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THE EFFECTS OF TEMPERATURE ON MIXED CULTURE PHA PRODUCTION USING WASTEWATERS

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การวิจัยนี้แบ่งการศึกษาเป็นสองส่วนดังนี้ ส่วนแรกเป็นการศึกษาผลกระทบของอุณหภูมิและการจำกัดธาตุอาหารที่มีต่อสมรรถนะการผลิตพีเอชเอ อุณหภูมิที่ใช้ในการทดลองได้แก่ 10 20 และ 30 องศาเซลเซียส โดยในแต่ละอุณหภูมิได้ทำการทดลองกับการจำกัดธาตุอาหาร 3 แบบ คือ 1) จำกัดเฉพาะไนโตรเจน 2) จำกัดเฉพาะฟอสฟอรัส และ 3) จำกัดทั้งไนโตรเจนและฟอสฟอรัส ใช้รูปแบบการเดินระบบแบบ 2 ขั้นตอน ได้แก่ ขั้นตอนการเพิ่มจำนวนแบคทีเรียที่ผลิตพีเอชเอ แล้วตามด้วยขั้นตอนการจำกัดธาตุอาหารเพื่อกระตุ้นการผลิตพีเอชเอให้มากขึ้น ส่วนที่สองเป็นการศึกษาศักยภาพของการใช้น้ำเสียอุตสาหกรรมลูกกวาด (คาร์โบไฮเดรต; ซีโอดีประมาณ 10000-20000 มก./ล.) เป็นสารอาหารเพื่อการผลิตพีเอชเอ อุณหภูมิที่ใช้ในการทดลองส่วนนี้ ได้แก่ 20 องศาเซลเซียส และอุณหภูมิห้องประเทศไทย (28-31 องศาเซลเซียส) การจำกัดธาตุอาหารใช้รูปแบบจำกัดทั้งไนโตรเจนและฟอสฟอรัส รวมทั้งศึกษาผลกระทบที่มีต่อการผลิตพีเอชเอจากการเพิ่มภาระบรรทุกสารอินทรีย์ในสารอาหารที่ป้อนให้

ในการทดลองส่วนแรก อุณหภูมิส่งผลต่ออัตราการผลิตและยีลด์ของพีเอชเออย่างเห็นได้ชัด นั่นคือ อัตราการผลิตพีเอชเอและยีลด์เพิ่มขึ้นที่อุณหภูมิต่ำ การจำกัดธาตุอาหารทั้งไนโตรเจนและฟอสฟอรัสให้ผลการผลิตพีเอชเอดีกว่าการจำกัดธาตุอาหารแบบอื่น ในขณะที่การจำกัดธาตุอาหารฟอสฟอรัสไม่ควรถูกนำไปประยุกต์ใช้ เนื่องจากให้อัตราการผลิตรวมทั้งยีลด์ค่อนข้างต่ำ ยกตัวอย่างเช่น ที่อุณหภูมิ 20 องศาเซลเซียส กับการจำกัดธาตุอาหารเฉพาะไนโตรเจน ค่าสัดส่วนพีเอชเอต่อน้ำหนักแห้งมีค่าสูงที่สุด คือ ร้อยละ 40 โดยค่าความเข้มข้นพีเอชเอในระบบ คือ 2830 มก./ล. ค่ายีลด์ของพีเอชเอที่อุณหภูมินี้คือ 0.2 มก.พีเอชเอ/มก.ซีโอดีที่ถูกใช้ไป ในการทดลองจำกัดธาตุอาหารเฉพาะฟอสฟอรัส ที่อุณหภูมิ 10 องศาเซลเซียส มีค่าสัดส่วนพีเอชเอต่อน้ำหนักแห้งมีค่าสูงที่สุด คือ ร้อยละ 52 ค่าความเข้มข้นพีเอชเอในระบบ คือ 1491 มก./ล. และมีค่ายีลด์ที่ค่อนข้างต่ำ คือ 0.05 มก.พีเอชเอ/มก. ซีโอดีที่ถูกใช้ไป สำหรับการทดลองจำกัดธาตุอาหารทั้งไนโตรเจนและฟอสฟอรัส ผลผลิตพีเอชเอในการทดลองที่ 10 และ 20 องศาเซลเซียส แทบจะไม่แตกต่างกัน โดยค่าสัดส่วนพีเอชเอต่อน้ำหนักแห้งสูงสุด คือ ร้อยละ 45 และความเข้มข้นพีเอชเอ 2133 มก./ล.

ในการทดลองส่วนที่สอง ค่าสัดส่วนพีเอชเอต่อน้ำหนักแห้งสูงสุดที่ผลิตได้ คือ ร้อยละ 23.4 ที่อุณหภูมิ 20 องศาเซลเซียส การเพิ่มภาระบรรทุกสารอินทรีย์ในสารอาหารที่ป้อนช่วยเสริมให้มีการผลิตพีเอชเอเพิ่มขึ้น ในการทดลองที่อุณหภูมิห้อง ค่าสัดส่วนพีเอชเอต่อน้ำหนักแห้งสูงสุดที่ได้ คือ ร้อยละ 17.8 ด้วยสารอาหารที่มีภาระบรรทุกสารอินทรีย์สูงถึง 5 กรัม/ลิตร-วัน อย่างไรก็ตาม อัตราการผลิตพีเอชเอที่ได้จากการทดลองในส่วนนี้เพิ่มมากกว่าที่ได้รับจากการทดลองในส่วนแรก ทั้งนี้เป็นเพราะความเข้มข้นสลัดจ์ในระบบค่อนข้างสูง

ผลจากการศึกษานี้สรุปได้ว่า แอกทิเวเต็ดสลัดจ์มีศักยภาพในการสะสมพีเอชเอในปริมาณมากได้ อุณหภูมิส่งผลกระทบอย่างเห็นได้ชัดต่อการผลิตพีเอชเอ รูปแบบการจำกัดธาตุอาหารทั้งไนโตรเจนและฟอสฟอรัส หรือ จำกัดเฉพาะไนโตรเจนสามารถกระตุ้นการผลิตพีเอชเอได้ดีใกล้เคียงกันและมากกว่ารูปแบบการจำกัดธาตุอาหารเฉพาะฟอสฟอรัส นอกจากนี้ น้ำเสียอุตสาหกรรมถือได้ว่ามีศักยภาพในการเป็นสารอาหารสำหรับการผลิตพีเอชเอได้ แต่ควรมีการศึกษาเพิ่มเติมเพื่อปรับปรุงการเดินระบบให้เหมาะสมยิ่งขึ้น

คำหลัก : โพลีไฮดรอกซีอัลคาโนเอต (พีเอชเอ), โพลีไฮดรอกซีบิวไทเรต (พีเอชบี), ผลกระทบของอุณหภูมิ, การจำกัดธาตุอาหาร, แอกทิเวเต็ดสลัดจ์

ภาควิชา	วิศวกรรมสิ่งแวดล้อม	ลายมือชื่อนิสิต	
สาขาวิชา	วิศวกรรมสิ่งแวดล้อม	ลายมือชื่ออาจารย์ที่ปรึกษา	
ปีการศึกษา	2547	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม	

4271829021 : MAJOR ENVIRONMENTAL ENGINEERING
KEY WORD: POLYHYDROXYALKANOATE (PHA)/ POLYHYDROXYBUTYRATE (PHB)/ TEMPERATURE EFFECT/ NUTRIENT LIMITATION/ ACTIVATED SLUDGE.

SOPA CHINWETKITVANICH : THE EFFECTS OF TEMPERATURE ON MIXED CULTURE PHA PRODUCTION USING WASTEWATERS. THESIS ADVISOR : PROF.DR. THONGCHAI PANSWAD, THESIS COADVISOR : PROF.DR.CLIFFORD W. RANDALL, 190 pp. ISBN: 974-17-4766-7

This study was divided into two parts; the first one was to investigate the effects of temperature and nutrients limitation on PHA production using synthetic wastewater. The pre-selected temperatures of 10, 20 and 30^{o}C were investigated under three conditions, i.e., only either N or P limitation and combined N&P limitation. The two-stage operation approach, i.e. a growth phase followed by a nutrients limitation phase was employed. The second was to investigate the potential of using high-strength candy industrial wastewater (carbohydrate; 10000-20000 mg/l COD) as a substrate for PHA production. Two tropical temperatures of 20^{o}C and Thai room temperature (28-31^{o}C) were investigated with combined N&P limitation condition. Also, the effect of increasing of COD loading on PHA production was studied.

In the first part, temperature clearly affected PHA productivities and yields, i.e., high productivities and yields could be obtained at low temperatures. Considering effect of nutrients limitation, combined N&P limitation provided the best results regarding PHA production, whereas P limitation was not recommended for the production of a large amount of PHA due to its low productivity and yield. For example, the highest PHA contents and concentrations obtained during N limitation were 40 %TSS and 2830 mg/l at 20^{o}C with the yield of 0.2 mg/mg COD_u. During P limitation, the 10^{o}C system showed higher PHA production; the PHA content of 52 %TSS and concentration of 1491 mg/l with low yield of PHA of 0.05 mg/mg COD_u. For the N&P limitation experiments, PHA productions in both of 10^{o}C and 20^{o}C systems were not much different. The highest was 45 %TSS PHA content and 2133 mg/l PHA concentration.

