.	85	64	75.29
	4. Glochidion eriocarpum Champ.	127	48	37.80
	5. Aporosa villosa (Lindl.) Baill.	38	32	84.21
	6. Quercus kerrii Craib	30	24	80.00
	7. <i>Lithocarpus polystachyus</i> (Wall. <i>ex</i> A. DC.) Rehd.	28	21	75.00
	8. Buchanania lanzan Spreng.	92	20	21.74
	9. Spatholobus parviflorus (Roxb.) O.K. 10. Tristaniopsis burmanica (Griff.)	37	17	45.95
	Wils. & Wat. var. <i>rufescens</i> (Hance) Parn. & Lug.	16	13	81.25

Table 15. (Continued)

Site	Species	Total number	Fire-damaged	% Fire
3			number	uannage
5	1. Dipterocarpus tuberculatus Roxb. var. tuberculatus	134	115	85.82
	2. Shorea obtusa Wall. ex Bl.	60	51	85.00
	3. <i>Dipterocarpus obtusifolius</i> Teijsm. <i>ex</i> Miq. var. <i>obtusifolius</i>	59	39	66.10
	4. Spatholobus parviflorus (Roxb.) O.K.	60	35	58.33
	5. Dalbergia cultrata Grah. ex Bth.	35	31	88.57
	6. Ochna integerrima (Lour.) Merr.	31	28	90.32
	7. <i>Ellipeiopsis cherrevensis</i> (Pierre <i>ex</i> Finet & Gagnep.) R.E.Fr.	35	20	57.14
	8. Gluta usitata (Wall.) Hou	20	18	90.00
	9. Shorea siamensis Miq. var. siamensis	26	15	57.69
	10. Quercus kerrii Craib	20	15	75.00
4		101	177	00 (7
	1. Dipterocarpus tuberculatus Roxb. var. tuberculatus	191	1//	92.67
	2. Shorea obtusa Wall. ex Bl.	122	113	92.62
	3. Dipterocarpus obtusifolius Teijsm. ex Miq. var. obtusifolius	84	54	64.29
	4. <i>Tristaniopsis burmanica</i> (Griff.) Wils. & Wat. var. <i>rufescens</i> (Hance) Parn.& Lug.	40	39	97.50
	5. Eugenia cumini (L.) Druce	33	32	96.97
	6. Ochna integerrima (Lour.) Merr.	23	22	95.65
	7. <i>Craibiodendron stellatum</i> (Pierre) W.W. Sm.	19	19	100.00
	8. Catunaregum spathulifolia Tirv.	21	17	80.95
	9. Gluta usitata (Wall.) Hou	12	12	100.00
	10. Spatholobus parviflorus (Roxb.) O.K.	19	7	36.84
5				
	1. Gardenia obtusifolia Roxb. ex Kurz	59	59	100.00
	2. Dipterocarpus tuberculatus Roxb. var. tuberculatus	52	51	98.08
	3. Buchanania lanzan Spreng.	53	48	90.57
	4. <i>Tristaniopsis burmanica</i> (Griff.) Wils. & Wat. var. <i>rufescens</i> (Hance) Parn.& Lug	35	35	100.00
	5. <i>Shorea obtusa</i> Wall. <i>ex</i> Bl.	15	15	100.00

Table 15.	(Continued)
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Site	Species	Total number	Fire-damaged number	% Fire damage
6				
	1. Cratoxylon formosum (Jack) Dyer ssp. pruniflorum (Kurz) Gog.	315	149	56.83
	 2. Tristaniopsis burmanica (Griff.) Wils. & Wat. var. rufescens (Hance) Parn.& Lug. 	108	40	37.04
	3. Hibiscus glanduliferus Craib	53	34	64.15
	4. Buchanania lanzan Spreng.	36	33	91.67
	5. Aporosa villosa (Lindl.) Baill.	22	22	100.00
	6. <i>Glochidion eriocarpum</i> Champ.	31	21	67.74
	7. Shorea obtusa Wall. ex Bl.	23	20	86.96
	8. Catunaregum spathulifolia Tirv.	18	18	100.00
	9. Dipterocarpus obtusifolius Teijsm. ex			
	Miq. var. obtusifolius	35	16	45.71
	10. Terminalia chebula Retz. var. chebula	14	12	85.71
7				
	1. <i>Lagerstroemia cochinchinensis</i> Pierre var. <i>ovalifolia</i> Furt. & Mont.	119	108	90.76
	2. Cratoxylon formosum (Jack) Dyer ssp. pruniflorum (Kurz) Gog.	118	86	72.88
	3. Millettia extensa Bth. ex Baker	71	68	95.77
	4. Shorea obtusa Wall. ex Bl.	65	61	93.85
	5. Colona flagrocarpa (Cl.) Craib	74	50	67.57
	6. Vitex peduncularis Wall. ex Schauer	43	42	97.67
	7. Dalbergia cultrata Grah. ex Bth.	60	36	60.00
	8. Catunaregum spathulifolia Tirv.	31	29	93.55
	9. Albizia odoratissima (L.f.) Bth.	41	24	58.54
	10. Celastrus paniculatus Willd.	96	13	13.54

Sucessional ecology and forest condition assessment

After fire disturbance, a degraded forest regenerates and various ecological changes occur. For an accurate forest condition assessment, we focused on ecological species groups, i.e. climax and pioneer species, which are two basic kinds of vegetation that appear, develop and often persist in a natural order of succession in disturbed or cleared areas. The percentage of fire damage, species diversity, and the tree size of the two species groups are discussed here.

Initial species which colonise an area are called pioneer species and include herbaceous flora (often weeds) and various kinds of woody plants. The latter group is called secondary growth and includes plants which are light demanding (shade intolerant), rapid-growing, and arrive by wind or are dispersed by small animals (especially birds). As these species develop, often annually or in several years, soil nutrients, moisture and shade increase. Eventually, conditions are suitable for the development of more permanent, i.e. climax or primary, vegetation. As long as fire, erosion and cutting do not occur, this vegetation can develop into a stable system which maintains itself naturally. In Appendix 3 the tree species found in the plots have been categorised as being either pioneer or climax. The dispersal mechanism of their fruits/seeds and number at each site are also noted.

From the survey results, the number of climax tree species was more than pioneer (secondary growth) species at all sites. This indicated that dof had reached a climax stage in the past but had been continuously degraded by humans. Periodic fire had maintained a stable fireclimax ecosystem. In this study, 3 species of Dipterocarpaceae: *Dipterocarpus obtusifolius* var. *obtusifolius*, *D. tuberculatus* var. *tuberculatus* and *Shorea obtusa* were the dominant components of the upperstorey while abundant non-Dipeterocarpaceae were *Buchanania lanzan*, *Quercus kerrii* and *Tristaniopsis burmanica* var. *rufescens*. Large trees of these climax species that were less damaged by fire than smaller trees remained conspicuous in the sites. In the understorey, a high number of woody climbers in sites 1 and 7 also indicated better forest conditions. Sites with high diversity, compared to those with lower diversity on the open slopes, were the consequence of more climax trees that were protected or survived fire. It is likely that vegetation on lower-angled slopes or in gullies is less susceptible to fire than that on steep slopes and ridges. Soil water content on flatter areas is also higher compared with that on on steeper slopes [20].

Early sucessional plant communities after fire may be dominated with some pioneer species. For example, sites 6 and 7 had the highest numbers of Cratoxylum formosum spp. Pruniflorum-a fire-tolerant, deciduous, wind-dispersed, pioneer tree. Site 7 also had the highest number and species richness of pioneer species in the open understorey. The abundance of pioneer species indicates that fire affects the vegetation composition with pioneer species increasing after repeated disturbance or decreasing if disturbance has been prevented [21]. More pioneer tree species having small seeds dispersed by wind or small birds are found on the steep slopes and ridges while most climax trees are found in flatter slopes or near a gully. The high number of pioneer trees and the high percentages of fire damage in sites 6 and 7 resulted from the topographic position of the plots. The same trend was reported in burned lowland dipterocarp rainforest in East Kalimantan, Indonesia [22]. Pioneer species also impede the regeneration of climax species by shading them during the first stage of succession [23]. Herbaceous ground cover, especially the fire-resistant grasses, Arundinella setosa var. setosa, Imperata cylindrica var. major and Pennisetum polystachyon, outgrow the surviving climax species in the early stage of recovery after fire. If subsequent fire ceases and succession continues, fast-growing pioneer species will provide suitable conditions for climax trees that slowly develop under shaded conditions.

Tree size can be used to assess the forest condition. Most of the large-sized trees are climax species which have a higher chance of survival after fire than small individuals. Large pioneer trees (>5 m high) are rare. Seedlings and saplings of pioneer trees on steep slopes tend to be suppressed by herbaceous flora that dominates the understorey after fire. Although degraded
sites are dominated by a few fire-tolerant species, protection of the remaining larger-sized trees from another fire will help reduce soil erosion and flash-flooding, and provide seed sources for forest recovery.

Key points for lowland decidouous forest recovery

Continuous human abuse of forests, especially with fire and logging, has had a degrading effect on plant diversity and resulted in loss of soil nutrients with accompanying erosion and siltation of streams. Fire kills all seedlings and most seeds while damaging saplings and understory vegetation. Forest recovery may be difficult after subsequent fires which lead to extirpation of some understorey tree species. Perennial herbs are not killed by fire since their underground rhizomes and tubers resist the heat. The seed bank of annual herbs as well as mushroom spores and mycelia are a direct indicator of fire damage and forest health. The early recovery of these species in burned forest crucially depends on surviving trees and coppicing regeneration [24]. When considering reforesting of demolished lowland deciduous or evergreen forests, soil improvement in degraded sites can be enhanced by planting mycorrhiza-associated species for nitrogen fixation and increased nutrient accumulation in the soil. Climax tree species which are animal-dispersed should have priority since their seed bank is usually absent in highy disturbed sites [25]. If subsequent fires continue, wind-dispersed species will invade more successfully. This will result in forest ecosystem dominated by relatively few pioneer species since the ability of animal-dispersed species to develop is negated due to the absence of large seed dispersers. Forest biodiversity can be more rapidly augmented by planting animal-dispersed climax species.

Conclusions

From this preliminary, rapid assessment of the lowland dof condition in Chang Kian Valley, Chiang Mai, several conclusions can be made:

- 1. All of the forest plots are in various stages of degradation, all caused by man.
- 2. Most areas are in poor condition as indicated by mostly sparse woody, often deformed or stunted growth and many bare areas where nothing grows and severe erosion has occurred.
- 3. Fires, all of which are intentionally started, are the main cause of this problem.
- 4. Fire not only destroys forest biodiversity and vegetation and retards forest growth, but also results in erosion, air pollution and flash-flooding.
- 5. Fire prevention has not been effective in the valley.
- 6. Unless serious and effective remedial action is taken to halt further forest degradation, the present situation will continue to decline, that is the environmental quality will get worse in Chiang Mai.
- 7. Proper replanting of severely degraded places is urgently required since natural regeneration has stopped in many places.

Recommendations

- 1. Professional assistance is required from many legitimate specialists to help alleviate the rapidly declining level of environmental quality in Chiang Mai.
- 2. The government must assist since legislation and enforcement of laws concerning forest encroachment are its responsibility.
- 3. All fires, logging, hunting and other abuses of forest resources (agricultural expansion, trash dumping and settlement) must cease.

- 4. Research proving that fire is actually detrimental to forest growth should be continued.
- 5. Public awareness must be promoted to gain the support of local people in understanding the problems and eventually help in protecting the forest.
- 6. Financial support must be provided by the government and perhaps also by the public.
- 7. Continued research on forest biodiversity must be done in zoology (insects, birds and mammals), fungi, vegetation, bryology, ecology, soil, erosion and hydrology.
- 8. A vegetation map of the area indicating the various conditions of growth, based on our work, should be produced for Chang Kian Valley.
- 9. An effective reforestation project should be started.
- 10. A plan must be implemented to indicate which forested areas require immediate restorative action, places where protection is needed, and sites that can be used for research, recreation and education.
- 11. Soil in the study sites should be professionally surveyed to show that the condition of the vegetation directly correlates with soil quality.
- 12. Competent research on the effects of fire on mushroom growth should be conducted.
- 13. These recommendations should be implemented as soon as possible.