In the second part, the highest PHA contents reached 23.4 %TSS obtained from experiment at 20^{o}C temperature. The increase of COD loading in the feeding could help promote the PHA production. The highest PHA content of 17.8 % of TSS was obtained with the fed COD loading of 5 g/l-d. However, the PHA productivities in this part were higher than those obtained in the first part due to much higher cell concentrations.

In conclusion, activated sludge biomass is capable of accumulating substantial amount of PHA. Temperature has a significant effect on PHA production. Also, combined N&P or only N limitations stimulate better PHA production than mere P limitation. Though industrial wastewater has a potential to be a substrate for PHA production, the optimum operation needs more investigation.

Department Environmental Engineering Student's signature .....

Field of study Environmental Engineering Advisor's signature .....

Academic year 2004 Co-advisor's signature .....

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CHAPTER 1

INTRODUCTION

1.1 Background

In both developed and developing countries, conventional plastics are extensively manufactured and used for numerous purposes such as production of commercial products, construction and manufacturing materials, packaging materials and numerous other goods which are meant to be disposable. In effect, they therefore become an essential material for mankind life and will be definitely more in the future. During this discussion, conventional plastics are defined as those synthesized from petroleum components. It is well known that these petrochemical plastics are resistant to microbial degradation. Their poor biodegradability when disposed into the environment is legendary, even for the so-called biodegradable plastics, which mostly fragment into small non-biodegradable pieces rather than undergo true biodegradation. The result of this resistance to biodegradation has resulted in massive environmental pollution, harm to both wildlife and humans, and has been a major contribution to the current landfill crisis. The problem leads to the efforts of scientists and engineers to develop biologically degradable plastics as substitutes.

A biodegradable plastic is a plastic designed to degrade by the action of microorganisms generally abundant in nature, such as bacteria, fungi and algae. As an alternative to non-biodegradable plastics, several kinds of biodegradable plastic materials have been researched and developed, e.g., polyhydroxyalkanoate (PHA), polylactide, polysaccharide, copolymers, aliphatic polyesters, poly(ε-caprolactone) and polyvinyl alcohol as listed in Table 1.1. Among these, PHA is the most attractive for the commercial scale production as their physical properties are similar to polypropylene and polyethylene, which are the raw materials for conventional plastics. Moreover, PHA can be biologically degraded to components that become a part of ecosystem carbon cycle. For example, it would degrade into water and carbondioxide

under aerobic condition or into methane under anaerobic condition by extracellular enzymes from microorganisms (Anderson and Dawes, 1990; Lee, 1996a).

Table 1.1: Commercial biodegradable plastics (reviewed by Booma et al., 1994)

Trade Name	Supplier	Chemical composition	Comments
	ICI Wilmington, DE, USA	Poly(β-hydroxybutyrate)	Isotactic, highly crystalline; similar to PP in properties but sinks in water.
BIOPOL	ICI Wilmington, DE, USA	Poly(β-hydroxybutyrate-co-β-hydroxyvalerate)	A range of properties can be obtained by varying the comonomer (HV) content.
	Cargill Minneapolis, MN, USA	Polylactic acid	Crystalline, rigid, biocompatible and compostable.
	EcoChem Wilmington, DE, USA	Polylactic acid	Compostable and recyclable
Tone polymers	Union Carbide Danbury, CT, USA	Polycaprolactone	Crystalline, resembles medium density PE; compostable but not water soluble.
Vinex	Air Products and Chemicals Allentown, PA, USA	Polyvinyl alcohol	Water soluble and compostable.
	Belland Solothurn, Switzerland	Acrylic copolymers	"Selectively soluble" polymers, dissolve in alkaline solution and can be reconstituted.
Enviro plastic	Planet Packaging Technologies (USA)	Polyethylene oxide and a compatible proprietary polymer	As water temperature increases, solubility decreases. Recyclable.

A few companies in Europe and United States have been trying to industrially develop the best production scheme for PHA and their downstream products (Table 1.2). However, at present one company, ZENECA Bio Products in United Kingdom, can

produce and market them commercially, under the trade name of BIOPOL™. Some products such as shampoo bottle (of Wella Company in Germany) and certain packaging in Japan use BIOPOL™ as a raw material.

Table 1.2: List of companies developing PHA (Lee, 1996a).

Companies	Products
Berlin Packaging Corp. (USA)	Marketing, sales, and distribution of ZENECA's BIOPOL™. Selling bottles made from BIOPOL™ to hair care company.
Bioscience Ltd. (Finland)	Medical applications of PHAs.
Bio Ventures Alberta Inc. (Canada)	Production of PHA by recombination *Escherichia coli.*
Metabolix, Inc. (USA)	Produrtion of PHA by transgenic plants. Licensing technology and joint ventures.
Monsanto (USA)	Production of PHA by transgenic plants (rapeseed and soybean).
Polyferm, Inc. (Canada)	Production of PHA from cheap substrate (hemicellulose). Production of PHA by *Pseudomonas cepacia* from xylose.
ZENECA Bio Products (UK)	Production of P(3HB) and P(3HB-co-3HV)(BIOPOL™) by fed-batch culture of *Alcaligenes eutrophus*.
ZENECA Seeds (UK)	Production of PHA by transgenic plants (rapeseed).

The possible uses from PHA are as the followings (Lee, 1996a).

- Packaging films, bags and containers
- Biodegradable carrier for long term dosage of drugs, medicines, insecticides, herbicides or fertilizers.
- Disposable items such as razors, utensils, diapers or feminine hygiene products.
- Starting materials for chiral compounds.
- Wound dressing
- Bone replacement and plates.

- Stimulation of bone growth and healing by piezoelectric properties.
- Blood vessel replacements.

Although this biodegradable polymer has great potential to be a substitute for conventional plastics, the production cost of biodegradable PHA plastics is unfortunately still not competitive with the plastics manufactured by the petrochemical industry. Some reviewers (Lee, 1996b; Braunegg et al., 1998) have reported that the price of BIOPOL™, a commercially marketed PHA plastic, is currently about 16-17 times higher than those of conventional plastics. BIOPOL™ is produced using aseptic pure culture conditions and purchased short-chain volatile fatty acids (VFAs), such as acetic acid, propionic acid, etc. One of major costs of commercial PHA production is therefore the expense of the substrate fed to bacteria in the fermentation process. As most of commercial PHA productions use pure culture strain of bacteria, the substrate supplied to those bacteria have to be specific and most of them unfortunately are relatively very expensive.

In the field of environmental engineering, it has been recently found that PHA plays the important role in activated sludge process, especially the anaerobic-aerobic system. That is, polyphosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO), dominant organisms in such a kind of activated sludge system, accumulated intracellular PHA as a metabolic intermediate during the anaerobic substrate uptaking period. Thereby, there have been several efforts to decrease the cost of PHA production by using mixed cultures such as activated sludge biomass (Satoh et al., 1998a; Takabatake et al., 1999; Punrattanasin, 2001). These studies have demonstrated that there is considerable potential for the utilization of mixed cultures and wastewater organics to produce PHAs for the production of fully biodegradable, yet versatile, plastics. Especially, Satoh et al. (1998a) attempted to produce PHA by using activated sludge instead of pure culture bacteria. They modified some of anaerobic-aerobic system by supplying a little amount of air to the system during anaerobic period, which was named as ‘microaerophilic’ period. The result was quite successful as they could accumulate PHA as high as 62 % of TSS.

Therefore, it is time to consider how wastewater treatment systems can be modified to permit utilization of activated sludge and organic wastewaters for PHA production, so that much less expensive plastics can be produced and the widespread use of conventional plastics can be reduced. If successfully applied, the financial liability of wastewater treatment could be reduced through the production of a commercially marketable by-product. This could prove to be specially attractive to industries that discharge high strength organic wastewaters.

1.2 The objectives

- To investigate the effects of temperature on PHA production by activated sludge cultures.
- To investigate the effects of nutrients limitation on PHA production by activated sludge cultures.
- To investigate the potential of using industrial wastewater as a substrate for PHA production by activated sludge.

1.3 Scope of works

This study was divided into two parts; one is to investigate the effects of temperature and nutrients limitation on PHA production using 660 mg/l COD (acetate and propionate) synthetic wastewater as a substrate. Three different temperatures were selected for this experimental part, i.e., 10, 20 and 30^{o}C. The limitations of only nitrogen (N), only phosphorus (P) and combined nitrogen and phosphorus (N&P) were investigated for each temperature. The second part is to investigate PHA production by using high strength (10000 mg/l COD) industrial (candy), glucose and sucrose based wastewater. Two tropical-ranged temperatures, 20^{o}C and Thailand room temperature were selected for this part.

CHAPTER 2

LITERATURE REVIEWS

2.1 Degradable plastics

Generally, conventional plastics are easily molded into any shape even fibers or thin films. In the past, much effort has been put to stabilize plastics and make them have high chemical and ultraviolet resistance; hence, they are very popular in many durable uses, disposable goods and packaging materials. However, recently, it is well aware that plastic litter and waste is a threat for a clean environment. Therefore, a degradable plastic is of major interest for the replacement of non-biodegradable plastics. Booma et al. (1994) has defined that "*a degradable plastic is a plastic designed to undergo a significant change in chemical structure under specific environmental conditions resulting in loss of some properties as measured by standard test methods appropriate to the plastic and the application in a period of time*". There are three types of degradable plastics introduced, that is, photodegradable, semi-biodegradable and completely biodegradable (Reddy et al., 2003).