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Appendix 1: Photos



Photo 1. Evidence of forest fires on the lower slopes of Doi Sutep-Pui in Chang Kian Valley including sites 5 and 6 (2 October 2008)



Photo 2. After a fire has stopped, the burned area on the right shows that dry organic matter has almost totally disappeared. (Site 3, 23 December 2008)



Photo 3. The burning of dry vegetation kills many plants and soil organisms, retards future growth, encourages soil erosion and flooding, and produces smoke which pollutes the air in Chiang Mai. Ground temperature during a fire averages 338°C and at 0.5-1 m high the average is 169°C. (Site 3, 23 December 2008)



Photo 4. Saplings up to 5 m tall (left) in an area near site 2 have their leaves killed by fire and seedlings cremated. Those individuals (right) able to survive burning are variously damaged and have their growth retarded. Loss of soil nutrients and soil erosion also result in impoverished plant development. (Site 2, 28 September 2008)



Photo 5. Uniform tree regrowth at site 3 is an indicator of original clearing of forest cover 25 years ago where frequent fires have caused biodiversity and forest regeneration to steadily decline. (Site 3, 23 December 2008)



Photo 6. The ground flora at site 4 is dominated by perennial, deciduous Cyperaceae and Gramineae. The aerial parts of these plants become dry and very combustible during the hot-dry season when fires are started. Bare areas, deformed tree growth and low biodiversity are indicative of fire damage. (Site 4, 23 December 2008)



Photo 7. Fire readily kills seedlings and damages saplings. The picture on the left shows how coppicing regrowth of several years has been destroyed by successive fires. *Dipterocarpus obtusifolius* Teijsm. *ex* Miq. var. *obtusifolius* (right) with a destroyed stem and *D. tuberculatus* Roxb. var. *tuberculatus* (Dipterocarpaceae) above and to the right are dominant surviviors in burned dof areas. (Site 4, 23 December 2008)



Photo 8. Site 1 (left) at Pah Laht Temple, the most intact and best developed place surveyed, has been protected from major disturbance for more than 50 years. In contrast, site 2 (right), prior to burning in 2009, clearly shows the ravages of previous fires. Continuous human disturbances have severely degraded all of the dof in Chang Kian Valley. (Pah Laht Temple, 21 September 2008; Site 2, 28 September 2008)

Appendix 2

Species diversity indices

Shannon's index = $H' = \sum p_i ln p_i$

where $p_i = proportion of individuals of the ith species$

Simpson's reciprocal index = 1 / D

where
$$D = \Sigma p_i^2 = \frac{\Sigma n_i (n_i - 1)}{N (N-1)}$$

 p_i = proportion of individuals of the i th species = n_i/N

 n_i = number of individual of the i th species

N = total number of individual

Tree species categorised as pioneer and climax species, their dispersal mechanisms and numbers at each site

N.	Determined memory	Deciduous/	Pioneer/Climax	Dispersal	Site						
NO.	Botanical name	Evergreen		mechanism	1	2	3	4	5	6	7
1	Albizia odoratissima (L.f.) Bth.	Deciduous	Pioneer	Wind	-	-	-	5	-	6	41
2	Anneslea fragrans Wall.	Deciduous	Climax	Animal	15	5	4	-	-	1	1
3	Antidesma acidum Retz.	Deciduous	Pioneer	Animal	14	2	-	-	-	-	23
4	<i>Aporosa octandra</i> (BH <i>ex</i> D.Don) Vick. var. <i>octandra</i>	Deciduous	Climax	Animal	3	-	-	-	-	-	29
5	Aporosa villosa (Lindl.) Baill.	Deciduous	Climax	Animal	8	38	13	9	1	22	5
6	Buchanania glabra Wall.	Deciduous	Climax	Animal	-	-	5	5	9	2	2
7	Buchanania lanzan Spreng.	Deciduous	Climax	Animal	29	92	5	8	53	36	-
8	Canarium subulatum Guill.	Deciduous	Climax	Animal	17	7	7	4	1	3	4
9	Catunaregum spathulifolia Tirv.	Deciduous	Climax	Animal	-	5	13	21	7	18	31
10	Catunaregum tomentosa (Bl. ex	Deciduous	Climax	Animal	-	1	-		-	-	-
	DC.) Tirv.							-			
11	<i>Clausena excavata</i> Burm. f. var.	Deciduous	Pioneer	Animal	7	-	-	-	-	-	22
12	<i>Clerodendrum serratum</i> (L.) Moon var. <i>wallichii</i> Cl.	Deciduous	Pioneer	Animal	-	4	5	-	-	-	10
13	Colona flagrocarpa (Cl.) Craib	Deciduous	Pioneer	Wind	-	-	-	-	-	-	24
14	<i>Craibiodendron stellatum</i> (Pierre) W.W.Sm.	Evergreen	Climax	Wind	28	18	-	19	-	-	-
15	Cratoxylon formosum (Jack) Dyer ssp. pruniflorum (Kurz) Gog.	Deciduous	Pioneer	Wind	-	-	-	-	21	315	118
16	Dalbergia cana Grah. ex Kurz	Deciduous	Pioneer	Wind	-	-	-	-	-	-	2
17	Dalbergia cultrata Grah. ex Bth.	Deciduous	Pioneer	Wind	45	1	35	-	1	-	60
18	Dalbergia oliveri Gamb. ex Prain	Deciduous	Pioneer, Climax	Wind	-	-	1	-	-	-	-
19	Desmodium oblongum Bth.	Deciduous	Climax	Wind	-	31	-	-	-	6	-
20	Dillenia obovata (Bl.) Hoogl.	Deciduous	Climax	Animal	-	-	-	-	-	-	3

Appendix 3. (Continued)

No	Dotonical name	Deciduous/	Pioneer/Climax	Dispersal	Site						
INO.	Botanical name	Evergreen		mechanism	1	2	3	4	5	6	7
21	Dillenia parviflora Griff.	Deciduous	Climax	Animal	-	8	30	6	1	13	-
22	Dimocarpus longan Lour. ssp.	Evergreen	Climax	Animal	1	-	-	-	-	-	2
	longan var. longan										
23	Diospyros ehretioides Wall. ex	Evergreen	Climax	Animal	2	4	-	1	-	1	2
	G.Don										
24	Dipterocarpus obtusifolius Teijsm.	Deciduous	Climax	Wind	66	151	59	84	1	35	4
	ex Miq. var. obtusifolius										
25	Dipterocarpus tuberculatus Roxb.	Deciduous	Climax	Wind	2	150	134	191	52	15	-
	var. tuberculatus										
26	Elaeocarpus floribundus Bl. var.	Evergreen	Climax	Animal	1	-	-	-	-	-	-
	floribundus										
27	Eugenia albiflora Duth. ex Kurz	Evergreen	Climax	Animal	4	-	-	-	-	-	2
28	Eugenia cumini Merr.	Deciduous	Climax	Animal	-	7	-	33	3	1	-
29	Eugenia grata Wight var. grata	Evergreen	Climax	Animal	3	-	-	-	-	-	-
30	Ficus hirta Vahl	Deciduous	Pioneer	Animal	3	-	-	-	-	-	-
31	Flacourtia indica (Burm.f.) Merr.	Deciduous	Pioneer	Animal	2	-	1	-	-	-	-
32	Garcinia cowa Roxb. ex DC.	Evergreen	Climax	Animal	-	-	-	2	-	-	-
33	Gardenia obtusifolia Roxb. ex Kurz	Deciduous	Climax	Animal	-	2	3	10	59	6	-
34	Gardenia sootepensis Hutch.	Deciduous	Climax	Animal	24	-	-	-	-	-	3
35	Garuga pinnata Roxb.	Deciduous	Climax	Animal	-	-	-	-	-	-	1
36	Glochidion eriocarpum Champ.	Deciduous	Pioneer	Animal	-	127	-	-	5	31	-
37	Gluta usitata (Wall.) Hou	Deciduous	Climax	Animal	-	-	32	12	-	-	5
38	Goniothalamus griffithii Hk.f. &	Evergreen	Climax	Animal	2	-	-	-	-	-	-
	Thoms.										
39	Grewia abutifolia Pers.	Deciduous	Climax	Animal	-	3	12	-	-	-	-
40	Grewia eriocarpa Juss.	Deciduous	Pioneer	Animal	-	1	-	-	-	-	10
41	Grewia lacei Drum. & Craib	Deciduous	Pioneer	Animal	-	-	2	-	-	-	1
42	Hibiscus glanduliferus Craib	Deciduous	Pioneer	Wind	-	-	-	-	4	53	4
43	Holarrhena pubescens Wall. ex	Deciduous	Climax	Wind	-	-	-	-	-	-	4
	G.Don										

Appendix 3.	(Continued)
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N.	Determined we we	Deciduous/	Pioneer/Climax	Dispersal	Site						
INO.	Botanical name	Evergreen		mechanism	1	2	3	4	5	6	7
44	Irvingia malayana Oliv. ex A.	Evergreen	Climax	Animal	3	-	5	-	-	-	24
	Benn.										
45	<i>Ixora cibdela</i> Craib	Evergreen	Climax	Animal	23	1	11	-	-	1	31
46	Lagerstroemia cochinchinensis	Deciduous	Climax	Wind	-	-	1	-	-	-	119
	Pierre var. ovalifolia Furt. &										
	Mont.										
47	Lagerstroemia macrocarpa Kurz	Deciduous	Climax	Wind	-	-	-	-	-	1	-
	var. macrocarpa										
48	Lannea coromandelica (Houtt.)	Evergreen	Climax	Animal	1	-	-	-	-	-	-
	Merr.										
49	Leea indica (Burm.f.) Merr.	Deciduous	Climax	Animal	11	13	9	2	-	6	16
50	Lithocarpus polystachyus (Wall. ex	Evergreen	Climax	Animal	114	28	1	-	-	-	-
	A. DC.) Rehd.										
51	Lophopetalum wallichii Kurz	Deciduous	Climax	Animal	-	6	5	2	-	-	-
52	Mammea siamensis (Miq.) T. And.	Evergreen	Climax	Animal	-	-	1	-	-	-	-
53	Memecylon scutellatum (Lour.)	Evergreen	Climax		4	-	10	1	-	1	-
	Hk. & Arn.			Animal							
54	Memecylon umbellatum Burm.f.	Evergreen	Climax	Animal	4	-	-	-	-	-	-
55	Meyna velutina Roby.	Deciduous	Climax	Animal	-	-	-	-	1	-	-
56	Mitragyna hirsuta Hav.	Deciduous	Climax	Wind	-	1	3	-	-	4	-
57	Mitragyna rotundifolia (Roxb.)	Deciduous	Climax	Wind	1	-	-		-	-	-
	O.K.							-			
58	Morinda tomentosa Heyne ex Roth	Deciduous	Climax	Animal	-	-	-	-	-	-	2
59	Ochna integerrima (Lour.) Merr.	Deciduous	Climax	Animal	8	6	31	23	2	2	21
60	Oroxylum indicum (L.) Kurz	Deciduous	Pioneer	Wind	-	-	-	-	-	-	1
61	Parinari anamense Hance	Evergreen	Climax	Animal	-	-	-	1	-	-	-
62	Pavetta tomentosa Roxb. ex Sm.	Deciduous	Climax	Animal	4	-	-	-	-	-	-
	var. tomentosa										
63	Phoenix loureiri Kunth var. loureiri	Evergreen	Climax	Animal	-	3	1	-	1	1	-

Appendix 3. (Continued)

Ma	Datanias I nome	Deciduous/	Pioneer/Climax	Dispersal	Site						
INO.	Botanical name	Evergreen		mechanism	1	2	3	4	5	6	7
64	<i>Phyllanthus emblica</i> L	Deciduous	Pioneer	Animal	1	-	1	7	4	2	-
65	Polyalthia cerasoides (Roxb.)	Deciduous	Pioneer	Animal	-	-	-	-	-	-	5
	Benth. ex Bedd.										
66	Premna nana Coll. & Hemsl.	Deciduous	Pioneer	Animal	-	4	16	2	-	-	-
67	Prismatomeris tetrandra (Roxb.)	Evergreen	Climax	Animal	16	-	-	-	-	-	-
	K.Sch. ssp. tetrandra	_									
68	Pterocarpus macrocarpus Kurz	Deciduous	Climax	Wind	-	-	-	-	-	1	16
69	Quercus kerrii Craib	Deciduous	Climax	Animal	36	30	20	3	1	3	10
70	Rothmannia sootepensis (Craib)	Evergreen	Climax	Animal	65	-	-	-	-	-	1
	Brem.	_									
71	Schima wallichii (DC.) Korth.	Evergreen	Pioneer,Climax	Wind	1	-	-	-	-	-	-
72	Schleichera oleosa (Lour.) Oken	Deciduous	Climax	Animal	-	-	-	-	-	-	2
73	Scleropyrum pentandrum (Denn.)	Evergreen	Climax	Animal	1	-	-	-	-	-	-
	Mabb.	_									
74	Shorea obtusa Wall. ex Bl.	Deciduous	Climax	Wind	10	85	60	122	15	23	65
75	Shorea siamensis Miq. var.	Deciduous		Wind	4	2	26	7	-	5	1
	siamensis		Climax								
76	Sterculia balanghas L.	Deciduous	Climax	Wind	-	-	-	-	-	-	2
77	Stereospermum colais (BH.ex	Deciduous		Wind	4	1	2	-	-	-	-
	Dillw.) Mabb.		Climax								
78	Stereospermum neuranthum Kurz	Deciduous	Climax	Wind	-	4	-	1	-	-	3
79	<i>Strychnos nux-vomica</i> L.	Deciduous	Climax	Animal	-	-	1	1	-	3	22
80	Symplocos racemosa Roxb.	Deciduous	Climax	Animal	1	-	1	1	2	7	3
81	Tarennoidea wallichii (Hk.f.) Tirv.	Evergreen		Animal	7	-	-	-	-	-	-
	& Sastre		Climax								
82	Terminalia alata Heyne ex Roth	Deciduous	Climax	Wind	1	7	1	1	15	9	14
83	Terminalia chebula Retz. var.	Deciduous			-	-	1	2	-	14	8
	chebula		Climax	Animal							
84	Terminalia mucronata Craib &	Deciduous			-	-	-	-	-	-	7
	Hutch.		Climax	Wind							

Appendix 3. (Continued)

Na	Deterior norma	Deciduous/	Pioneer/Climax	Dispersal	Site						
INO.	Botanical name	Evergreen		mechanism	1	2	3	4	5	6	7
85	Tristaniopsis burmanica (Griff.)	Evergreen	Climax	Wind	2	16	12	40	35	108	-
	Wils. & Wat. var. rufescens										
	(Hance) Parn.& Lug.										
86	Vernonia parishii Hk. f.	Evergreen	Climax	Wind	24	-	-	-	-	-	1
87	Vitex canescens Kurz	Deciduous	Pioneer	Animal	-	-	13	-	-	-	1
88	Vitex limoniifolia Wall. ex Kurz	Deciduous	Pioneer, Climax	Animal	3	-	-	11	-	-	-
89	Vitex peduncularis Wall. ex	Deciduous	Climax	Animal	7	3	3	-	1	8	43
	Schauer										
90	Walsura trichostemon Miq.	Evergreen	Climax	Animal	-	-	-	-	-	1	-
91	Wendlandia tinctoria (Roxb.) DC.	Evergreen	Pioneer	Wind	-	15	1	-	6	4	1
	ssp. <i>floribunda</i> Cow.										
92	<i>Xylia xylocarpa</i> (Roxb.) Taub. var.	Deciduous	Climax	Wind	-	-	-	-	-	-	31
	kerrii (Craib & Hutch.) Niels.										