Photodegradable plastics have UV sensitizing groups such as carbonyl, ethylenic or aromatic groups incorporated into the polymer. These groups are sensitive to sunlight by absorbing the extensive UV radiation (several weeks to months) and undergo photochemical reactions, resulting in the reduction of molecular weight and disintegration of their polymeric structure. However, when this kind of plastics disposed into landfills that usually lack of exposed sunlight, they therefore could not degrade via photochemical oxidation and remain non-degraded.

Plastics incorporated with starch and starch derivatives as fillers and additives belong to the group of semi-biodegradable plastics. The idea of starch-linked plastics is that once disposed into landfills, the starch component is biodegraded, consequently releasing the plastic fragments porous and susceptible to further biodegradation.

However, the degradation mechanism of starch/polyethylene studied by Krupp and Jewell (1992) has shown that bacteria attack only the starch component and the left behind polyethylene fragments plays the role of turning off the biodegradation, which thereby remain non-degradable.

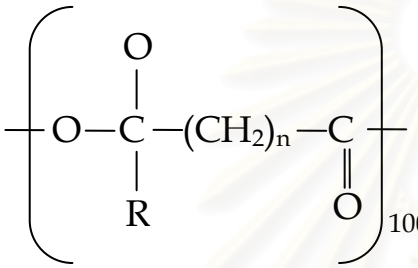
The third group is completely biodegradable plastics, which entirely degrade via microbial attack. This type of plastics is rather new and promising because bacteria can utilize and degrade it into CO_2 and biomass in a composting infrastructure. The various biodegradable plastics include polyhydroxyalkanoates (PHA), polylactide (PLA), poly(ε-caprolactone) and water solubles such as polyvinyl alcohol. The commercial biodegradable plastics are summarized in Table 1.1.

2.2 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are polyesters of hydroxyalkanoate intracellularly stored by numerous types of bacteria. PHAs act as carbon and energy storage for living cell, similarly to glycogen in animals or starch in plants. The storage materials are defined as substances which are synthesized by the organism under specific conditions such as unfavorable growth conditions. Dawes and Senior (1973) stated that PHA provided an excellent storage material because of its high reduced state or its function as an "electron sink". PHAs are homopolymer or copolymer that their molecular weight ranging from 2×10^5 to 3×10^6 daltons. PHAs are stored in bacteria cells in the form of granules within cytoplasm, which their number and size are varied and related to bacteria strain, and they are generally spherical in shape. *Alcaligenes eutrophus* could store about 8 to 13 PHA granules per cell with the diameter of granule ranging from 0.2 to 0.5 μm. (Anderson and Dawes, 1990).

The primary molecular structure of PHA is shown in Figure 2.1. The number of repeating units may be ranged from a number of 100 to 30000. Lafferty et al. (1988) noted that this variation of the number of repeating units is affected by:

- the method of extraction of PHA granules from the bacteria cell,
- the strain of cultured bacteria,
- the type of substrate employed,
- the time of harvesting the cells from a batch culture process,
- what factors were limiting growth,
- the actual fermentation conditions such as temperature.



n=1	R=hydrogen	Poly(3-hydroxypropionate)
	R=methyl	Poly(3-hydroxybutyrate)
	R=ethyl	Poly(3-hydroxyvalerate)
	R=propyl	Poly(3-hydroxyhexanoate)
	R=pentyl	Poly(3-hydroxyoctanoate)
	R=nonyl	Poly(3-hydroxydodecanoate)
n=2	R=hydrogen	Poly(4-hydroxybutyrate)
n=3	R=hydrogen	Poly(5-hydroxyvalerate)

Figure 2.1: Molecular structure of polyhydroxyalkanoates.

Monomers of PHA have been recently found up to 90 species. A group of 3-hydroxyalkanoates (3-HAs) contained 3-14 carbon atoms of carbon chain length in a molecule are found the most. 3-HAs have many different species, that is, some have only single bonds for an entire molecule while some have one or even two double bonds (so-called unsaturated 3Has) in the side chain. Furthermore, 3-HAs with a branched R-pendant group were also discovered and not only methyl groups or halogens (such as bromine, chlorine and fluorine) were detected as the R-pendent group, but also cyano groups. The molecular structure of 3-HAs could be linear or branch with aliphatic or aromatic side groups. PHAs are divided into two groups by the number of carbon atoms contained in their molecules (de Koning, 1995; Reddy et al., 2003), i.e., short chain length PHAs (SCL-PHAs) have 3-5 carbon atoms in a molecule and medium chain length PHAs (MCL-PHAs) have 6-14 carbon atoms. Polyhydroxybutyrate (PHB) was the most simply representatives of SCL-PHAs group. There are only a few organisms accumulating MCL-PHAsi, of which *Pseudomonas oleovorans* has been extensively studied. The biosynthetic PHA has several properties, which make them attractive for applications, such as they are thermoplastic

and/or elastomeric, high degree of polymerization, generally lipophilic, insoluble in water and biodegradable (Steinbüchel, 1996).

2.3 Physical properties

PHA has thermoplastic properties, in which it becomes highly viscous and moldable at temperature close to or above their melting points (T_m). The T_m values and glass transition temperature (T_g) or crystallinity depend on the composition of PHAs. Most of the studies on physical properties of PHAs have been conducted with homopolymers, polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV), and copolymer, polyhydroxybutyrate-co-polyhydroxyvalerate (P(HB-co-HV)). PHB is an aliphatic polyester homopolymer consisted of 23,000 – 25,000 molecules of 3-hydroxybutyrate monomer and has asymmetric carbon atoms with D-pattern structure. The conformational structure of PHB and P(HB-co-HV) consists of a right handed helix with a double screw along the chain axis. PHB is highly crystalline (80%) with a melting point of 179^{o}C, which is similar to polypropylene's melting point. Besides, its crystallinity and glass transition temperature (T_g) of 10^{o}C are also closed to those of polypropylene despite their chemical properties are completely different from each other (Anderson and Dawes, 1990). In addition, the other difference between PHB and polypropylene is PHB has a higher density and does not float in an aquatic environment, while polypropylene does. Also, PHB has poor chemical resistance than polypropylene.

PHB has several useful properties such as moisture resistant, piezoelectricity and UV resistant, but not resist to solvents. PHB will be decomposed at temperature of 200^{o}C, which is closed to its melting point, therefore, during melting stage, some of PHB could be decomposed and vaporized, then, lose its mass. Although PHB has more strength than polypropylene, PHB is also more brittle. Hence, there are efforts to lessen brittleness of PHB by adding other monomers such as 3-hydroxyvalerate (3HV) or by blending with other polymers. The copolymer of 3HB and 3HV has lower crystallinity and melting temperature, thus, it becomes stiffer and more flexible, which

widen its application area. A 3(HB-co-HV) polymer contained 25% of 3HV has a melting point of 137°C and a T_g of -6°C (Booma et al., 1994). Table 2.1 shows that polymers become tougher when the fraction of 3HV monomer is higher. Also, the lower melting point without affecting the temperature of decomposition helps prevent the degradation during melting processing. By varying the comonomer content, the properties of polymer can be well balanced for particular applications. The polymer contained low 3HV is hard and brittle like PVC, while medium level of 3HV content provides good balance of toughness like polypropylene (PP) and higher 3HV content makes the polymer be soft and tough, resembling polyethylene (PE).

Table 2.1: Comparison of polymers properties (Lee, 1996a)

Polymer	Melting temp. (°C)	Young's modulus (GPA)	Tensile strength (MPA)	Elongation to break (%)	Notched izod impact strength (J/m)
P(3HB)	179	3.5	40	5	50
P(3HB-co-3HV)					
3 mol% 3HV	170	2.9	38	-[a]	60
9 mol% 3HV	162	1.9	37	-	95
14 mol% 3HV	150	1.5	35	-	120
20 mol% 3HV	145	1.2	32	-	200
25 mol% 3HV	137	0.7	30	-	400
P(3HB-co-4HB)[b]					
3 mol% 4HB	166	-	28	45	-
10 mol% 4HB	159	-	24	242	-
16 mol% 4HB	-	-	26	444	-
64 mol% 4HB	50	30	17	591	-
90 mol% 4HB	50	100	65	1080	-
P(4HB)[c]	53	149	104	1000	-
P(3HHx-co-3HO)[d]	61	-	10	300	-
Polypropylene	170	1.7	34.5	400	45
Polyethylene-terephthalate	262	2.2	56	7300	3400
Polystyrene	110	3.1	50	-	21

Note : [a] Data not available.
[b] Poly(3-hydroxybutyrate-co-4-hydroxybutyrate).
[c] Poly(4-hydroxybutyrate).
[d] Poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate).

P(HB-co-HV) has been used in several applications such as in orthopedic devices, personal hygiene products, some packaging and controlled drug released. P(HB-co-HV) can undergo degradation in environments like activated sewage sludge or anaerobic sewage sludge. Moreover, P(HB-co-HV) is found to slowly degrade in sea water (Doi, 1990; Booma et al., 1994). The depolymerization of P(HB-co-HV) will involve two steps of degradation the high molecular weight polymer to a smaller fragment, thence, depolymerizes it into a monomer.