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Full Paper

Influence of pH, sucrose concentration and agitation speed on exopolysaccharide production by *Lactobacillus confusus* TISTR 1498 using coconut water as a raw material substitute

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Abstract: Coconut water (CW) was used as a complex nitrogen source for exopolysaccharide (EPS) production by *Lactobacillus confusus* TISTR 1498 to reduce the cost of fermentation medium. EPS production was carried out in a bioreactor using $(0.5\times)$ modified MRS-sucrose-CW medium, in which three relatively expensive complex nitrogen sources (peptone, yeast extract and beef extract) were halved relative to those present in $(1\times)$ modified MRS-sucrose medium. Fermentation parameters (pH, sucrose concentration and agitation speed) were varied in the process of optimisation. Under an optimised condition (pH 5.5, sucrose concentration of 100 g/L and agitation rate of 50 rpm), the maximum EPS level of 38.2 g/L was produced at 35°C after 30 h of cultivation. This EPS concentration (38.2 g/L) from the $(0.5\times)$ medium was higher than those produced in the $(1\times)$ modified MRS-sucrose and $(1\times)$ modified MRS-sucrose-CW media (21.3 and 31.5 g/L respectively), both having the original concentration of all three complex nitrogen sources. Thus, in a bioreactor where the pH level was properly controlled, the EPS production was greatly enhanced. Sugar concentration also played an important role in the production of EPS.

Keywords: exopolysaccharide, Lactobacillus confusus, coconut water, fermentation

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Introduction

Exopolysaccharides (EPS) are self-produced biopolymers by a number of microorganisms and are important as a natural additive in food industry. In particular, those produced by lactic acid bacteria (LAB) have gained an increased attention due to the GRAS (generally recognised as safe) status of the microbes. In the past, EPS production by LAB generally involved the optimisation of growth condition and level of generated EPS [1-11]. The EPS concentrations obtained from LAB such as *Lactobacillus* spp. were reported with the variation in productivity that could span between 0.06 to 60 g/L [6, 12-17]. To be economically feasible, the EPS production should be in the range of 10-15 g/L [13].

EPS production by *Lactobacillus confusus*, recently known as *Weissella confusa*, has been reported, which is related to characteristics and product improvement of wheat sourdough [18-19]. A research article described the structure of EPS, produced by *Lactobacillus confusus*, which was suitable as an alternative for a linear dextran produced by *Leuconostoc mesenteroides*, and also for the production of prebiotic gluco-oligosaccharides [20].

Most LAB are fastidious microorganisms requiring complex media for optimal growth and bioactivity. Besides carbon source and other supplementary nutrients for LAB cultivation, the widely used complex nitrogen sources such as expensive yeast extract, beef extract and peptone are important necessities for growth and inevitably raise the EPS production cost. It has been estimated that the yeast extract expense accounts for 30% or more of the total production cost [21-22]. Consequently, a cheaper alternative nitrogen source should be sought after in order to minimise the cost of fermentation medium.

Coconut water has been considered as worthless by-product or inexpensive raw material in Thailand and most of it is discarded to waterways. In 2001, nearly 200,000 tons of coconut water was produced in Thailand [23]. It has also been reported by the Office of Trade Policy that coconut production rose up to 1.72 million tons in 2008 [24]. Coconut water is a nutritive source of different sugars, organic acids and trace elements. It contains about 10 mg of nitrogen per litre, in comparison to 1 g of nitrogen in 10 g of yeast extract [23]. The utilisation of coconut water as a low-cost carbon source for the production of EPS by *Agrobacterium* sp. and of scleroglucan by *Sclerotium rolfsii* MTCC 2156 was reported previously [25-26].

Earlier, we reported a preliminary study on the employing of coconut water to reduce the use of complex nitrogen sources for EPS production by *L. confusus* CMU 198 in a modified MRS-sucrose medium [27]. In that study, the concentrations of three expensive medium components (peptone, yeast extract, and beef extract) in the modified MRS-sucrose-CW medium were halved, which resulted in a maximum EPS production of 11.7 g/L in unshaken Erlenmeyer flasks without pH control. In order to enhance the EPS production further, this study was thus conducted in a bioreactor in which the pH level, agitation speed and sucrose concentration were controlled and optimised.

Materials and Methods

Bacterial strain and inoculum preparation

Lactobacillus confusus TISTR 1498 (identical to L. confusus CMU 198), isolated from traditional northern Thai fermented pork (Nham), was used. The strain was obtained from Thailand

Institute of Scientific and Technological Research (TISTR) culture collection (accession number TISTR 1498). It was maintained in the MRS medium [28] plus 60% glycerol at -80°C until use. The inoculum was prepared by recovering the frozen culture stock in 10 ml of modified MRS-sucrose broth and incubating for 24 h. In the economic point of view corresponding to the temperature in Thailand and energy saving, the temperature of incubation was selected at 35°C. The optical density measured at 650 nm (OD₆₅₀) of the resulting suspension was adjusted to 0.8, which was experimentally equivalent to 2.32×10^8 CFU/ml before use.

Media and fermentation conditions

The MRS medium was used to maintain and recover the strain from frozen state. However, in the study on the optimisation of pH, sucrose concentration and agitation speed for EPS production, the $(0.5\times)$ modified MRS-sucrose-CW medium [27] was used. The medium consisted of (g/L): peptone (Difco Laboratories, U.S.A.) 5.0; beef extract (Difco Laboratories, U.S.A.) 2.5; yeast extract (Difco Laboratories, U.S.A.) 2.5; sucrose (Mitrphol Group, Thailand) 20; K₂HPO₄ (Fisher Chemical, NJ) 2.0; di-ammonium hydrogen citrate (Fisher Chemical) 2.0; CH₃COONa. H₂O (Fisher Chemical) 7.6; MgSO₄.7H₂O (Fisher Chemical) 0.1; and MnSO₄ (Fisher Chemical) 0.4. Tween 80 (Ajax Chemical, Australia) (1 ml/L) was also added and coconut water obtained from a local market and stored in the freezer at -25° C was used as a replacement of deionised water in the medium preparation.

Under optimised conditions, other two media, viz. the $(1\times)$ modified MRS-sucrose [27] and the $(1\times)$ modified MRS-sucrose-CW, were also used. The amounts of peptone, yeast extract and beef extract in both media were double those in the $(0.5\times)$ medium. The only difference between the pair was that coconut water was used in the latter medium in stead of deionised water to dissolve the ingredients of the medium. All media were sterilised by autoclaving at 121°C for 15 min. Batch fermentations were carried out in a 5-L bioreactor (B. Braun Biostat B., Biotech International, Pennsylvania, USA) with 3 L working volume at 35°C. The bioreactor was inoculated with 10% (v/v) inoculum. Samples were taken every 6 h during 30 h of cultivation period and analysed for EPS, biomass, sucrose and lactic acid concentrations.

The pH was controlled at 5.0, 5.5, 6.0, 6.5 and 7.0 with 1M HCl or 5M NaOH as necessary. The initial sucrose concentrations were 40, 60, 80, 100 and 120 g/L while the pH and agitation speed were kept constant at 5.5 and 50 rpm respectively. The agitation speed was varied at 25, 50 and 75 rpm while the pH was controlled at 5.5 and the initial sucrose concentration kept at 100 g/L.

Analytical methods

The pH was measured using a pH meter (model SA 230; Orion Research, USA). Lactic acid and sucrose concentrations were determined using HPLC (Shimadzu LC-10ATvp, Shimadzu Co., Japan) under the following conditions—column: Aminex HPX-87X (300 mm×7.8 mm) incubated in column oven (model CTO-10ASvp) at 38°C; mobile phase: 5 mM H₂SO₄ in deionised water; flow rate: 0.75 ml/min; detectors: UV–VIS (model SPD-10Avp) at 210 nm and RI (model RID-10). The absorbance of each sample for biomass determination was quantitated at 650 nm by a spectrophotometer (Shimadzu UV-1700, Shimadzu Co., Japan) and the corresponding biomass concentration was calculated from a standard calibration curve. The EPS concentrations were measured as described by Duenas et al. [7]. In brief, the supernatant obtained by centrifugation (10,000 \times g for 10 min) of the fermented broth at 4°C in order to separate the cells was used for EPS determination. To inactivate EPS degrading enzymes and to precipitate proteins, the supernatant was added with 30%(v/v) trichloroacetic acid and stored for 30 min at 4°C. The crude EPS was then isolated by cool ethanol precipitation (using 3 volumes of cool ethanol). After centrifugation (3,500×g, 15 min, 4°C), the EPS pellets were dispersed in aqueous 80% ethanol and centrifuged again (three

times). The final precipitates were dried to a constant weight at 55°C. All determinations were

Results

Effect of pH

performed in triplicate.

pH is one of the most important factors which can influence growth and production of particular products by LAB. In this study, the EPS production was investigated in the (0.5×) modified MRS-sucrose-CW medium at pH 5.0, 5.5, 6.0, 6.5 and 7.0. Fermentation was performed at a temperature of 35°C, agitation speed of 50 rpm and initial sucrose concentration of 40 g/L, which corresponded to the level present in coconut water and in the modified MRS-sucrose medium. The favourable pH level for EPS production was found to be 5.5, the EPS production being at a maximum of 12.95 g/L (μ = 0.06 L/h, Y_{P/S} = 0.3 g/g and q_P = 0.2 g/L/h) after 24 h incubation period (Figure 1a and Table 1). The maximum production level of lactic acid also occurred at this pH (Figure 1c) but not the biomass production (Figure 1b). All sugars were consumed after 30 h at pH 5.5, 6.0, 6.5 and 7.0 (Figure 1d).

Effect of initial sucrose concentration

The sugar concentration is another essential factor that influences EPS production. In this study, the effect of initial sucrose concentration was investigated at 40, 60, 80, 100 and 120 g/L by adjustment with crystalline sucrose. Fermentation was performed at 35°C, pH 5.5 and agitation speed of 50 rpm. EPS production was enhanced with the increase in initial sucrose concentration at 40-100 g/L, whereas an adverse effect on EPS production was evident at 120 g/L (Figure 2a). Maximum EPS (38.17 g/L), minimum biomass (1.5 g/L) and minimum lactic acid (19.1 g/L) were observed at the initial sucrose level of 100 g/L ($\mu = 0.19$ L/h, Y_{P/S} = 0.39 g/g and q_P = 3.7 g/L/h) (Figures 2a-c and Table 1). The use of initial sugar concentrations of 60, 80 and 100 g/L resulted in complete sugar consumption within 30 h while at 120 g/L residual sugar of about 42.5 g/L was detected (Figure 2d).

Effect of agitation speed

Mixing is provided to keep the fermentation broth homogeneous as well as enhance mass transfer of nutrients and air. The effect of agitation speed on EPS production was investigated in the (0.5×) modified MRS-sucrose-CW medium at 25, 50 and 75 rpm. Fermentation conditions were maintained at the optimum (35°C, pH 5.5 and 100 g/L initial sucrose concentration). The highest EPS level of 38.17 g/L was produced at the speed of 50 rpm ($\mu = 0.19$ L/h, Y_{P/S} = 0.39 g/g and q_P = 3.7 g/L/h) (Figure 3a and Table 1) with accompanying lowest biomass and lactic acid concentrations (Figures 3b-c). Sugar was almost depleted after 30 h of incubation period (Figure 3d).



Figure 1. Concentrations of EPS (a), biomass (b), lactic acid (c) and sucrose (d) during the cultivation of *L. confusus* TISTR 1498 in the $(0.5\times)$ modified MRS-sucrose-CW medium (40 g/L of initial sucrose) with agitation speed at 50 rpm at different pH levels (\bullet : uncontrolled pH fermentation, O: pH 5, $\mathbf{\nabla}$: pH 5.5, \triangle : pH 6, \blacksquare : pH 6.5 and \square : pH 7)

EPS production in the $(1 \times)$ modified MRS-sucrose medium

To investigate the effect of the medium on EPS production, a similar experimental set-up was used with $(1\times)$ modified MRS-sucrose and $(1\times)$ modified MRS-sucrose-CW as media under optimised conditions (pH 5.5, 100 g/L sucrose and 50 rpm agitation speed) at 35°C. The EPS concentrations produced were 21.35 g/L and 30.51 g/L in the two media respectively (Figure 4a).

Discussion

This investigation focuses on the use of coconut water as a partial substitute for complex nitrogen sources, namely peptone, yeast extract and beef extract, for the production of EPS by a strain of lactic acid bacterium. It is evident that coconut water can be used to decrease the initial concentrations of the nitrogen sources in the $(1\times)$ modified MRS-sucrose medium by 50%.

The utilisation of several alternative nitrogen sources such as waste materials and agroindustrial by-products for the cultivation of lactic acid bacteria have been reported. These include malt



Figure 2. Concentrations of EPS (a), biomass (b), lactic acid (c) and sucrose (d) during the cultivation of *L. confusus* TISTR 1498 in the $(0.5\times)$ modified MRS-sucrose-CW medium, with pH = 5.5 and agitation speed = 50 rpm, at different initial sucrose concentrations (\bullet : 60 g/L, O: 80 g/L, ∇ : 100 g/L, and \triangle : 120 g/L)

combing nuts [21] and corn steep liquor-supplemented acid hydrolysate of soybean meal [22] for lactic acid production, as well as whey permeate [6] for exopolysaccharide production, while ram horn hydrolysate was applied to achieve both lactic acid and exopolysaccharide production [29-30].

With regard to the effect of pH on EPS production, higher EPS concentrations are usually produced under controlled pH condition [e.g. 1-2, 4-5, 10]. According to this study, the most favourable pH level for fermentation by *L. confusus* TISTR 1498 is 5.5, which resulted in the EPS yield of 12.95 g/L (at sucrose concentration of 40 g/L). The amount of EPS produced might or might not have any link with the biomass production [31-32]. From this finding, the optimal pH level of EPS production by *Lactobacillus confusus* TISTR 1498 was not found to be in the range between 6-6.7 as reported elsewhere [2-3, 8, 11, 27].

The type and concentration of sugar plays an important role in the production of EPS [8-11, 25]. In our previous study, the strain CMU 198 (identical to TISTR 1498) only produced EPS in the presence of sucrose as carbon source [27]. The optimal initial sucrose concentration of 100 g/L for the



Figure 3. Concentrations of EPS (a), biomass (b), lactic acid (c) and sucrose (d) during the cultivation of *L. confusus* TISTR 1498 in the (0.5×) modified MRS-sucrose-CW medium (100 g/L of sucrose) with pH = 5.5 at different agitation rates (\bullet : 25 rpm, O: 50 rpm, and ∇ : 75 rpm)

strain TISTR 1498 is at the same level as that for *Lactobacillus sp.* strain LB80 [33] and *L. reuteri* ATCC 55730 [34]. The optimal sugar concentration achieved by several lactic acid bacteria ranges between 20-100 g/L [12, 25, 33]. Higher level of sugar concentration can be detrimental to the microbial growth due to unfavourable osmotic pressure [35]

Mixing is provided in order to keep the fermentation broth homogeneous and, in some cases, aerated. In this study, the agitation rate of 50 rpm was enough to provide proper mixing. The agitation rate of 100 rpm was used for the cultivation of *Streptococcus thermophilus* LY03 [3] and *L. reuteri* ATCC 55730 [31]. Continuous agitation at 150 rpm showed a negative effect on growth and production of EPS by *Pediococcus damnosus* IOEB 8801 [9] but a maximum production of the exobiopolymer by *Cordycep militaris* was achieved at this speed [36]. An elevation in the agitation speed also decreased EPS production by *Aureobasidium pullulans* [37] and *Enterobacter cloacae* WD7 [38].

Table 1. Kinetic parameters at different pH, agitation speeds and sucrose concentrations for EPS production and growth of *Lactobacillus confusus* TISTR 1498 in $(0.5\times)$ modified MRS-sucrose-CW medium (30 h of incubation)

Variable	EPS	μ	$Y_{X/S}$	$Y_{P/S}$	$q_{\rm S}$	q_P
	(g/L)	(L/h)	(g/g)	(g/g)	(g/L/h)	(g/L/h)
\mathbf{pH}^1						
Uncontrolled	4.69 <u>+</u> 0.29	0.21 <u>+</u> 0.14	0.18 <u>+</u> 0.060	0.22 <u>+</u> 0.040	1.17 <u>+</u> 0.037	0.26 <u>+</u> 0.030
5	4.81 <u>+</u> 0.34	0.04 <u>+</u> 0.003	0.06 <u>+</u> 0.007	0.09 <u>+</u> 0.002	0.66 <u>+</u> 0.038	0.06 <u>+</u> 0.002
5.5	12.95 <u>+</u> 1.07	0.06 <u>+</u> 0.002	0.09 <u>+</u> 0.004	0.30 <u>+</u> 0.011	0.67 <u>+</u> 0.073	0.20 <u>+</u> 0.017
6	8.81 <u>+</u> 0.82	0.09 <u>+</u> 0.012	0.10 <u>+</u> 0.005	0.20 <u>+</u> 0.012	0.90 <u>+</u> 0.051	0.18 <u>+</u> 0.031
6.5	3.10 <u>+</u> 0.21	0.05 <u>+</u> 0.001	0.10 <u>+</u> 0.030	0.01 <u>+</u> 0.003	0.50 <u>+</u> 0.008	0.01 <u>+</u> 0.008
7	1.90 <u>+</u> 0.09	0.03 <u>+</u> 0.002	0.08 <u>+</u> 0.002	0.01 <u>+</u> 0.001	0.40 <u>+</u> 0.017	0.01 <u>+</u> 0.005
Agitation speed (rpm) ²					
20	36.42 <u>+</u> 2.74	0.16 <u>+</u> 0.014	0.02 <u>+</u> 0.002	0.38 <u>+</u> 0.012	8.00 <u>+</u> 0.287	3.04 <u>+</u> 0.147
50	38.17 <u>+</u> 3.21	0.19 <u>+</u> 0.008	0.02 <u>+</u> 0.003	0.39 <u>+</u> 0.025	9.50 <u>+</u> 0.139	3.70 <u>+</u> 0.028
75	36.40 <u>+</u> 3.78	0.16 <u>+</u> 0.006	0.02 <u>+</u> 0.001	0.43 <u>+</u> 0.034	8.00 <u>+</u> 0.248	3.44 <u>+</u> 0.219
Initial sucrose con	ncentration (g	g/L) ³				
40	12.95 <u>+</u> 1.47	0.06 <u>+</u> 0.002	0.09 <u>+</u> 0.008	0.30 <u>+</u> 0.012	0.67 <u>+</u> 0.041	0.20 <u>+</u> 0.011
60	16.38 <u>+</u> 1.82	0.09 <u>+</u> 0.001	0.07 <u>+</u> 0.004	0.29 <u>+</u> 0.019	1.28 <u>+</u> 0.098	0.37 <u>+</u> 0.026
80	20.55 <u>+</u> 2.98	0.10 <u>+</u> 0.009	0.06 <u>+</u> 0.003	0.30 <u>+</u> 0.027	1.67 <u>+</u> 0.053	0.51 <u>+</u> 0.037
100	38.17 <u>+</u> 3.89	0.19 <u>+</u> 0.018	0.02 <u>+</u> 0.001	0.39 <u>+</u> 0.032	9.50 <u>+</u> 0.182	3.70 <u>+</u> 0.042
120	32.65 <u>+</u> 2.41	0.06 <u>+</u> 0.001	0.04 <u>+</u> 0.002	0.38 <u>+</u> 0.024	1.50 <u>+</u> 0.002	0.57 <u>+</u> 0.021

Notes :

X = Microbial biomass; S = Substrate; P = Product

μ = Specific growth rate	=	$\frac{1}{X}\frac{dX}{dt}$
$Y_{X/S}$ = Yield coefficient for cells	=	$\Delta X / \Delta S$
$Y_{P/S}$ = Yield coefficient for EPS	=	$\Delta P / \Delta S$
q_s = Specific substrate consumption rate	=	$\mu/Y_{_{X/S}}$
q_P = Specific EPS production rate	=	$Y_{P/S} \times q_S$

¹ Initial sucrose concentration = 40 g/L, agitation speed = 50 rpm

 2 pH = 5.5, initial sucrose concentration = 100 g/L

 3 pH = 5.5, agitation speed = 50 rpm



Figure 4. Concentrations of EPS (a) and biomass (b) during the cultivation of *L. confusus* TISTR 1498 (pH = 5.5, sucrose = 100 g/L, agitation rate = 50 rpm) in (1×) modified MRS-sucrose (\bigcirc) and (1×) modified MRS-sucrose-CW (\bigcirc)

The effect of pH control is strongly pronounced again here when the $(1\times)$ modified MRSsucrose and $(1\times)$ modified MRS-sucrose-CW media gave higher levels of EPS production (Figure 4a) when compared to our previous study under uncontrolled fermentation, which gave a maximum of only 4.6 g/L of ESP in similar media [27]. However, the $(0.5\times)$ modified MRS-sucrose-CW medium used in this study was observed to further increase ESP yield. Presertsan et al. [38] suggested that a high C/N ratio may play an important role in producing a high yield of EPS with lower cell growth.

Since the concentrations of peptone, beef extract and yeast extract could be decreased by half as shown in this study, the cost of the $(0.5\times)$ cultivation medium in such a system in turn could be demonstrated to decrease from 3.47 to 1.88 THB/g EPS or nearly twofold. In Table 1, under the condition of 100 g/L initial sucrose concentration, it is seen that high EPS yield with Y_{P/S} of 0.39 reflects a low biomass production with Y_{X/S} of 0.02. These results are similar to those in the report of Presertsan et al [38].

Conclusions

Regarded as an agricultural waste that can cause pollution of the environment, coconut water, one of the naturally renewable resources, was used to partially replace complex nitrogen sources (peptone, yeast extract and beef extract) in the modified MRS-sucrose medium for EPS production by *Lactobacillus confusus* TISTR 1498. Under optimised and controlled conditions (pH 5.5, 100 g/L total sugar concentration and 50 rpm agitation speed), a high amount of ESP (about 38.2 g/L) was produced in a bioreactor. The cost of such fermentation could thus be significantly reduced.

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Communication

Effect of feeding *Spirulina platensis* on growth and carcass composition of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*)

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Abstract: The present study was designed to evaluate the use of spirulina (*Spirulina platensis*) as a protein source for hybrid red tilapia. Spirulina was incorporated into four isonitrogenous and isocaloric diets at 0, 5, 10 and 20% of the fishmeal-based diet. The test diets were fed to the fish at 2% body weight twice a day for 120 days. The final weight gain, specific growth rate, feed conversion ratio and survival rate of fish were not affected by spirulina supplementation (p>0.05). There was no significant difference (p>0.05) in carcass proximate composition of the fish fed on spirulina diets as compared to those on control diet. This study demonstrates that up to 20% of spirulina can be substituted for fishmeal in a fishmeal-based diet for hybrid red tilapia without any adverse effect on fish growth.

Keywords: spirulina, *Spirulina platensis*, hybrid red tilapia, fishmeal-based diet, carcass composition

Introduction

Fishmeal, a major ingredient of fish feed, has to be imported, especially that with a protein content over 60%. Low domestic fishmeal production is the result of many factors such as decreases in fishing area and catch. The high protein content of spirulina is one of the main reasons for it being considered as an unconventional source of protein. In addition, the amino acid pattern of the alga compares favourably with that of other food proteins, its cells being capable of synthesising all amino acids, thus providing all the essential ones to humans and animals [1].

Earlier studies involved examination of how supplementing a diet with dry spirulina powder affects the taste and quality of fish. Hirano and Suyama [2] reported that using spirulina in the feed of ayu results in a good growth with better flavour, firmer flesh and brighter skin colour. Other studies suggested that a 5% dietary spirulina supplementation depresses the lipid in the muscle and improves the taste and texture of striped jack [3-4]. As a pigmentation additive, spirulina was also found to improve the colour of red tilapia. Feeding raw spirulina as uni-feed to tilapia resulted in a slightly better evaluation of colour, texture and fatness than that obtained from commercial diets [5].

Other studies suggested that spirulina supplemented in the feed of freshwater fish has the effect of improving growth and promoting gonad development and maturation [6]. Tilapia fed solely on raw spirulina could maintain normal reproduction from parents to progeny throughout three generations [7]. It has been verified that larval tilapia fed solely on raw spirulina cultivated in photo-bioreactors can grow normally from the onset of exogenous feeding without any nutrient supplements [8]. Nandeesha et al. [9] reported that the final weight gain, specific growth rate and food conversion ratio of common carp are not affected by spirulina supplementation. A 3% supplementation of the algal meal in moist pellets produced a significant enhancement of growth and feed utilisation efficiency in red sea bream [10]. Therefore, an innovation of cost-effective technologies for cultivation of spirulina should help in the exploitation of this alga as a fish feed ingredient.

This study was designed to evaluate the use of spirulina as a partial replacement for fishmeal protein in practical diets for hybrid red tilapia in terms of growth and carcass proximate composition.

Materials and Methods

Experimental diets

Spirulina (*Spirulina platensis*) was produced from the culture medium consisting of dry egglaying chicken manure collected from a closed-system house supplemented with urea [11].

Four isonitrogenous (30% crude protein) and isocaloric (16.3 kJ/g) practical diets were formulated with different percentages of spirulina (0, 5, 10 and 20% of dry matter) as presented in Table 1. All ingredients were blended in a mixer and pelleted by a commercial meat grinder. The pellets were dried in a convection oven for 8 h at 65°C before being sealed in plastic bags and stored at -18°C until use. Each diet was fed to fish twice daily (0900 and 1500 h) at 2% body weight, and feeding rate was adjusted fortnightly.

Experimental units

Twelve $4m \times 5m \times 1.2m$ (width × length × depth) polyethylene net cages (1-cm mesh size) were set up by attaching the net to staked bamboo poles in a 1,000-m² earthen pond. The net was suspended at a 30-cm height above the water level and the cage bottom was kept at least 50 cm above the pond floor.

Experimental fish

Sex-reversed hybrid red tilapia (*Oreochromis mossambicus* \times *O. niloticus*) were obtained from a commercial hatchery in Chiang Mai. At the beginning, fingerlings with an initial weight of 10.89 ± 0.16 g were soaked in NaCl solution (5 g of NaCl/litre of water) before being randomly assigned to the

twelve net cages at a density of 50 fish/ m^2 . Commercial catfish pellets (30% crude protein) were fed during the first week to help the fish adapt to their new environment before the experiment.