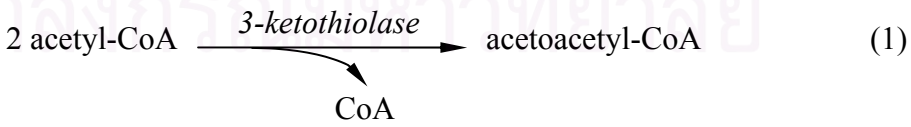
2.4 PHA biosynthesis

Studies with numerous organisms so far have shown that PHA is synthesized from the precursor, acetyl coenzyme A (acetyl-CoA), with a sequence of three reactions and related enzymes as the followings (Anderson and Dawes, 1990; Yamane, 1993);

1) 3-ketothiolase (acetyl-CoA acetyltransferase; EC 2.3.19)
2) Acetoacetyl-CoA reductase (hydroxybutyryl-CoA dehydrogenase; EC 1.1.1.36)
3) Poly(3-hydroxalkanoate) synthase (or PHA synthase)

This three-step pathway belongs to the bacterium *Ralstonia eutropha* (formerly know as *Alcaligenes eutrophus*). Three reactions during PHA synthesis are described in order as the followings;

<u>*Firs step*</u>: Two molecules of acetyl-CoA are condensed into acetoacetyl-CoA catalyzed by 3-ketothiolase enzyme as shown in the equation (1).



(1)

Second step: NADPH reduces acetoacetyl-CoA to D-(-)-3-hydroxybutyryl-CoA with a catalyst, acetoacetyl-CoA reductase, as shown in the equation (2).

$$\text{acetoacetyl-CoA} + \text{NADPH} + \text{H}^+ \xrightarrow{\textit{acetoacetyl-CoA reductase}} \text{D-(-)-3-hydroxybutyryl-CoA} + \text{NADP}^+ \qquad (2)$$

Third step: Poly (3-hydroxybutyrate) synthase polymerize D-(-)-3-hydroxybutyryl-CoA molecules to become PHB polymer as shown in the equation (3).

$$\text{D-(-)-3-hydroxybutyryl-CoA} \xrightarrow{\textit{poly (3-hydroxybutyrate) synthase}} \text{PHB} + \text{CoA} \qquad (3)$$

Therefore, during cell growth, intracellular NAD(P)H was oxidized quickly, resulting in low cofactors concentration and the activities in the TCA cycle remained high. Most of acetyl-CoA usually entered TCA cycle for generation of energy and formation of anabolic precursors essential for the synthesis of cell constituents. The reaction of citrate synthase in TCA cycle results in free CoASH released. The high free CoASH inhibited the reaction of condensation of acetyl-CoA into acetoacetyl-CoA as shown in Figure 2.2. Thereby, the PHA synthesis is expected to be low under such growth conditions. During the nutrient limitation, if protein synthesis is impaired or inhibited, acetyl-CoA will not enter TCA cycle, resulting in high concentration of acetyl-CoA and low concentration of free CoASH. 3-ketothiolase will not be inhibited and leading to synthesis of PHA.

Most of the organisms synthesize PHA using the above pathway; however, some bacteria have a little different detail of reactions from this pathway, such as, *Rhodospirillum rubrum* has five steps of PHA biosynthesis pathway, which the difference is this bacterium reduces acetoacetyl-CoA during the second step with NADH instead of NADPH and the product is L-(+)-3-hydroxybutyryl-CoA. There

must be enoyl-CoA hydratase enzyme to catalyze L-(+)-3-hydroxybutyryl-CoA to become D-(-)-3-hydroxybutyryl-CoA before entering the third step of synthesis process.

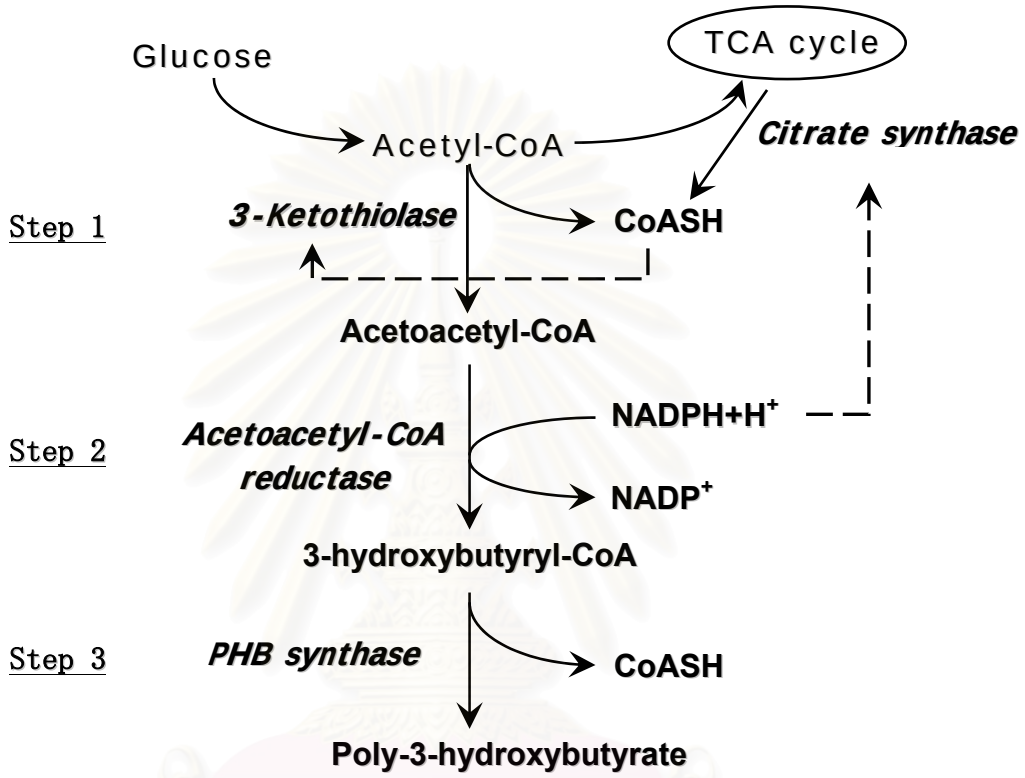


Figure 2.2: Regulation of PHB metabolism in *R. eutropha* (Du et al., 2001)

Pseudomonas oleovorans also has a different PHA biosynthesis pathway as the intermediates from the β-oxidation of fatty acids are directed to MCL-PHA biosynthesis, which contains 3-hydroxyoctanoate (3HO) and 3-hydroxyhexanoate (3HHx) as the main components when cultivated on octane, octanol or octanoate. Other strains in the genus of *Pseudomonas* except *Pseudomonas oleovorans* have a little more difference of PHA biosynthesis pathway, which is copolymers containing of MCL-hydroxyalkanoates are synthesized from acetyl-CoA. The precursors for PHA synthesis are originated from de novo fatty acid synthetic pathways.

In addition, in the studies of *Azotobacter beijerinckii* and *Alcaligenes eutrophus* bacteria have found that there is the degradation of PHA catalyzed by PHA depolymerase occurred simultaneously during the synthesis process; their PHA metabolism was illustrated as a cyclic pathway shown in Figure 2.3.

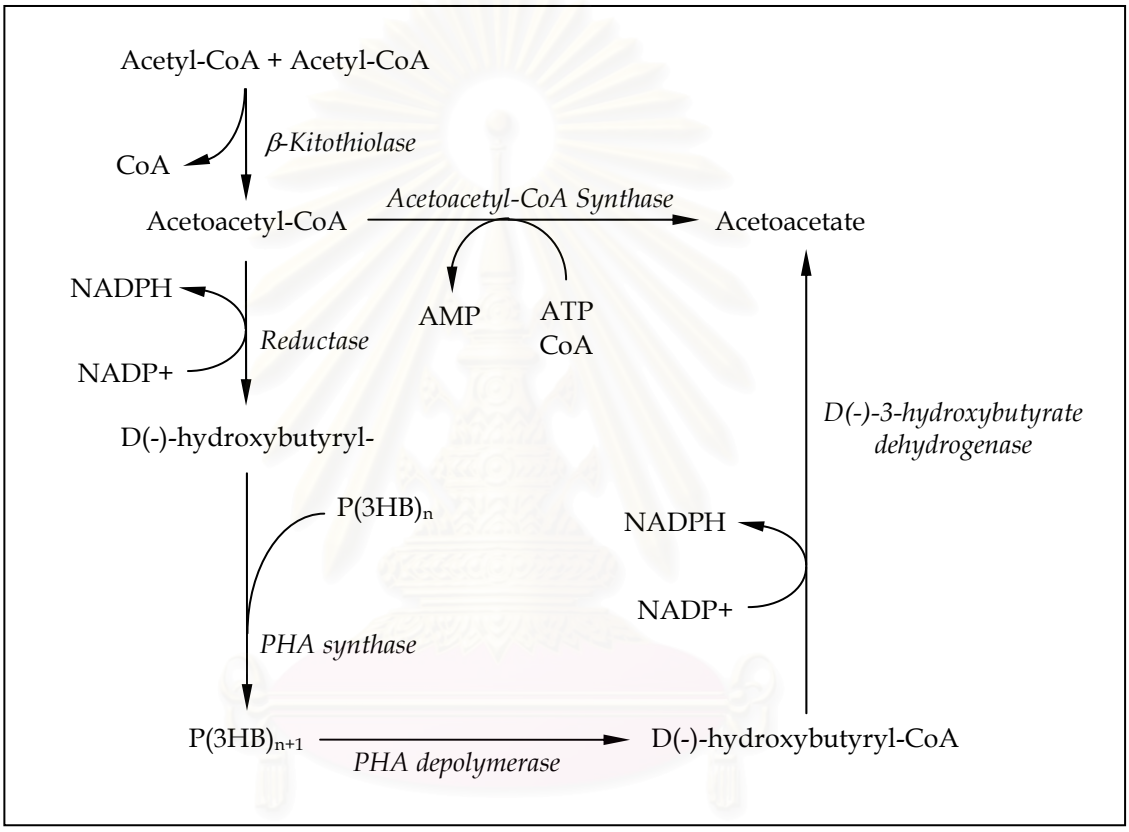


Figure 2.3: Metabolic pathway involved in the synthesis and degradation of PHB in *Alcaligenes eutrophus* (Lee, 1996a)

2.5 Microorganisms related to PHA accumulation

There are more than 300 different bacterial species capable of PHAs synthesis. The genera of these bacteria listed in Table 2.2. Only relatively few groups of bacteria cannot synthesize intracellular PHA, e.g., methanogenic bacteria and lactic acid bacteria (Steinbuchel, 1996). Among this variety of microorganisms, only some bacterial species have been employed for the commercial production of PHAs, e.g., *Alcaligenes eutrophus*, *Alcaligenes latus, Azotobacter vinelandii*, some strains of methylotrophs, *Pseudomonas oleovorans* and, recently, recombinant *A. eutrophus*, recombinant *Escherichia coli* and recombinant *Klebsiella aerogenes*. These bacteria have a capability of being cultivated to high cell concentration with high PHA content in a short period of time, resulting in high productivity.