Ingredient (%)		D	iet	
Ingredient (%)	S00	S05	S10	S20
Spirulina powder ^a	0.00	5.00	10.00	20.00
Fishmeal ^b	27.70	23.96	20.22	12.74
Rice bran	35.00	35.00	35.00	35.00
Soybean meal	20.00	20.00	20.00	20.00
Broken rice	16.80	15.54	14.28	11.76
Vitamin C	0.50	0.50	0.50	0.50
Proximate composition	(% of dry matter,)		
Crude protein	30.18 ± 0.14	30.10 ± 0.20	30.12 ± 0.15	30.19 ± 0.15
Crude lipid	8.74 ± 0.18	8.89 ± 0.14	8.63 ± 0.21	8.86 ± 0.20
Ash	10.24 ± 0.04	10.25 ± 0.10	10.06 ± 0.02	9.86 ± 0.02
Crude fibre	5.87 ± 0.30	5.38 ± 0.08	5.83 ± 0.34	5.42 ± 0.16
NFE °	44.96 ± 0.51	45.37 ± 0.41	45.36 ± 0.18	45.35 ± 0.22
Gross energy (KJ/g)	16.32 ± 0.04	16.37 ± 0.03	16.33 ± 0.06	16.37 ± 0.06

Table 1. Ingredients and proximate composition of experimental diets on dry weight basis

^a Crude protein 45.2, crude lipid 2.7%

^b Crude protein 57.9, crude lipid 11.1%

^c Nitrogen-free extract

Analytical methods

At the end of the experimental period of 120 days, nine whole fish from each cage were minced together and a sample was taken from the mince for proximate analysis of the carcass composition. The proximate composition of the experimental diets and fish carcasses was determined according to AOAC methods [12]. Crude protein was determined by micro Kjeldahl method; total lipid by Soxhlet extraction; crude fibre by acid detergent method; and ash by combustion at 550°C for 12 h. Carbohydrate as nitrogen-free extract (NFE) was calculated as weight of sample less crude protein, total lipid, crude fibre and ash. The water quality in the cage was monitored fortnightly by recording of water temperature (at 1600 h), dissolved oxygen (at 0600 h), total ammonia (at 1600 h), nitrite (at 1600 h) and pH (at 0600 h). An oxygen meter (YSI model 59), a spectrophotometer (Hach DR/2000) and a pH meter (Schott-Gerate CG 840) were utilised in acquiring such data.

Statistical analysis

Statistical analyses were performed on the data obtained from completely randomised design with three replications after 120 days of the experimental period. One-way ANOVA was used to test the effect of the dietary treatment. Tukey's test was also applied to compare the means when a significant difference (p<0.05) was detected by ANOVA.

Results and Discussion

The water quality in the present study (Table 2) was within an acceptable range for tilapia culture [13]. The growth performance of hybrid red tilapia fed on the experimental diets were not significantly different (p>0.05) (Table 3). Furthermore, fish survival was not statistically different among dietary treatments. Lu and Takeuchi [7] and Lu et al. [14] reported that compared to those fed on a commercial diet, tilapia fed solely on raw spirulina can maintain normal growth for 150 days to 30 weeks of the rearing period. Nandeesha et al. [9,15] reported that fishmeal protein can be effectively replaced by spirulina powder without sacrificing the growth of common carp, catla and rohu. El-Sayed [16] recorded that dried *Spirulina maxima* is an excellent substitute for fishmeal in silver seabream diets, even at a high substitution level (75%). Because it is rich in proteins, vitamins, minerals, essential amino acids and fatty acids [17], spirulina has been identified as a potential protein source for fish feed [9]. However, Takeuchi et al. [18] found that juvenile tilapia fed solely on the alga show a lower feed efficiency and protein efficiency ratio than commercial-diet-fed tilapia.

Diet	Temperature (°C)	pН	DO (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)
S00	30.77 ± 0.03	7.50 ± 0.00	3.29 ± 0.08	0.736 ± 0.031	0.279 ± 0.005
S05	30.83 ± 0.09	7.43 ± 0.03	3.14 ± 0.10	0.734 ± 0.024	0.288 ± 0.002
S10	30.63 ± 0.03	7.43 ± 0.03	3.17 ± 0.01	0.722 ± 0.005	0.276 ± 0.004
S20	30.63 ± 0.07	7.40 ± 0.00	3.00 ± 0.03	0.754 ± 0.010	0.282 ± 0.007
P-value	0.111	0.085	0.084	0.748	0.443

Table 2. Water quality in the experimental cages (Mean \pm SE^{*})

* Standard error

Fable 3. Growth	performance and	% survival	of hybrid red tila	pia fed on ex	perimental diets	$(Mean \pm SE)$
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Diet	Initial weight (g)	Final weight (g)	Weight gain (WG) (g)	Specific growth rate (SGR) (%/day)	Feed conversion ratio (FCR)	Survival (%)
S00	11.00 ± 0.00	158.61 ± 3.41	147.61 ± 3.41	1.78 ± 0.01	1.76 ± 0.01	91.11 ± 1.11
S05	10.89 ± 0.11	152.49 ± 8.09	141.60 ± 8.02	1.76 ± 0.03	1.78 ± 0.08	91.11 ± 1.11
S10	10.89 ± 0.11	143.49 ± 1.34	132.61 ± 1.36	1.72 ± 0.01	1.86 ± 0.01	92.22 ± 1.11
S20	10.78 ± 0.11	155.71 ± 2.26	144. 93 ± 2.16	1.78 ± 0.00	1.82 ± 0.03	90.00 ± 1.92
P-value	0.487	0.186	0.182	0.139	0.483	0.728

Table 4 shows the proximate composition of carcasses of the hybrid red tilapia fed on experimental diets. All proximate constituents of the carcasses were not significantly affected (p>0.05) by the different dietary treatments. In a previous study, spirulina meal incorporation up to 75% did not affect the body composition of silver seabream [16]. Nandeesha et al. [9] recorded no difference in the moisture and protein content in carcasses of common carp fed on diets incorporated with up to 55% spirulina powder. However, the fat content decreased concomitant with an increased spirulina supplementation level. In contrast, the feeding of algae was reported to elevate the body lipid in red

seabream [19]. For Nile tilapia (*O. niloticus*), Puwastein et al. [20] and Justi et al. [21] found similar values of moisture (78.1% and 79.0%), crude protein (19.8% and 18.2%), lipid (1.8% and 1.1%) and ash (1.0% and 1.36% respectively). For *Oreochromis* sp., Chou and Shiau [22] obtained different results from those present in this study for moisture (79.50%), crude protein (12.67%), lipid (2.05%) and ash (3.89%).

Diet	Moisture	Protein	Lipid	Ash	Fibre	NFE ^a
S00	73.89 ± 0.25	$17.82 \pm$	3.04 ± 0.14	2.76 ± 0.34	0.60 ± 0.20	1.88 ± 0.40
		0.21				
S05	73.61 ± 0.35	$18.54 \pm$	2.79 ± 0.42	2.16 ± 0.29	1.03 ± 0.16	1.88 ± 0.52
		0.33				
S10	73.60 ± 0.20	$18.52 \pm$	2.17 ± 0.28	2.65 ± 0.40	0.34 ± 0.10	2.72 ± 0.15
		0.30				
S20	74.72 ± 0.35	$18.82 \pm$	2.74 ± 0.70	1.68 ± 0.24	0.84 ± 0.20	1.20 ± 0.39
		0.16				
P-value	0.081	0.12	0.574	0.153	0.086	0.128

Table 4. Proximate composition (% wet weight) of carcasses of hybrid red tilapia fed on experimental diets (Mean \pm SE)

^a Nitrogen-free extract

Conclusions

The present study suggests that up to 20% of spirulina can be substituted for fishmeal in a fishmeal-based diet for hybrid red tilapia without any adverse effects on fish growth and proximate composition of carcasses. The use of spirulina in the diet can thus reduce the amount of incorporated fishmeal, which presently is the main protein source for the culture of most fish species.

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Full Paper

Season- and year-wise distribution of some trace metals and anions in Gulshan Lake, Bangladesh

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Abstract: A study was conducted to quantify some trace metals and anions in Gulshan Lake, Bangladesh over five years. The heavy metals, i.e. Pb, Cd, Cr, Co, Ni and Cu, were found below detection limit. The concentration ranges of other metals including anions were 170-1280 μ g/L for Fe, 30-190 μ g/L for Mn, 24-63 μ g/L for Zn, 4-6 mg/L for phosphate, 10-15 mg/L for sulphate, 17-39 mg/L for chloride and 0.20-0.30 mg/L for fluoride. No clear trend in concentration of detected metals and anions, sampling-site-, season- or year-wise, was observed for the period of 2002 and 2004 - 2007. All measured concentrations were found to be well below drinking water standards.

Keywords: Gulshan Lake, trace metals, anions, time-wise distribution, lake water, water pollution

Introduction

Inorganic and organic constituents are the most common environmental contaminants originating from human activities and have continuously been detected in waters and biota. The presence of some trace metals in aquatic environment causes serious problems on plants, animals and human health as well as the ecosystem [1-2]. As a result, water analysis has always attracted interest of scientists.

Organisms need trace amounts of many metals to survive. However, optimal nutritional requirements for the metals of a species generally lie in narrow concentration ranges. Severe imbalance

of metal proportions caused by exposure to elevated concentrations can induce death of an organism [3]. Metals such as Pb, Cd and As exhibit extreme toxicity even at trace levels [4]. Rivers are the dominant pathways for transport of metals [5] and trace elements may become significant pollutants of many small river banks [6]. The behaviour of a metal in natural waters is a function of the substrate sediment composition, the suspended solid composition and the water chemistry [7-8]. During their transport, the trace metals undergo numerous changes in their speciation due to dissolution, precipitation, sorption and complexation phenomena [9-10], which affect their behaviour and bioavailability. A recent study revealed that the accumulation and distribution of hydrocarbons, trace metals and chlorinated compounds in soil, water and the environment are increasing at an alarming rate, causing deposition and sedimentation in water reservoirs and affecting aquatic organisms [11]. The list of sites contaminated with trace metals grows every year, presenting a serious problem for human health and a grave danger to the environment [12].

In this investigation, the water of Gulshan Lake was studied. Gulshan Lake is one of the largest lakes and a great recreational place/centre in Dhaka, the capital city of Bangladesh. Due to the discharge of sewage and untreated effluents from different factories, the quality of the water bodies around Dhaka has deteriorated [13-14]. During 1980-2006 some, major construction work had also been undertaken along the valley of Gulshan Lake, which might have adversely affected the quality of the lake water. Moreover, mass tourism around the lake is a common fashion and can also affect the water quality. The use of polluted water can lead to several problems for human health and aquatic plant lives [15]. Back in 2002, a large number of fish were found dead in Gulshan Lake. It was then a big public concern and there was a general demand to monitor the concentration levels of pollutants in the lake water to explore the possible causes of death of the aquatic organisms. In our other short-term studies [16-20], surface and drinking water quality was monitored, which revealed that levels of some ionic species were higher than Bangladesh standard. This long-term research program, initiated in 2002 and covering a period of 5 years, was designed to monitor the concentration of some trace metals and anions in water collected from various spots of Gulshan Lake. The background levels of some important trace metals (Mn, Fe, Zn, Cr, Ni, Co, Cu, Cd and Pb) and anions (phosphate, sulphate, chloride and fluoride) in the lake water were established and the season- and year-wise variation of the levels of the detected ionic species in the lake water was examined between March 2002 and September 2007.

Materials and Methods

Standard chemicals and equipment

Nine individual standard solutions (Pb, Cd, Cr, Co, Ni, Cu, Fe, Mn and Zn) and certified reference materials used were purchased from Spex Certiprep. (NJ, USA). Concentrated sulphuric acid, concentrated hydrochloric acid, barium chloride, sodium nitrate, gum acacia, glacial acetic acid and ammonium molybdate, all of extra pure or Analar grade, were purchased from E. Merck (Germany). Standard solutions for anions and concentrated nitric acid were purchased from Sigma-Aldrich (USA). Ionic strength adjuster and total ionic strength adjustment buffer were supplied by Orion Research Inc. (USA). Atomic absorption spectrophotometers (Perkin-Elmer models 3110 and 560) were used to determine the concentrations of the metals. A UV-visible spectrophotometer

(Shimadzu, model 1201, Japan) was used to determine the concentrations of phosphate and sulphate. A digital ion-selective electrode meter (Orion ion analyser, model 470 A) was used for determination of chloride and fluoride. Electrical conductivity was measured by a conductivity meter (Jenway, model No. 4070) and pH was determined by a WTW Multiline P4 universal pH meter.

Selection of sampling spots and collection of water samples

Gulshan Lake is approximately 2.0 km long and 0.2 km wide. Three sampling stations were selected to cover the whole lake. Sampling points were at least 0.5 km distant from each other. The sampling stations are shown in Figure 1. A volume of 2 L of water sample was collected from each sampling station at a depth of 25 cm from the surface using a previously-cleaned 2L-capacity white polyethylene plastic bottles. The sampling was done on March (pre-monsoon), July (monsoon) and September (post-monsoon) starting from the year 2002 to 2007 with the exception of year 2003. On the year 2003, samples were not collected because of sample collection and analysis problems. After collection, samples were transported immediately (~ 1 hr) to the laboratory where each water sample was divided into two portions. One portion assigned for metal analysis was acidified (pH < 2.0) with concentrated nitric acid to prevent adsorption of metals onto the bottle. Measurement of anions was performed with the non-acidified portion quickly after filtration. Samples not immediately analysed were kept in the refrigerator at 4°C until further analysis.



Figure 1. Location of the three sampling sites

Sample preparation and analysis

A volume of 250 mL of the acidified sample was taken into a 300-mL beaker and then heated on a hotplate with 2 mL of conc HNO₃ until the total volume was reduced to approximately 5 mL. The concentrated sample was then transferred to a 10-mL volumetric flask and made up to volume for metal analysis by AAS.

Determination of phosphate [13,16,19] was performed by taking a known volume of water sample (depending on phosphate concentration on trial basis) in a 100-mL volumetric flask. $2N H_2SO_4$

(20 mL), 2% ammonium molybdate (2.5 mL) and 0.35% BaCl₂ (1 mL) were added successively into the flask, which was then heated for 15-20 min in a water bath. After cooling down to ambient temperature, the final volume was made up to 100 mL with distilled deionised water. Four standard solutions (0.1, 0.2, 0.4 and 0.8 ppm) were prepared in the similar way and the absorbance of the solutions were measured at 578 nm. A calibration curve was made and the concentration of phosphate in water sample was determined using the calibration curve.

For sulphate determination [13,16,19], 50 mL of water sample was taken in a 100-mL volumetric flask. Then 1% gum acacia (1 mL) prepared in 1:1 glacial acetic acid: deionised water, 6N HCl (1 mL) and 10% BaCl₂ (5 mL) were added successively into the flask and the final volume of 100 mL was made up with distilled deionised water. Standard solutions (1.0, 2.0, 4.0, 6.0, 8.0 and 10 ppm) were prepared in a similar fashion and their absorbance measured at 420 nm. Concentration of sulphate in water sample was measured using the calibration curve.

The concentrations of chloride and fluoride in water samples were measured using ion selective electrodes based on direct calibration [21]. A volume of each of the calibration standard solutions (0.01, 0.10, 1.0, 10.0 and 100 ppm) was added with the required amount of an ionic strength adjuster. For chloride, this was 2% of 5M NaNO₃. For fluoride, an equal volume of a total ionic strength adjustment buffer was used. The electrode potential (mV) of the standards was measured using the target electrode connected the ion-selective electrode meter. A calibration curve was prepared by plotting the electrode potential versus concentration. The concentration of the anions in the real sample was determined directly from the slope and the measured electrode potential value of the sample.

Calibration curve and quality assurance/quality control

A series of calibration levels were prepared from known concentrations of each reference standard solution. Calibration curves were prepared for all target elements using at least four different concentration levels of each standard solution. A regression analysis was carried out and a coefficient of determination (R^2 value) of 0.9995 was obtained for all elements. Individual calibration curve was used to determine the concentrations of each element/metal. Quality assurance includes measurement of detection limits (Table 1), recovery and analysis of standard certified reference materials. Procedural blanks were used throughout sample preparations to evaluate contamination from reagent, container, etc. No contamination was detected in the blanks. Procedures for sample preparations and analyses were validated by carrying out all operations in triplicate.

Results and Discussion

General

In this investigation, heavy metal concentrations and other physicochemical properties of the water of Gulshan Lake are presented for the period from 2002 to 2007 (excepting year 2003). In general, the levels of the different ionic species including toxic metals were much lower than the Bangladesh drinking-water standards [22]. Table 2 reports the concentrations of metals and anions observed in the lake water above the detection limit. The annual average values of phosphate were at borderline or only slightly lower than the standard value of 6 mg/L. The annual average chloride concentrations were 17-39 mg/L and were much lower than drinking water standard of 150-600 mg/L.
Metals/	LOD	Technique
anions	(µg/L)	used
Pb	20	AAS
Cd	3	
Cr	4	
Со	4	
Ni	10	
Cu	4	
Fe	5	
Mn	3	
Zn	3	
Cl	1000	ISE [*]
F	20	
SO4 ²⁻	1000	UV/VIS
PO4 ³⁻	60	

Table 1. Limit of detection (LOD) for metals and anions analysed by AAS and other techniques

* Ion-selective electrodes

The annual average concentrations of fluoride were 0.20-0.30 mg/L, which were much lower than the standard at 1.0 mg/L. Annual mean sulphate concentrations ranged from 9.6 to 15 mg/L and were much lower than the drinking water standard (400 mg/L). The concentrations of toxic metals (Pb, Cd, Cr, Co, Ni and Cu) were below the limits of detection and the drinking water standard levels. The average concentration ranges of the essential elements were 170-1280 μ g/L for Fe, 30-190 μ g/L for Mn and 24-63 μ g/L for Zn. Although the majority of samples provided Fe levels that meet Bangladesh drinking water standard (300-1000 μ g/L), those collected on 2002 and 2007 showed Fe concentrations below the standard (Table 2). The Mn concentrations were higher than the drinking water standard of 100 μ g/L with the exception of the 2007 value (Table 2). The Zn concentrations were much lower than the Bangladesh drinking water standard (5000 μ g/L).

Location- and season-wise variation

The distribution of trace metals and anions in surface water of Gulshan Lake was not uniform. Figure 2 shows concentrations of Fe, Mn, Zn, phosphate, sulphate, chloride and fluoride, and water electrical conductivity measured at three sampling stations (Figure 1) throughout the study period. Attempt was made to correlate the concentrations of the metals and anions observed in the sampling stations. However, due to their irregular trends of highest or lowest concentrations in a particular season, correlation efforts were not fruitful for Fe, Mn, Zn and phosphate. This shows that the origins of contamination that caused these differences were not uniform and could be related to tourist activities. In the case of sulphate, chloride, fluoride and electrical conductivity, no significant variation was observed among the sampling stations. Season-wise variation of concentrations of water quality characteristics are also shown in Figure 2. Inconsistency of observed results in season-wise

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Sample	Sam-		Concentration of anions (mg/L)							Concentration of metals				Elect	rical		
grabb-	pling															Condu	ctivity
ing	time	PO	4	SO.	1	Cl		F		Fe (m	g/L)	Mn (m	g/L)	Zn (µg	;/L)	(μS/	cm)
year		Avg ±	Yr	Avg ±	Yr	Avg ±	Yr	Avg ±	Yr	Avg ±	Yr	Avg ±	Yr	Avg ±	Yr	Avg±	Yr
		S.D.	avg	S.D.	avg	S.D.	avg	S.D.	avg	S.D.	avg	S.D.	avg	S.D.	avg	S.D.	avg
	Mar	4.00±		19.6±		19.2±		0.25±		0.21±		0.09±		9.03±		391±	
	IVIAI	0.19		2.62		3.37		0.10		0.10		0.00		2.98		5.03	
2002	Iul	1.86±	37	6.27±	12	1.85±	17	0.13±	0.2	0.10±	0.2	0.04±	0 1	7.30±	26	400±	397
2002	501	0.25	5.7	1.16	12	0.09	17	0.05	0.2	0.02	0.2	0.02	0.1	1.68	20	22.3	
	Son	5.12±		8.67±		29.2±		0.24±		0.29±		0.25±		60.2±		396±	
	Sep	1.42		0.58		2.43		0.02		0.21		0.08		32.1		9.71	
	Mar	5.40±		12.5±		12.8±		0.27±		0.65±		0.17±		51.9±		494±	
	IVIdi	1.15		1.01		1.36		0.00		0.26		0.01		12.7		11.1	
2004	Lul.	4.03±	27	12.8 ±	12	29.0±	22	0.21±	0.2	0.95±	0 0	0.19±	0.2	31.9±	11	376±	272
2004	Jui	0.94	5.7	0.19	12	5.59	22	0.02	0.2	0.70	0.8	0.04	0.2	15.6	41	48.5	372
	Son	1.57±		9.48±		23.7±		0.09±		0.83±		0.08±		38.1±		247±	
	Seh	0.43		1.15		0.55		0.01		0.72		0.02		2.15		21.0	
	Mar	9.90±		23.2±		46.2±		0.33±		1.69±		0.24±		128±		543±	
	IVIdI	1.96		7.04		4.81		0.00		1.03		0.06		56.1		37.6	
2005	Int	1.95±	11	9.65±	11	31.9±	20	0.26±	0.2	1.10±	1 2	0.17±	0.2	30.7±	62	413±	267
2005	Jui	0.57	4.1	1.43	11	2.75	50	0.02	0.2	0.85	1.5	0.08	0.2	8.02	05	32.2	507
	Sen	0.42±		1.44±		11.4±		0.13±		1.05±		0.16±		30.7±		145±	
	Jep	0.10		0.19		0.67		0.03		0.40		0.04		18.9		3.06	
	Mar	9.35±		11.3±		46.0±		0.27±		0.52±		0.16±		72.6±		370±	
	IVIdi	4.27		1.31		6.30		0.01		0.16		0.05		10.3		26.8	
2005		6.20±		9.53±		40.9±		0.25±		0.62±		0.17±		50.3±		369±	2.52
2006	Jul	1.16	6.1	1.16	9.6	17.9	39	0.04	0.3	0.05	0.6	0.05	0.2	37.3	51	31.6	368
	Sen	2.67±		7.94±		29.1±		0.24±		0.71±		0.19±		28.6±		365±	
	Jep	0.85		0.62		3.59		0.00		0.05		0.05		2.87		17.4	
	Mor	5.31±		21.8±		49.9±		0.27±		0.26±		0.06±		27.1±		510±	
	IVIdI	0.76		1.27		4.89		0.01		0.04		0.04		11.6		39.3	
		3.60±		15.5±		35.8±		0.23±		0.16±		0.04±		24.3±		373±	
2007	Jul	0.88	3.6	2.86	15	14.2	36	0.03	0.2	0.05	0.2	0.01	.04	8.70	24	13.2	373
	6	2.02±		8.84±		21.9±		0.14±		0.09±		0.02±		20.5±		235±	
	Sep	0.07		0.47		2.98		0.01		0.06		0.00		0.88		21.8	

|--|

Note: S.D. represents standard deviation calculated from the measurements at three sampling sites (P1, P2 and P3) for each sampling time. Avg and Yr represent average and year respectively. Year average was calculated from averages of three sampling times for each year.



Figure 2. Variation of water quality of Gulshan Lake at three sampling points over five years (2002, 2004-2007)

variation leads to a similar explanation with regard to sampling stations, indicating no particular sources of contaminants from local pollution events or other local anthropogenic origins other than tourism influence.



Figure 3. Seasonal variation of water quality of Gulshan Lake over five years (2002, 2004-2007)

Year-wise variation

Figure 3 represents time-course-dependent average concentrations of phosphate, sulphate, chloride, fluoride. Fe, Mn and Zn in Gulshan Lake water including its electrical conductivity. Yearwise phosphate and sulphate concentrations were almost constant excepting year 2006 and 2007. For chloride, approximately 1.4 times year-wise increment was observed starting from 17.0 mg/L in year 2002 (but excluding year 2007 in which there was a decrease). In the case of fluoride, there was virtually no year-wise variation throughout the study duration. For Fe, Mn and Zn, the year-wise highest and lowest average concentrations were all found in 2005 and 2007 respectively. Specifically, Mn concentration had dropped dramatically about 3 to 5 times in 2007 compared to other years' results. Year-wise distributions of Fe and Zn can be observed in the plots (Figure 3), which depict an initial increase and subsequent decrease of concentrations of both Fe and Zn. The unplanned discharges of untreated effluents from different factories such as tanneries, pharmaceutical industries, textile plants, sulfuric acid production and metal-working industries, apart from sewage, could be contributing to the variation of the above monitored items.

Conclusions

A five-year water quality monitoring program was conducted for Gulshan Lake water, which indicates that the lake water was still not polluted by of different metals and anions that might have caused the death of aquatic organisms such as fish in 2002. Neither sampling-site-wise, season-wise nor year-wise concentrations of all the studied variables showed any clear pattern of change. Thus, a possible explanation might be that the lake has been an acceptor of both regular and non-regular pollution pulses. Investigation of organic pollutants may be worthy.

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Full Paper

Optimisation of weld-metal chemical composition from weldingflux ingredients: A non-pre-emptive goal programming approach

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Abstract: A non-pre-emptive goal programming (NGP) model for weld-metal chemical composition optimisation from welding-flux ingredients is proposed. The proposed model, which is the first multi-objective mathematical programming approach to welding-flux formulation, provides increased flexibility to the welding-flux formulator in several ways: (1) several conflicting objectives can be simultaneously considered, (2) the best compromise for welding-flux formulation can be achieved with minimal expenditure of resources and experimental efforts, (3) the welding-flux designer can explore various trade-off options, and (4) the lead time and cost of developing welding consumables can be drastically reduced. This paper has extended the work of Kanjilal and co-investigators by coupling it with the NGP optimisation technique so as to prescribe the welding-flux ingredient levels that will achieve optimum performance for the flux at minimum experimental efforts and cost. The feasibility and suitability of the model is illustrated with data from the literature.

Keywords: welding flux, weld-metal composition, non-pre-emptive goal programming, multi-objective optimisation, multi-response

Introduction

Weld-metal mechanical properties and metallurgical features depend on the chemical composition of the as-deposited weld metal. Service requirements and progress in steel technology constantly demand new developments in welding process and consumables to deposit the weld metal with chemical composition equivalent to that of the base metal. The chemical composition of the weld

is influenced by the composition of the base metal, filler metal and flux [1-5]. A common practice in welding-consumable design is to use a filler metal with the same composition with the base metal. However, in some situations such as joining of dissimilar metals or for economic reasons, it may be necessary that a cheaper filler metal with composition different from that of the base metal is used. In such situations, the flux ingredients and their respective proportions are carefully selected so that the welding process can deposit the weld metal with the required chemical composition. The first step in welding-consumable design is to determine the filler metal to be used. Once the choice of the filler metal is made, the next stage of welding-consumable design is the development of the welding flux that can be used with the filler metal to deposit the weld metal with chemical composition that meets service requirements.

The challenge confronting the welding-flux designer (WFD) is not trivial even when the filler metal has the same composition with the base metal. This is due to the complex interaction between the flux ingredients and the weld pool during the welding process. In addition, the flux is required to perform several other functions such as metal refining, good arc striking and restriking, maintenance of stable arc and minimum spatter, and good slag detachability. All these characteristics combine to determine the weld-metal quality and the productivity of the welding process. Since filler metals are costly, it is cost-effective to use cheaper filler metals which may not have the same composition with the base metal. The required composition of the weld deposit is achieved by proper design of the welding flux. Often, the same type of filler metal is used to deposit the weld metal with different chemical composition through the manipulation of the flux composition. Hence it is possible to use the same type of filler metals if the fluxes are designed to suit the welding of such metals. A well designed flux will reduce the cost of welding and enhance the productivity of the welding process.