Table 2.2: Poly(3-hydroxybutyrate)-Accumulating Microorganisms (Doi, 1990)

Acinetobacter	*Gamphosphaeria*	*Photobacterium*
Actinomycetes	*Haemophilus*	*Pseudomonas*
Alcaligenes	*Halobacterium*	*Rhizobium*
Aphanothece	*Hyphomicrobium*	*Rhodobacter*
Aquaspirillum	*Lamprocystis*	*Rhodospirillum*
Azospirillum	*Lampropedia*	*Sphaerotilus*
Azotobacter	*Leptothrix*	*Spirillum*
Bacillus	*Methylobacterium*	*Spirulina*
Beggiatoa	*Methylocystis*	*Streptomyces*
Beijerinckia	*Methylosinus*	*Syntrophomonas*
Caulobacter	*Micrococcus*	*Thiobacillus*
Chlorofrexeus	*Microcoleus*	*Thiocapsa*
Chlorogloea	*Microcystis*	*Thiocystis*
Chromatium	*Moraxella*	*Thiodictyon*
Chromobacterium	*Mycoplana*	*Thiopedia*
Clostridium	*Nitrobacter*	*Thiosphaera*
Derxia	*Nitrococcus*	*Vibrio*
Ectothiorhodospira	*Nocardia*	*Xanthobacter*
Escherichia	*Oceanospirillum*	*Zoogloea*
Ferrobacillus	*Paracoccus*	

Bacteria used in PHA production can be categorized into two groups considered from the culture conditions required for PHA synthesis. The first group of bacteria needs the limitation of some essential nutrients such as nitrogen, phosphorus, potassium, magnesium, sulfur or oxygen for stimulating PHA synthesis, concurrently, with the ample availability of supplied carbon substrate, for example, *Alcaligenes eutrophus, Protomonas extorguens, Pseudomonas oleovorans, etc.* The second group of bacteria does not require nutrient limitation condition for PHA production; they can accumulate intracellular PHA during growth such as *Alcaligenes latus, Azotobacter vinelandii* and recombinant *Escherichia coli*

2.5.1 *Alcaligenes eutrophus*

Among various PHA accumulating microorganisms, *Alcaligenes eutrophus* (or another name is *Ralstonia eutropha*) has been most extensively used in both of commercial PHA production and regarding research due to its ability to accumulate large amount of PHA, up to 80% of cell dry weight (Anderson and Dawes, 1990; Lee, 1996a) with good molecular mass and that PHA can be relatively easily extracted. Fed-batch cultures are usually carried out with the nutrient limitation in order to achieve a high PHA production from this bacterium. Bitar and Underhill (1990) and Shimizu et al. (1994) could accumulate PHA content in *Alcaligenes eutrophus* bacterium higher than 70% w/w with nitrogen limitation condition while the first group of researchers used butyric acid and the latter used glucose as substrates. In addition, Ryu et al. (1997) reported that their fed-batch fermentation of *Alcaligenes eutrophus* with phosphate limitation could obtained PHB content as high as 80% w/w and high PHB productivity of 3.14 g/l-h. Besides PHB, *Alcaligenes eutrophus* can also synthesize other copolymers of PHA such as P(3HB-co-3HV) when both glucose and propionic acid (Byrom, 1992) or both butyric acid and valeric acid (Shimizu et al., 1994) are fed as carbon sources. This bacterium was also tested for its ability to produce P(3HB-co-4HB) copolymers by Renner et al. (1996). Furthermore, Madden et al. (2000) cultivated *Alcaligenes eutrophus* for the production of PHA terpolymers, poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-4-hydroxybutyrate) or P(3HB-co-3HV-co-

4HB), on the feed composed of glucose, propionic acid and either γ-butyrolactone or sodium 4-hydroxybutyric acid. The bacterium in this study accumulated PHA contents ranging from 46 to 64% w/w.

There are several attempts to develop the efficient PHA production employing *Alcaligenes eutrophus*. Kim et al. (1994a) stated that the concentration of carbon source must be controlled at the level of 10-20 g/l for the efficient production of PHB by *Alcaligenes eutrophus*. They suggested two methods to maintain glucose concentration, one of which is a method using CO_2 evolution rate (CER) and another is an automatic on-line glucose monitoring system. In comparison between two methods, higher PHB concentration of 121 g/l and productivity of 2.42 g P(3HB)/l-h and 76% w/w of PHB content were obtained in 50 hrs from the process using an on-line glucose monitoring method. Furthermore, they found that prolongation of culturing phase, resulting in higher cell concentration, could increase the final PHB concentration and productivity. With the same research group (Kim et al., 1994b) and the same culturing strategy, the highest P(3HB-co-3HV) concentration of 118 g/l and productivity of 2.55 g/l-h were obtained.

The use of hydrogen and carbon dioxide as substrate for P(3HB) production has been considered. Another group of researchers (Sugimoto et al., 1999) has developed a new concept for cultivation method, that is, there have to be a heterotrophic growth stage followed by an autotrophic P(3HB) accumulation stage. *Alcaligenes eutrophus* had been heterotrophically grown on acetic acid automatically fed by pH-stat continuous feeding system. Subsequently, the autotrophic production of P(3HB) from CO_2 was carried out under oxygen-limited condition. P(3HB) concentration of 12.6 g/l obtained after 83 hrs of autotrophic cultivation was not quite high level. However, CO_2 is considered as a more economical substrate than organic acids if the problem of explosion of flammable gas is not accounted.

To date, the bacterium *Alcaligenes eutrophus* was employed in the commercial production of copolymers, P(3HB-co-3HV) with 0 to 24 mol% 3HV, marketed with

the trade name of BIOPOL® outside North America by Monsanto (Braunegg et al., 1998). BIOPOL® is also marketed in USA under another trade name of PHBV by ICI Chemicals (Lindsay, 1992).

2.5.2 *Alcaligenes latus*

Besides *Alcaligenes eutrophus, Alcaligenes latus* was considered as a remarkable candidate for the production of PHA because of its fast-growing nature and ability to accumulate the homopolymer, P(3HB), at substantial rates during normal cell growth (Lee, 1996a; Braunegg et al., 1998). The PHA content in this bacterium can be as high as 80% of cell dry weight (Hrabak, 1992). With the advantage of accumulating polymer during cell growth, the fermentation using *Alcaligenes latus* can be carried out in one step. Furthermore, *Alcaligenes latus* can utilize sucrose directly as well as glucose syrup or a mixture of glucose and fructose. Therefore, further cost reductions would be possible if cheap beet or cane molasses can be used as carbon source. Industrial P(3HB) production by *Alcaligenes latus* was performed by Chemie Linz GmbH in Australia in the late of 1980s (Hrabak, 1992; Braunegg et al., 1998). Yamane et al. (1996) cultivated *Alcaligenes latus* with fed-batch fermentation using sucrose as a sole carbon substrate. The PHB content in this bacterium was maintained at ca.50% throughout the fermentation, whereas harvested cell concentration was raised as high as 142 g/l and PHB concentration of 68.4 g/l was obtained in a short period of culture time of 18 hrs, resulting in high productivity of 4.0 g P(3HB)/l-h. Although *Alcaligenes latus* can accumulate a substantial amount of P(3HB) without need of nutrient limitation, Wang and Lee (1997) did the contrary in their work by employing the nitrogen limitation condition during the accumulation of P(3HB). They, however, could enhance the P(3HB) content as high as 88.3% of cell dry weight and obtain the highest P(3HB) productivity of 4.94 g P(3HB)/l-h reported up to date. Lee and Choi (1998) designed and analyzed the process for the large scale production of 100,000 tonnes of P(3HB) per year in according to fermentation strategy and results obtained from Wang and Lee (1997). They stated that the production cost of P(3HB) by *Alcaligenes latus* could be lessen to 2.6 US$ per kg P(3HB).

In addition to homopolymer of P(3HB), Hrabak (1992) reported that *Alcaligenes latus* has a potential to produce copolymers, e.g., P(3HB-co-3HV), P(3HB-co-4HB) and terpolymer of P(3HB-co-3HV-co-4HB) at least in lab scale experiments. Ramsay et al. (1990) compared the production of P(3HB-co-3HV) copolymer among six bacterial strains, including *Alcaligenes eutrophus* and *Alcaligenes latus*. They found that under the same dilution rate of substrate feeding with nitrogen limitation, *Alcaligenes latus* accumulated about 40% (wt/wt) P(3HB-co-3HV) while *Alcaligenes eutrophus* did a maximum value of 33% (wt/wt). The authors also suggested that though *Alcaligenes latus* did not required nutrient limitation for the accumulation of PHA and was suited for single-stage-chemostat PHA production, the addition of a second stage resulted in an increase of PHA content in the cells.

2.5.3 *Azotobacter vinelandii*

Azotobacteraceae is a family of aerobic nitrogen-fixing bacteria, of which several strain have been widely studied for the accumulation of P(3HB) polymer. *Azotobacter* species were actually the first microorganism cultivated by ICI for P(3HB) production (Byrom, 1992). However, these bacteria also accumulated large amounts of polysaccharide and were difficult to control; they therefore were rejected for further development (Steinbüchel, 1996). A mutant strain of *Azotobacter vinelandii*, named UWD, has been developed and studied by Page and Knosp (1989). The authors mentioned that the mutation enhanced the formation of P(3HB) during the exponential growth phase of strain UWD. Also, the synthesis of P(3HB) affected the formation of cell mass in the way for oxidizing NADH. Furthermore, the production of P(3HB) could be increased by employing with a high cell mass; this mutant strain could accumulate P(3HB) up to 75 % of cell dry weight. Page (1992) demonstrated that in addition to acetate, mutant *Azotobacter vinelandii* was be able to accumulate a large amount of P(3HB) (over 70% w/w of P(3HB) content) from inexpensive, unrefined of carbon sources such as glucose, sucrose, beet molasses. Later, it was shown that mutant UWD was also be able to synthesize P(3HB-co-3HV) by feeding odd-chain fatty acids (valeric, heptanoic or nonanoic acids) to the culture during growth on a

primary carbon source, usually a sugar. Page and Manchak (1995) showed that fed-batch glucose cultures with 10 mM (final concentration) valerate promoted the formation of the copolymer P(3HB-co-3HV) up to 94 % of cell dry weight with 18% HV fraction.

Another similar genus bacterium, *Azotobacter beijerinckii*, had been studied by Senior and Dawes (1971). Their collaborators (Senior et al., 1972) obtained P(3HB) content of 74% of the dry weight towards the end of exponential growth. Further studies about the regulation of P(3HB) synthesis by *Azotobacter beijerinckii* were carried out by the same group of researchers (Senior and Dawes, 1971; Senior and Dawes, 1972; Jackson and Dawes, 1976). In addition, *Azotobacter chroococcum* was investigated by Savenkova et al. (1999). The addition of ammonium increased both cell growth and P(3HB) accumulation in *Azotobacter chroococcum*. The effects of phosphate supply and aeration were investigated with the aim of attaining high productivity of P(3HB) in this study. High P(3HB) content of 75 % of cell dry weight and the yield of 0.24 g/g were achieved.

2.5.4 *Pseudomonas*

Pseudomonas oleovorans does not accumulate P(3HB) under the same conditions as other microorganisms does. It accumulates medium-chain-length (MCL) PHAs when grown on carbon sources such as medium-chain-length alkanes or alkanoic acids (Anderson and Dawes, 1990; Doi, 1990; Braunegg et al., 1998). It can also grow on simple carbon source such as glucose but there is no substantial PHA accumulation reported (Haywood et al., 1989). The composition of polymer synthesized by this bacterium depends on the growth substrate used in cultivation. For example, when it grows on n-decane, a polymer contained 3-hydroxydecanoate, 3-hydroxyoctanoate and 3-hydroxyhexanoate is formed. Nevertheless, Huisman et al. (1989) noted that 3-hydroxyoctanoate and 3-hydroxynonanoate were the major components of the copolymer when substrate larger than octane or nonane was used. Preusting et.al. (1993) established an ammonium-limited continuous culture of *Pseudomonas*

oleovorans for the PHA production using n-octane as a sole carbon source. The P(3HHx-co-3HO) productivity of 0.58 g PHA/l-h was reached with high cell density of 11.6 g/l. *P. oleovorans* in this work remained stable, without being contaminated, in continuous cultures for at least 30 days. Not only under nutrient limitation phase, but also MCL-PHA accumulation by *P. oleovorans* occurred under carbon-limited growth conditions. However, in the work of Durner et al. (2001), the PHA content from nitrogen-limited culture was always higher than from carbon-limited one. The best PHA content obtained from this work was 42 % of cell dry weight with PHA yield of 0.23 g C-PHA /g-$C_{substrate}$ when *P. oleovorans* was cultivated on octanoate under nitrogen-limited condition.

Huijberts et al. (1992) investigated the biosynthesis of PHAs by *Pseudomonas putida* growing on carbohydrates, i.e., glucose, fructose and glycerol. Synthesized PHAs composed of 3-hydroxydecanoate (3HD) as the major constituent and six other monomers, including units of 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO) and saturated and mono-unsaturated monomers of 12 and 14 carbon atoms. PHA contents from this bacterium were quite low, ranging from 17 to 25 % of cell dry weight. The authors indicated that when *Pseudomonas putida* grew on nonrelated substrates such as glucose, it synthesized PHA monomers from intermediates of de novo fatty acid biosynthesis. Kim et al. (1997) tried to enhance the production of PHA by *Pseudomonas putida* with a two-step fed-batch cultivation. The cultivation strategy was designed to grow bacterial cells on glucose and octanoate in the first step and subsequently subjected to nutrients (N & O_2) limiting conditions in the second step with an addition of octanoate as the sole carbon source. PHA content was improved from less than 40 to 65.5 % of cell dry weight when the first one grown on glucose only and the latter grown on combined glucose and octanoate as main carbon sources.

2.5.5 Recombinant *Escherichia coli*

With the advancement of genetic engineering nowadays, the attempts of cloning the genes involved in P(3HB) biosynthesis by *A.eutrophus* and introducing into

Escherichia coli have been successful. It was detected that recombinant *E. coli* expressing these genes could accumulate a large amount of P(3HB) (Lee 1996a). Steinbüchel (1996) stated that all three PHA biosynthesis genes of *A. eutrophus* must be expressed thence the formation of P(3HB) in *E. coli* can occur. *E. coli* has several advantages, e.g., fast growth and high cell density cultures, accumulation of a large amount of polymer (as shown in Figure 2.4), ability to use various inexpensive carbon sources, the lack of a PHA depolymerase and nutrient limitation is not required.

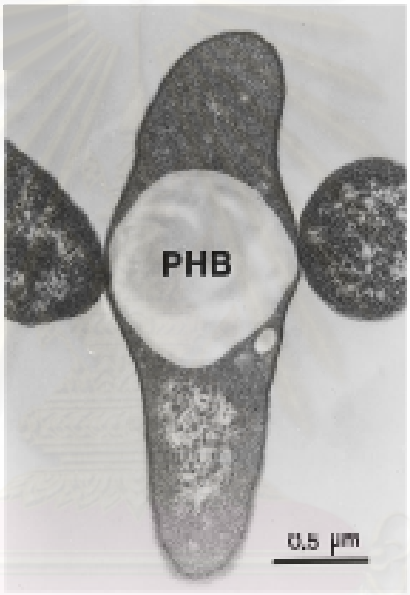


Figure 2.4: Recombinant *E. coli* cell with large P(3HB) granule (Resch et al., 1998).

Furthermore, PHA recovery from *E. coli* is easier than *A. eutrophus* due to PHA granules in *E. coli* are larger and more crystalline (reviewed by Yim et al., 1996). The authors demonstrated that recombinant *E. coli* could accumulate P(3HB-co-3HV) copolymer using glucose and propionate as carbon substrates. Valentin and Dennis (1997) also demonstrated the accumulation of P(3HB-co-4HB) copolymer by a recombinant *E. coli* when grown on glucose. P(3HB-co-4HB) copolymer contained 2.8 mol.% 4HB was accumulated up to the content of 52 % of cell dry weight. The P(3HB-co-4HB) copolymer was also produced by recombinant *E. coli* using γ-aminobutyrate and glutamate as carbon substrates in Valentin et al. (2000). However, recombinant *E. coli* cells experienced filamentation during the synthesis and

accumulation of polymer due to the inactivation of an essential cell division protein FtsZ (Lee, 1996a). Wang and Lee (1998) employed recombinant *E. coli* with filamentation suppression by the overexpression of this protein FtsZ. The authors reported P(3HB) concentration and content reached 104 g/l and 69.5 % of cell dry weight, respectively, resulting in the P(3HB) productivity of 2.0 g/l-h. This is the highest P(3HB) concentration obtained by recombinant *E. coli* reported up to date. Liu et al. (1998) have constructed a recombinant *E. coli*, which can produce P(3HB) on glucose, and use beet molasses instead of glucose as a carbon source. The fermentation with hydrolyzed molasses produced P(3HB) content of as high as 80 % of cell dry weight and P(3HB) concentration and productivity of 39.5 g/l and 1 g/l-h, respectively.

The advantage of *E. coli* in ability to utilize inexpensive carbon source led to the possibility to reduce production cost of P(3HB). Van Wegen et al. (1998) has presented an economic analysis of PHA production in large scale by using recombinant *Escherichia coli*. Base on an annual production of 4300 tonnes of P(3HB), the price per kilogram of plastic reduced to US$6.08. Furthermore, for a scenario of 60% glucose substitution with diary whey, the production cost could be reduced to US$2.67 per kilogram of plastic.