The objectives a welding flux is expected to achieve are often mutually incompatible. The incompatibility arises because the improvement in one objective or quality characteristic can only be made to the detriment of one or more of the other quality characteristics. Compromises and balances are often provided and designed into the flux by the WFD such that as many as possible of the quality characteristics or desirable objectives are met. The traditional method employed by the WFD to achieve these compromises and balances is by lengthy trial-and-error experiments. The drawbacks of the conventional welding-flux design approach are many [6-7]: (i) there is usually a very long lead time because of the extensive experimental weld production and testing, (ii) it is costly because of the labour requirements and consumption of a considerable amount of costly materials and energy during the lengthy experiments, (iii) the welding flux developed by the conventional method has a random character and it is difficult to guarantee optimum formulation or determine the flux with the best compromise formulation, and (iv) the feasibility or otherwise of achieving a desired compromise formulation. These drawbacks have persisted in the state-of-the-art welding-flux design because of a paucity of multi-objective optimisation models in the welding-flux technology.

Recently, Kanjilal et al. [8] proposed a methodology of developing analytical models for the prediction of the weld-metal chemical composition from welding-flux ingredient levels using data from experiments conducted according to a type of design-of-experiment (DoE) method known as

mixture experiments. Regression models for the prediction of oxygen, manganese, nickel, silicon and sulphur contents as a function of flux ingredient levels were proposed. The models can only predict the values of individual responses when the levels of flux ingredients are chosen. In practice, the WFD is interested in determining the flux ingredient levels that optimise all the responses or quality characteristics simultaneously and not individually. For instance, he may wish to achieve predetermined target values for some responses while at the same time he may also want to maximise desirable responses and minimise undesirable ones. The WFD therefore needs modelling tools that can handle multiple objectives and also assist him in exploring various trade-off options in order to be able to determine the best compromise for flux formulation. Goal programming is a versatile multi-objective optimisation modelling tool that has found wide-scale applications in various industries and in other areas of arc-welding technology except in welding-flux design where such applications are scanty [9]. This study seeks to extend the work of Kanjilal et al. [8] by coupling it with the non-preemptive goal programming (NGP) technique for the determination of flux ingredient levels that give the best compromise for flux formulation. As far as we know, NGP applications to the welding-flux design have not appeared in the open literature. In the following section we describe the systematic

procedure a WFD can follow in the application of NGP to welding-flux design. Next, numerical examples are solved using data from the work of Kanjilal and co-investigators to illustrate the application of goal programming to welding-flux design.

The Proposed NGP Approach

The key conditions for the application of the NGP methodology are: (a) all the response variables defining the quality of the welding flux depend on the same set of predictor variables and (b) prediction equations that describe the relationship between the response variables and the predictor variables can be assumed over the domain of interest. The procedure for developing such response equations is beyond the scope of this paper. The procedure has been described elsewhere [6-8]. Once these conditions are met the procedure outlined below may be followed to arrive at the flux formulation that will give the best balance between the responses. The procedure is in two phases. Phase I is the determination of the feasible solution space and feasibility while phase II is the development and solution of the NGP model.

Phase I: Determination of the solution space and feasibility

The WFD needs to know the solution space so that the feasibility or otherwise of achieving the desired performance level for the welding flux can be known early. The steps the WFD may follow are:

Step 1: The WFD determines, for each of the responses, the minimum and maximum values, $f_i^{\min}(x)$ and $f_i^{\max}(x)$ respectively, within the experimental domain by solving equations (1a and 1b) below.

<i>Minimise</i> , $\eta_i = f_i(x)$	
subject to:	(1a)
$x \in C_{*}$	

Maximise, $\eta_i = f_i(x)$ subject to: $x \in C_s$ (1b) where

 $\eta_i = \text{response } i, \text{ for each } i \in I$ $f_i(x) = \text{regression equation/function for response } i, \text{ for each } i \in I$ x = n-dimensional decision/predictor variables $C_s = \text{ set of feasible constraints}$ $f_i^{\min}(x) = \text{ minimum value of the regression equation for response } i, \text{ for each } i \in I$ $f_i^{\max}(x) = \text{ maximum value of the regression equation when solved individually for response } i, \text{ for each } i \in I$

I = set of responses, quality characteristics or objectives

Step 2: The WFD writes the values of $f_i^{\min}(x)$ and $f_i^{\max}(x)$ for each $i \in I$. The solution space is defined by $[f_i^{\min}(x), f_i^{\max}(x)]$ and any point within or on the boundary is theoretically feasible.

Step 3: The WFD writes the desired or target value, T_i , for each $i \in I$ and compares them with their respective $[f_i^{\min}(x), f_i^{\max}(x)]$ interval. If the T_i values for all $i \in I$ fall within or on the boundary of the solution space, then it is feasible to achieve the needed flux with the present flux ingredients. If one or more of the T_i values fall outside the solution space, then it is not feasible for the WFD to achieve the desired flux with the present flux ingredients without some changes.

Step 4: If it is feasible to achieve the desired flux, the WFD proceeds to Phase II. Otherwise he goes back to experiments and makes necessary changes such as the addition of ferroalloys, addition or substitution of flux ingredients or any other actions based on experience and principles of metallurgy.

Phase II: Development and solution of the NGP model

It is not feasible to achieve all the target values simultaneously because of the conflicting nature of the quality characteristics. Therefore, there will be deviations from the target values of the quality characteristics. The deviations are undesirable and should be minimised. The unwanted deviations are assigned weights according to their relative importance to the WFD and then minimised as an Archimedian sum. The basic approach of NGP consists of the following steps [10]:

Step 1: The WFD assigns weights, w_i^+ and w_i^- , to each quality characteristic's deviation $(d_i^+ \text{ and } d_i^-)$, where w_i^+ and w_i^- are the respective weights for the positive and negative deviations from the target value of response *i* for each $i \in I$ ($w_i^+ = w_i^-$ if positive and negative deviations are weighted equally), and d_i^+ and d_i^- are the respective positive and negative deviations from the target value of response *i* for each $i \in I$. The weights w_i^+ and w_i^- take the value zero if the minimisation of the corresponding deviation is not important to the WFD.

Step 2: The WFD constructs the goal constraints of the problem as: $f_i(x) + d_i^- - d_i^+ = T_i$

Step 3: The WFD determines the deviational variable(s) to be minimised for each response. The four possibilities are the following. If $f_i(x) \ge T_i$, then solutions that fall below T_i are undesirable. Hence negative deviations (d_i^-) are to be minimised. Similarly, if $f_i(x) \le T_i$, then minimize d_i^+ and if $f_i(x) = T_i$, minimise both d_i^+ and d_i^- . If $T_{Li} \le f_i(x) \le T_{Ui}$, then minimise d_{Li}^- and d_{ui}^+ , where T_{Li} and T_{Ui} are the respective lower and upper limits while d_{Li}^- and d_{ui}^+ are the negative and positive deviations from the respective lower and upper limits.

Step 4: The WFD writes the complete NGP model to minimise the weighted sum of the unwanted deviations, subject to the technological and goal constraints as:

Minimise,
$$asum = \sum_{i \in I} (w_i^- d_i^- + w_i^+ d_i^+)$$

subject to:
 $f_i(x) + d_i^- - d_i^+ = T_i$ (3)

$$x \in C_s$$

where 'asum' is the achievement function or the Archimedian sum of the deviations.

Step 5: The WFD uses the appropriate algorithm or software to solve the problem.

Application of the Proposed Methodology

This section illustrates how the proposed NGP methodology can be integrated with the models of Kanjilal et al.[8] to establish feasibility and prescribe the flux ingredient levels that give the best balance between the conflicting objectives. Kanjilal and co-investigators developed prediction models for manganese, silicon, sulphur, oxygen and nickel contents in the weld deposit as a function of flux ingredient levels for submerged arc welding of C-Mn steel. The empirical models are shown in Table 1. The flux ingredients used were the reagent-grade CaO, MgO, CaF₂ and Al₂O₃. The experiments were conducted with a low-carbon filler wire with a diameter of 3.15 mm at fixed welding parameters (current 400A, voltage 26V, speed 4.65 mm/s and electrode extension of 25mm). The composition of the base metal and filler wire is given in Table 2 while the flux formulations as per the mixture experiment design are given in Table 3.

Constraints

The constraints of the NGP model are lower and upper limits of the flux ingredients:

$15 \le x_{CaO} \le 35$	(4)
$10 \le x_{MgO} \le 32.40$	(5)
$10 \le x_{CaF_2} \le 40$	(6)
$8 \le x_{Al_2O_3} \le 40$	(7)

where, x_{CaO} , x_{MgO} , x_{CaF_2} and $x_{Al_2O_3}$ are the respective proportions of CaO, MgO, CaF₂ and Al₂O₃ in the flux. These proportions in the Kanjilal experiments always sum up to 80% of the flux composition.

(2)

The balance (20%) was made up of SiO_2 , Fe-Mn, Ni and bentonite, all of which are of constant amounts throughout the experiment (Table 3). Hence there is an additional constraint given by:

$$x_{CaO} + x_{MgO} + x_{CaF_2} + x_{Al_2O_3} = 80$$
(8)

Element	Prediction equation
Manganese	$f_{\rm Mn}(x) = -0.0244 x_{\rm CaO} + 0.059 x_{\rm MgO} + 0.0012 x_{\rm CaF_2} + 0.0024 x_{\rm Al_2O_3} - 0.0004 x_{\rm CaO} x_{\rm MgO}$
content	$+ 0.0012 x_{CaO} x_{CaF_2} + 0.0013 x_{CaO} x_{Al_2O_3} - 0.0013 x_{MgO} x_{CaF_2} - 0.0014 x_{MgO} x_{Al_2O_3}$
	$-0.0002 x_{CaF_2} x_{Al_2O_3}$
Silicon	$f_{\rm Si}(x) = 0.0107 x_{\rm CaO} + 0.0520 x_{\rm MgO} + 0.0083 x_{\rm CaF_2} + 0.0128 x_{\rm Al_2O_3} - 0.0011 x_{\rm CaO} x_{\rm MgO}$
content	$-0.0001 x_{\text{CaO}} x_{\text{CaF}_2} - 0.00008 x_{\text{CaO}} x_{\text{Al}_2\text{O}_3} - 0.0012 x_{\text{MgO}} x_{\text{CaF}_2} - 0.0013 x_{\text{MgO}} x_{\text{Al}_2\text{O}_3}$
	$+0.0002 x_{CaF_2} x_{Al_2O_3}$
Sulphur	$f_{\rm S}(x) = 0.00312 {\rm x}_{\rm CaO} + 0.00471 {\rm x}_{\rm MgO} + 0.00181 {\rm x}_{\rm CaF_2} + 0.0022 {\rm x}_{{\rm Al}_2O_3}$
content	$-0.00015 x_{_{CaO}} x_{_{MgO}} - 0.00007 x_{_{CaO}} x_{_{CaF_2}} - 0.00008 x_{_{CaO}} x_{_{Al_2O_3}} - 0.00009 x_{_{MgO}} x_{_{CaF_2}}$
	$- 0.00011 x_{MgO} x_{Al_2O_3} - 0.00002 x_{CaF_2} x_{Al_2O_3}$
Oxygen	$f_{O_2}(x) = 63.305 x_{CaO} - 12.42 x_{MgO} + 6.457 x_{CaF_2} + 16.775 x_{Al_2O_3} - 0.945 x_{CaO} x_{MgO} - 1.557 x_{CaO} x_{CaF_2}$
content	$- 2.061 x_{CaO} x_{Al_2O_3} + 0.835 x_{MgO} x_{CaF_2} + 0.767 x_{MgO} x_{Al_2O_3} + 0.378 x_{CaF_2} x_{Al_2O_3}$
Nickel	$f_{\rm Ni}(x) = -0.0776 x_{\rm CaO} + 0.0556 x_{\rm MgO} - 0.0181 x_{\rm CaF_2} - 0.0058 x_{\rm Al_2O_3} + 0.0006 x_{\rm CaO} x_{\rm MgO} + 0.0006 x$
content	$0.0030 x_{\text{CaO}} x_{\text{CaF}_2} + 0.0026 x_{\text{CaO}} x_{\text{Al}_2\text{O}_3} - 0.0015 x_{\text{MgO}} x_{\text{CaF}_2} - 0.0018 x_{\text{MgO}} x_{\text{Al}_2\text{O}_3} + 0.0018 x_{\text{MgO}} x_{\text{Al}_2\text{O}_3} + 0.0018 x_{\text{MgO}} x_{\text{Al}_2\text{O}_3} + 0.0018 x_{\text{MgO}} x_{\text{A}_2\text{O}_3} + 0.0018 x_{M$
	$0.0004 x_{CaF_2} x_{Al_2O_3}$

Table 1. Prediction equations for the chemical elements in the weld deposit

Source: Kanjilal et al. [8]

Table 2. Base metal and filler wire composition

Element	Carbon	Manganese	Silicon	Sulphur	Phosphorus	Nickel	Oxygen	Nitrogen
	(wt.%)	(wt.%)	(wt.%)	(wt.%)	(wt.%)	(wt.%)	(ppm)	(ppm)
Base metal	0.22	0.77	0.25	0.03	0.02	-	350	50
Filler wire	0.10	0.56	0.05	0.02	0.01	-	380	60

Source: Kanjilal et al. [8]