2.5.6 Methylotrops

Several strains of methylotrophs were screened for their capability to produce a large amount of P(3HB) from methanol as a substrate. Suzuki et al. (1986a) has investigated *Pseudomonas* sp. K and achieved the high P(3HB) concentration of 136 g/l after 175 hrs. The maximum P(3HB) content also reached 66 % of cell dry weight with the yield of 0.18 g P(3HB) /g methanol. Further investigation with the same bacterium (Suzuki et al., 1986b), better PHA production was obtained with controlled carbon /nitrogen feeding. Another methylotrop, *Pseudomonas* 135, was cultivated on methanol as a sole carbon source for the accumulation of P(3HB) by Daniel et al. (1992). The maximum content of P(3HB) in this work could reach 55 % of cell dry

weight in ammonium-limited fed-batch culture. The bacterium *Protomonas extorquens* was also able to produce P(3HB) from methanol in fed-batch culture by Suzuki et al. (1986c). With a small quantity of ammonia feeding, the P(3HB) content was promoted up to 60 % of cell dry weight.

Methylobacterium extorquens could also accumulate P(3HB) from methanol as a sole carbon source. Cells contained P(3HB) content between 40 % and 46 % of cell dry weight. P(3HB) material obtained from this work was claimed to have high molecular mass in the 900-1800 kDa range (Bourque et al., 1995). Kim et al. (1996) has performed high production of P(3HB) by *Methylobacterium organophilum* cultivated on methanol under potassium (K) limitation. High P(3HB) productivity of 1.8-2.0 g P(3HB)/l-h and content of 52-56 % of cell dry weight were resulted from this work. *Methylobacterium rhodesianum* MB126 is as well a methylotrophic strain using the serine pathway for methanol assimilation. This strain usually accumulates P(3HB) on methanol only when ammonium becomes limited, but P(3HB) content of 30 % of cell dry weight was also found when fructose was used as a substrate even though ammonium was still present in the medium (Ackermann and Babel, 1997). Moreover, in the same genus, a strain of *Methylobacterium* sp. ZP24 has been shown to produce P(3HB) from cheap substrates such as sucrose and molasses though with a low efficiency. Yellore et al. (1999) has performed the enhancement of P(3HB) production by *Methylobacterium* sp. ZP24 when sucrose substrate was partially substituted with low concentrations of several organic acids.

Since methanol is an inexpensive substrate, bacteria of the genus *Methylobacterium* used to be considered for the commercial production of PHA by ICI because this company is a major methanol producer (Byrom, 1992). However, there are too much disadvantages in PHA production by these bacteria such as the difficulties in the extraction of polymer from the cells, the low molecular weight of polymer, slow fermentation, etc. Therefore, they were not further considered as a good candidate for industrial production of PHA.

2.5.7 PHA accumulation in other microorganisms

Brandl et al (1989) have investigated the ability of the phototrophic, purple, non-sulfur bacterium *Rhodospirillum rubrum* to produce PHA. This *R. rubrum* was grown anaerobically in the light on various carbon sources such as β-hydroxycarboxylic acids and different n-alkanoic acids. PHA content up to 45% of cell dry weight was detected while nitrogen was limiting. *R. rubrum* produced a copolymer containing β-hydroxyhexanoate (HHx) in addition to HB and HV monomers when grown on n-alkanoic acids and additional monomer of β-hydroxyheptanate acid when grown on β-hydroxyheptanoic acid. A strain of *Rhodococcus* sp. NCIMB 40126 has the capability to synthesize copolymer of P(3HB-co-3HV), containing mainly 3HV monomer units, from only single carbon sources in contrast with *Alcaligenes eutrophus* (Anderson et al., 1992). *Rhodococcus* sp. NCIMB 40126 was investigated and shown to produce a P(3HB-co-3HV) copolymer with 75 mol% of 3HV from carbohydrate substrate such as glucose (Haywood et al., 1991). As well, polymer containing various monomers such as 4HB, 5HV or 3HHx in addition to 3HB and 3HV was also produced by this organism. *Chromatium vinosum* was mostly used in the studies of purified PHA synthase (Liebergesell et al., 1994; Jossek et al., 1998). Liebergesell et al. (1994) succeeded to localize PHA synthase within cytoplasm in cells of *C. vinosum* grown under non-storage conditions and at the surface of the PHA granules in cells grown under storage conditions.

Fast-growing *Rhizobium* are able to synthesize several intracellular polymers, as well P(3HB) under specific conditions. The coproduction of exopolysaccharide and P(3HB) was investigated in two strains of *Rhizobium meliloti* by Tavernier et al. (1997). The authors recommended that the M5N1 strain of *Rhizobium meliloti* was more promising organism than another due to it accumulated P(3HB) up to 85 % of cell dry weight from yeast extract-containing medium supplemented with fructose.

The accumulation of PHA by the newly isolated *Aeromonas hydrophila* is reported by Lee et al. (2000). The authors isolated this *Aeromonas hydrophila* strain from raw

sewage samples. The production of PHA by this bacterium was studied on various carbon sources, i.e., glucose, sodium gluconate, lauric acid, oleic acid, etc. Phosphorus limiting could induce the maximum PHA content of 45 % of cell dry weight and high yield of 0.51 g PHA/ g oleic acid when using oleic acid as a carbon substrate.

Mutant strains of *Burkholderia* sp. isolated by the collaborators of Silva et al. (2000) were studied. These strains have properties, which should help facilitating the industrial P(3HB) production. These properties are the ability to utilize sucrose, accumulating P(3HB) in high amounts with an efficiency of higher that 80% of the maximum theoretical yield. P(3HB-co-3HV) content of around 40 % of cell dry weight was produced from carbohydrate and propionic acid.

The methanotrophic strain of *Methylocystis* sp. GB25 DSMZ 7674 was cultivated in two-stage process; a continuous growth phase and a P(3HB) accumulation phase in batch culture, by Wendlandt et al. (2001). Methane was used as a carbon substrate. The PHB content of 51 % of cell dry weight and the very high yield of P(3HB) on substrate of 0.55 g/g were obtained. The author presented that P(3HB) extracted from this bacterial cells has a high molecular mass of up to 2.5 x 10^6 Da.

There are efforts on PHA production by photoautotrophic microorganism in order to make the possibility to reduce the substrate cost of PHA production. A strain of *Synechocystis* sp. PCC6803 is a member of cyanobacteria, known as blue-green algae. Wu et al. (2001) has studied the PHA accumulation in this strain and report that the level of accumulated PHA was up to 4.1 % of cell dry weight under nitrogen-limited photoautotrophic conditions. Though it is a very low amount of accumulated PHA in comparison with other organisms, the advantage is still present since no carbon source was required for the polymer accumulation.

2.5.8 Transgenic plant

The present of β-ketothiolase in the cytoplasm of higher plants initiated several researches on the transfer and expression of PHA biosynthesis genes in plants. Transgenic plants harboring the bacterial PHA synthesis genes have been aimed to ultimately reduce the price of PHA to close to that of starch. Steinbüchel (1996) and Braunegg et al. (1998) reviewed that the first successful expression of the *Alcaligenes eutrophus* genes encoding acetoacetyl-CoA reductase and PHA synthase was in *Arabidopsis thaliana*, a small oil seed plant. This resulted in P(3HB) synthesis in the cytoplasm, nucleus and vacuoles of all plant tissue, but only in small amounts. The further studies need to be carried out in order to divert the carbon flow from starch, sucrose or lipid synthesis to PHA synthesis. The recent studies (reviewed by Lee, 1996b; Braunegg et al., 1998) can improve PHA accumulation in transgenic plant, *Arabidopsis thaliana*, up to 14 % of dry weight in the form of granules similar to those of bacterial PHA inclusion.

2.6 Fermentation strategies

As mentioned above, PHA accumulating microorganism are divided into two groups based on culture conditions needed for PHA synthesis; one requires the unbalanced growth condition to stimulate PHA synthesis, whereas another accumulates PHA during normal growth. Fed-batch culture or continuous cultivation techniques has been considered for the production of PHA with high productivity. In batch culture, Durner et al. (2001) reviewed that this technique is not ideally suited for PHA production because the accumulation of polymer has to occur concurrently in a cultivation phase in which the cells are not fully active and partially deteriorate. Also, there is a limit of initial substrate concentration used in cultivation, which affects low biomass concentration production in simple batch system.

Fed-batch culture was basically aimed to retain the growth-associated component of PHA production (Raje and Srivastava, 1998). The fed-batch culture is most often developed, especially when microorganisms in the first group were employed. As

these microorganisms need nutrients limitation condition for synthesizing PHA, a two-step cultivation method was mostly developed. In contradiction to batch culture, bacterial cells are allowed to grow to a desired concentration with optimum nutrients supplied in the first step, after which an essential nutrients for growth is limited to allow the accumulation of PHA.