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Sample	Mixture	variables c	ompositio	n (wt %)	Constant composition (wt %)					Measured responses from experiments				
No.														
	CaO	MgO	CaF_2	Al_2O_3	SiO ₂	Fe-	Fe-	Ni	Bentonite	Mn	Si	S	0	Ni
						Mn	Si			(%)	(%)	(%)	(ppm)	(%)
P1	15.00	15.00	10.00	40.00	10.0	4.0	3.0	1.0	2.0	0.560	0.340	0.042	560	0.21
P2	15.00	15.00	40.00	10.00	10.0	4.0	3.0	1.0	2.0	0.520	0.210	0.042	570	0.11
P3	15.00	32.40	10.00	22.60	10.0	4.0	3.0	1.0	2.0	0.620	0.280	0.040	520	0.20
P4	15.00	17.00	40.00	8.00	10.0	4.0	3.0	1.0	2.0	0.470	0.170	0.034	500	0.17
P5	15.00	32.40	24.60	8.00	10.0	4.0	3.0	1.0	2.0	0.600	0.248	0.044	530	0.27
P6	35.00	15.00	10.00	20.00	10.0	4.0	3.0	1.0	2.0	0.670	0.229	0.028	380	0.24
P7	17.00	15.00	40.00	8.00	10.0	4.0	3.0	1.0	2.0	0.488	0.270	0.040	490	0.32
P8	35.00	15.00	22.00	8.00	10.0	4.0	3.0	1.0	2.0	0.580	0.200	0.028	480	0.29
Р9	29.60	32.40	10.00	8.00	10.0	4.0	3.0	1.0	2.0	0.690	0.260	0.027	330	0.23
P10	35.00	27.00	10.00	8.00	10.0	4.0	3.0	1.0	2.0	0.540	0.193	0.034	480	0.31
P11	24.43	23.14	24.43	8.00	10.0	4.0	3.0	1.0	2.0	0.700	0.120	0.021	300	0.50
P12	15.67	15.67	40.00	8.66	10.0	4.0	3.0	1.0	2.0	0.601	0.150	0.037	350	0.34
P13	25.92	24.36	10.00	19.72	10.0	4.0	3.0	1.0	2.0	0.620	0.160	0.016	320	0.30
P14	23.40	15.00	24.40	17.20	10.0	4.0	3.0	1.0	2.0	0.748	0.258	0.031	300	0.78
P15	19.87	32.40	14.86	12.87	10.0	4.0	3.0	1.0	2.0	0.800	0.370	0.020	320	0.59
P16	15.00	22.36	24.92	17.72	10.0	4.0	3.0	1.0	2.0	0.507	0.200	0.024	600	0.05
P17	35.00	19.00	14.00	12.00	10.0	4.0	3.0	1.0	2.0	0.595	0.273	0.015	470	0.33
P18	22.67	21.63	21.63	14.07	10.0	4.0	3.0	1.0	2.0	0.517	0.160	0.023	540	0.29

Table 3. Flux formulations determined by mixture design and results of the experiments

Source: Kanjilal et al. [8]

Determination of solution space and feasibility

For manganese content in the weld deposit, the $\left[f_{Mn}^{\min}(x), f_{Mn}^{\max}(x)\right]$ values are determined as follows:

$$\begin{aligned} &Minimise, (f_{Mn}(x)) = -0.0244x_{CaO} + 0.059x_{MgO} + 0.0012x_{CaF_2} + 0.0024x_{Al_2O_3} - 0.0004x_{CaO}x_{MgO} + 0.0012x_{CaO}x_{CaF_2} + 0.0013x_{CaO}x_{Al_2O_3} - 0.0013x_{MgO}x_{CaF_2} - 0.0014x_{MgO}x_{Al_2O_3} - 0.0002x_{CaF_2}x_{Al_2O_3} \\ &\text{subject to constraint equations (4)-(8)} \end{aligned}$$

and

 $Maximise, (f_{Mn}(x)) = -0.0244x_{CaO} + 0.059x_{MgO} + 0.0012x_{CaF_2} + 0.0024x_{Al_2O_3} - 0.0004x_{CaO}x_{MgO} + 0.0012x_{CaO}x_{CaF_2} + 0.0013x_{CaO}x_{Al_2O_3} - 0.0013x_{MgO}x_{CaF_2} - 0.0014x_{MgO}x_{Al_2O_3} - 0.0002x_{CaF_2}x_{Al_2O_3}$

subject to constraint equations (4)-(8)

Similarly, the $[f_i^{\min}(x), f_i^{\max}(x)]$ for Si, S, O and Ni are determined to establish the feasible solution space.

Development and solution of the NGP model

Suppose the WFD wants a flux that will deposit a weld metal with the composition in Table 4.

Table 4. Desired weld-metal composition

Element	Mn (%)	Si (%)	S (%)	O (ppm)	Ni (%)
Amount desired	0.760	≥ 0.200	≤ 0.035	250-350	0.460

We consider two welding-flux design situations:

- (i) All deviations are of equal importance to the WFD (Example 1);
- (ii) Some deviations are of greater concern to the WFD than others (Example 2).

Example 1: All deviations are of equal concern to the WFD. Hence all the deviations are assigned equal weights $(w_i^+ = w_i^- = 1)$ for each $i \in I$. The goal constraints of the problem may be stated as (Tables 1 and 4):

$f_{Mn}(x) + d_{Mn}^{-} - d_{Mn}^{+} = 0.760$	(manganese content goal constraint)	(11)
$f_{Si}(x) + d_{Si}^{-} - d_{Si}^{+} = 0.200$	(silicon content goal constraint)	(12)
$f_{S}(x) + d_{S}^{-} - d_{S}^{+} = 0.035$	(sulphur content goal constraint)	(13)
$f_{O_2}(x) + d_{LO_2}^{-} - d_{LO_2}^{+} = 250$	(oxygen content lower-bound goal constraint)	(14)
$f_{O_2}(x) + d_{UO_2}^{-} - d_{UO_2}^{+} = 350$	(oxygen content upper-bound goal constraint)	(15)
$f_{Ni}(x) + d_{Ni}^{-} - d_{Ni}^{+} = 0.460$	(nickel content goal constraint)	(16)

It is required that the Mn content be 0.760; therefore, deviations above and below the target are unwanted and must be minimised. Si content above 0.200 is acceptable to the WFD but values below

(10)

the lower limit are not desirable and must be minimised. Sulphur content in the weld must not exceed 0.035; therefore, deviation above this value is minimised. For oxygen, deviations above the upper limit and below the lower limit are minimized, while for Ni both the positive and negative deviations are minimised. The complete NGP model to minimise the Archimedian sum of the unwanted deviations is:

$$Minimise, asum = d_{Mn}^{-} + d_{Mn}^{+} + d_{Si}^{-} + d_{S}^{+} + d_{LO_{2}}^{-} + d_{UO_{2}}^{+} + d_{Ni}^{-} + d_{Ni}^{+}$$

subject to: (17)

technological constraints (the constraint equations (4)-(8)) goal constraints (the constraint equations (11)-(16))

Example 2: The WFD wants to achieve the same weld-metal content as in example 1 but the deviations are not of equal concern to him. Many methods exist by which the WFD may assign weights to the deviations to reflect his concern. In this study, the pairwise comparison method is used (Table 5) [11]. The normalised scores (weights) from Table 5 are:

$$w_{Mn}^{-} = 0.30$$
, $w_{Mn}^{+} = 0.06$, $w_{Si}^{-} = 0.17$, $w_{S}^{+} = 0.26$, $w_{LO_2}^{-} = w_{UO_2}^{+} = 0.02$, $w_{Ni}^{-} = 0.15$ and $w_{Ni}^{+} = 0.02$

The NGP model is:

 $Minimise, asum = 0.3.d_{Mn}^{-} + 0.06d_{Mn}^{+} + 0.17d_{Si}^{-} + 0.26d_{S}^{+} + 0.02d_{LO_{2}}^{-} + 0.02d_{UO_{2}}^{+} + 0.15d_{Ni}^{-} + 0.02d_{Ni}^{+}$ subject to: (18)

technological constraints (the constraint equations (4)-(8)) goal constraints (the constraint equations (11)-(16))

Deviation	d_{Mn}^{-}	d_{Mn}^{+}	d_{Si}^{-}	d_{S}^{+}	$d_{\scriptscriptstyle LO_2}{}^-$	$d_{UO_2}^{+}$	d_{Ni}^{-}	d_{Ni}^{+}	Total score (t)	Normalised score $t/T - Weight$
									score (i)	i / I – Weight
d_{Mn}^{-}	_	2	2	2	2	2	2	2	14	0.30
d_{Mn}^{+}	0	_	0	0	1	1	0	1	3	0.06
d_{Si}^{-}	0	1	_	0	2	2	1	2	8	0.17
d_s^+	0	2	2	_	2	2	2	2	12	0.26
$d_{\scriptscriptstyle LO_2}^{-}$	0	0	0	0	_	0.5	0	0.5	1	0.02
$d_{UO_2}^{+}$	0	0	0	0	0.5	_	0	0.5	1	0.02
d_{Ni}^{-}	0	1	0	0	2	2	_	2	7	0.15
d_{Ni}^{+}	0	0	0	0	0.5	0.5	0	_	1	0.02
Grand total (T) 47										1.00

Table 5. Pairwise comparison of deviations for weight determination

Note: Scores for WFD's relative concern: high concern = 2; moderate concern = 1; equal concern = 0.5; less concern = 0

Discussion

The models were solved with the Lingo 11 software. The ranges of chemical composition possible for each of the elements in the weld metal within the experimental domain define the feasible solution space, which is presented in Table 6, while the NGP result for flux formulation that gives the best balance between the elements is presented in Table 7. Any weld-metal composition that falls within this range is achievable with the filler wire. The appropriate welding-flux ingredient levels are determined by solving the NGP model. The WFD need not embark on extensive and expensive experiments when it is required that a consumable that will deposit the weld metal with different composition be made provided such composition falls within the feasible solution space.

The capability to establish feasibility is a great advantage of this methodology because of the cost saving in terms of time, labour, materials and energy. In the case of the conventional welding-flux design approach, the feasibility or otherwise of achieving the desired flux performance level is not easy to ascertain until a lot of time, labour and resources have been expended on trial-and-error experiments. For the case under study, the feasible solution space can be identified with only 18 experiments (Tables 3 and 6). The WFD can know how far a given desired composition deviates from the feasible solution space in situations where the desired weld-metal composition does not fall within the feasible solution space. This knowledge can serve as a useful guide for the WFD in determining the next line of action.

Element	Lower limit	Upper limit		
Mn	0.453 %	0.762 %		
Si	0.175 %	0. 374 %		
S	0.024 %	0.049 %		
0	249 ppm	633 ppm		
Ni	0.104 %	0.615 %		

 Table 6.
 Feasible solution space

The NGP model solutions are presented in Table 7. Without any further experiments the flux levels that will give the best balance between the various chemical elements are established. In example 1, where all deviations are equally weighted, the flux formulation that will give the best balance or compromise between the elements is 28.45% CaO, 32.40% MgO, 10.00% CaF₂ and 9.15% Al₂O₃ (Table 7). Manganese target is underachieved by 2.0% while sulphur content is 5.7% above the target value. Oxygen content is within the specified range (250-350 ppm). Nickel target in weld metal is achieved exactly while silicon content value is at an acceptable level.

In the case of example 2, where the negative deviation from the manganese content target is of the highest concern to the WFD and is assigned the highest weight, the manganese target is achieved, unlike in example 1 where it is underachieved by 2.0%. The corresponding welding-flux formulation is 26.43% CaO, 32.40% MgO, 10% CaF₂ and 11.17% Al_2O_3 . Positive deviation from the sulphur content target is next in terms of the concern of the WFD with a corresponding weight of 0.26. The positive

	TargetNGP modelvaluevalue			Deviation						Best compromise for welding-flux formulation (%) from NGP model	
		s)		Case 1			Case 2			Example 1	Example 2
Element		Case 1 (equal weight	Case 2	(-ve)	(+ve)	(%)	(-ve)	(+ve)	(%)		
Mn	0.760	0.745	0.760	0.015	-	2.0	0.0	0.0	0.0	CaO (28.45)	CaO (26.43)
Si	≥ 0.200	0.370	0.365	0.0		0.0	0.0		0.0	MgO (32.40)	MgO (32.40)
S	≤ 0.035	0.037	0.036		0.002	5.7		0.001	2.9	CaF ₂ (10.00)	CaF ₂ (10.00)
0	250-350	299	284							Al ₂ O ₃ (9.15)	Al ₂ O ₃ (11.17)
Ni	0.460	0.460	0.486	0.0	0.0	0.0	0.0	0.026	5.7		

Table 7. NGP model solution for flux formulation

deviation from the sulphur content target decreases from 5.7% in example 1 to 2.9% in example 2. The nickel content target is achieved in example 1 while in the case of example 2, there was a positive deviation of 5.7%. Because of the mutual incompatibility of the objectives, it is not possible to improve one quality characteristic without negatively affecting one or more of the other quality characteristics. When nickel content target is achieved in example 1, the manganese content is underachieved, while in example 2 the manganese content target is achieved but the nickel content target is overachieved. The NGP approach provides flexibility to the WFD, who can use different weight structures for the deviations from the targets to explore various trade-off options before choosing the one that best meets his needs.

Apart from weld-metal chemical composition optimisation, NGP method may also be useful in other multiple-objective welding-flux design situations. The determination of welding-flux ingredient levels that will achieve the desired values of acicular ferrite, polygonal ferrite, bainite and grain boundary ferrite contents in the weld-metal microstructure, or give the desired balance between mechanical properties such as yield strength, tensile strength, Charpy impact strength, hardness and elongation are such examples.

Conclusions

The NGP approach for multi-response optimisation of weld-metal chemical composition from welding-flux ingredients is proposed. The major conclusions are:

• It is feasible for the WFD to simultaneously consider many mutually incompatible responses or objectives with the NGP method.

• If all the responses depend on the same set of predictor variables and the models that capture the relationship between the response and predictor variables can be assumed over the experimental

domain, then the proposed methodology can be used to determine the best balance between the responses.

• The proposed methodology can be used to establish the feasible solution space and the feasibility or otherwise of achieving the desired performance level of the welding flux before a lot of resources are expended on experiments.

• The random character of the welding flux developed by traditional approach is eliminated because the NGP model ensures that the flux that gives the best balance between the objectives of the WFD is formulated.

• The WFD can use different weight structures to explore trade-off options before choosing the formulation that best suits his needs.

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