For the fed-batch culture of microorganism belonging to the second group that can accumulate PHA during cell growth, the success of the fermentation depends on the strategy of nutrient feeding. Complex nitrogen sources such as fish peptone, proteose peptone, yeast extract or corn syrup could further promote cell growth as well as polymer accumulation (Page, 1992) due to PHA synthesis in these bacteria is not stimulated by nutrient limitation condition. However, cell growth and PHA accumulation need to be well balanced in order to obtain both high amount of PHA and cell concentration. Lee (1996b) explained the relationship between PHA content and the residual cell concentration, used to determined how much PHA can potentially be produced; that is, low amount of either PHA content or residual cell concentration will result in low PHA concentration and productivity, the best result will obtain when both of them are in high level. Therefore, it is crucial to decide when to stop the cultivation. Mostly, PHA productivity was considered for terminating the fermentation, the prolonged cultivation may achieve higher PHA concentration, but resulting in low overall productivity.

The technique of continuous cultivation should be the only method allowing organisms grow under a defined limitation for prolonged periods of time. Theoretically, the continuous culture should give the highest volumetric productivities. The continuous process should be attractive to the commercial production of PHA since it eliminates the downtime occurring during batch or fed-batch culture fermentation, resulting in more production time. To date, however, there have been little studies of PHA accumulation using continuous technique, e.g., *Alcaligenes latus* (Ramsay et al., 1990) and *Pseudomonas oleovorans* (Preusting et al., 1993) have been cultivated continuously and succeeded in the production of substantial amount of

PHA. Surprisingly, Henderson and Jones (1997) cultivated a mutant strain of *Alcaligenes eutrophus* in continuous culture under various conditions. The maximum P(3HB) content of 79.3 % of cell dry weight was obtained when the bacterium was grown on glucose with dilution rate of 0.025 h^{-1} and ammonia as the growth-limiting nutrient. The continuous cultures can be employed for both groups of microorganisms; a two-stage chemostat for the microorganisms required unbalanced growth and a one-stage chemostat for the microorganisms, which do not need a great change in the limiting nutrient concentration between cell growth phase and polymer accumulation phase.

However, strain degeneration in continuous cultures is a well-known problem, particularly for recombinant strains. The studies of culture instability and contamination need to be more carried out in order to prove that continuous cultures can truly give higher productivity than fed-batch cultures. Also, a careful economic analysis has to be performed as PHA yields are generally lower in continuous cultures.

2.7 Carbon substrates in PHA production

Excluding the recover process, substrate cost and PHA yield contribute largely to the economics of PHA production. As PHA is made by a range of different bacteria, a number of carbon sources, including carbohydrate, oils, alcohols, organic acids and hydrocarbons, can be used.

Methanol

As methanol is a cheap carbon source and ICI is the producer of methanol, methylotrophs were first attracted to the industrial PHA production by ICI (Byrom, 1992). In methylotrophs, PHA synthesis from methanol has been derived in the facultative serine pathway. There are some strains of bacteria utilizing methanol during PHA accumulation such as *Pseudomonas* sp. (Suzuki et al., 1986a, 1986b; Daniel et al. 1992), *Protomonas extorquens* (Suzuki et al., 1986c), *Methylobacterium*

extorquens (Bourque et al., 1995), *Methylobacterium organophilum* (Kim et al., 1996), *Methylobacterium rhodesianum* (Ackermann and Babel, 1997), *Methylobacterium* sp. ZP24 (Yellore et al., 1999). The highest reported PHA content for methylotroph is about 66 % of cell dry weight by Suzuki et al. (1986a). The yield of polymer on this article is 0.18 g/g methanol, which is quite far from theoretical yield of 0.54 g/g methanol calculated by Yamane (1993).

Hydrogen and CO_2

Hydrogen may be considered as an alternative substrate to methanol. Hydrogen oxidizing bacteria, *Alcaligenes eutrophus*, have been reported to produce substantial amount of P(3HB) from CO_2, H_2 and O_2 (Sugimoto et al., 1999). Although hydrogen and CO_2 was accounted as inexpensive substrates, consumption cost is only part of the economic assessment of hydrogen. Due to the flammability, low solubility and large oxygen requirement for the fermentation, the overall cost must include the safety handling, plant construction and materials.

Carbohydrates

Carbohydrate is a readily accessible substrate for PHB production, but the yield cannot be higher than 1 mol monomer per mol glucose because of the loss of CO_2 during the reaction of pyruvate into acetyl-CoA. *Alcaligenes eutrophus* is still the organism of choice because it can store high PHA when grown on simple sugars. The wild-type of *Alcaligenes eutrophus* grows only on fructose (Steinbüchel and Pieper, 1992), but mutants are capable to grow on glucose (Marchessault and Sheppard, 1997). The combination of carbohydrate and other types of carbon substrates could help increase the PHA production. For example, glucose and γ-butyrolactone were fed together for a strain of Alcaligenes eutrophus G^{+3} to help accumulating P(3HB-co-3HV) as high as 78 % of cell dry weight (Braunegg et al., 1995). *Alcaligenes latus* also utilizes sucrose most efficiently (Yamane et al., 1996), and so cheap molasses. In addition, recombinant *Escherichia coli* utilize glucose (Valentin and Dennis, 1997) or molasses

(Liu et al., 1998) as a carbon substrate for PHA production was studied. Moreover, Azotobacter vinelandii was investigated on PHA production by utilizing carbohydrate substrates such as glucose and beet molasses (Page, 1992).

Organic acids

Organic acids are substrates, which are metabolically closer to PHA such that acetyl-CoA is not derived from pyruvate or is bypassed, resulting no loss of carbon via CO_2 released. Crotonate is incorporated into PHA almost directly and should yield the high amount of PHA on substrate, but it was not widely used as its price is quite high. Butyric acid might be converted directly to P(3HB), but is still expensive. For the production of P(3HB-co-3HV) copolymer by *Alcaligenes eutrophus*, Doi et al. (1988) suggested that cosubstrates of butyric and pentanoic acids are incorporated into the copolymer without decomposition of the carbon skeletons in the cell. Braunegg et al. (1995) supplied glucose and propionic acid for the P(3HB-co-3HV) production by *Alcaligenes latus* can stimulate the PHA content of 72 % of cell dry weight. Doi et al. (1992) has accumulated PHA by using a bacterium *Alcaligenes eutrophus* with only pentanoic acid as the sole carbon source, the copolymer of P(3HB-co-3HV) was still obtained with the content of 46 % of cell dry weight. Acetic acid is a source of acetyl-CoA obviously, but unfortunately, acetic acid is a relatively oxidized substrate and is poor energy sink for biomass synthesis. Therefore, acetic acid is not suitable to be a sole carbon source for two-stage cultivation of PHA. Long-chain fatty acids such as n-alkanoic acid of carbon numbers ranging from C_2 to C_{22} were also used in production of short-chain-length PHA by using a new strain of *Alcaligenes*, relatively high PHA contents of around 60 % of cell dry weight were reported by Akiyama et al. (1992).

Other carbon substrates

Plant oils and animal fats such as rapeseed oil, olive oil, soybean oil, palm oil, etc., were also used in Akiyama et al. (1992). The P(3HB) contents obtained from these carbon substrate were in the range of 31 to 47 % of cell dry weight. In addition, Kahar

et al. (2004) also investigated the PHA production from renewable inexpensive soybean oil by *Ralstonia eutropha* and achieved the high P(3HB) content of 72-76 % of cell dry weight. The yields of PHA on soybean oil were also high up to 0.72 to 0.76 g PHA/g soybean. This high yield could lead to the possibility of cost reduction for PHA production from renewable substrate.

Lee and Choi (1999) recommended that PHA production from waste should be considered as a coupled process for reducing the amount of organic waste. There are several studies tried to use waste or wastewater as substrates for PHA production. For example, Rusendi and Sheppard (1995) used hydrolysed potato waste, which mostly containing glucose, as substrate for P(3HB) production by *Alcaligenes eutrophus*. They received the PHA content of 77 % of cell dry weight and the yield of 0.38 g/g glucose. Lee and Yu (1997) has demonstrated the production of PHA by using supernatant of digested municipal sludge supplied to *Alcaligenes eutrophus* and reported that *A. eutrophus* could produce PHA from this substrate (34 % of cell dry weight) as well as from the mixture of pure propionic and butyric acids (33 % of cell dry weight). Jin et al. (1999) used acidified wastewater from UASB system for PHA production by *A. eutrophus* and obtained the maximum PHA content of 63.3 % of cell dry weight. Yu (2001) has also performed a similar study of using acidified wastewater for the production of PHA by *A. eutrophus*. The author first digested starchy organic waste in UASB reactor to form volatile fatty acids of totally 4000 mg/l. The acids were further utilized and incorporated into PHA by bacterium *A. eutrophus* with the PHA content of 34 % of cell dry weight. Maskow and Bable (2000) has investigated a single-stage continuous process for producing PHB from toxic substrates using two strains of *Variovorax paradoxus* JMP 116 and *Ralstonia eutropha* JMP 134. Phenol and sodium benzoate were used as the sole carbon sources. The higher PHA content of 50 % of cell dry weight was obtained from *Ralstonia eutropha*.

2.8 PHA production by using activated sludge biomass

PHA synthesis in EBPR process

Although cultivation strategies to achieve high PHA concentration, high cell density and high PHA productivity in pure culture and mixed of pure culture have been extensively studied and well defined, the knowledge for production of PHA using activated biomass is still rare. PHA has been first extracted from activated sludge by Wallen and Rohwedder (1974) and this discovered PHA is not a homopolymer, but a copolymer composed of 3-hydroxyvaleric and 3-hydroxybutyric acids as the major constituents. The knowledge that PHA is an intermediate metabolic product in activated sludge initiated the idea of massive PHA production by using activated sludge biomass.

To date, it is known that PHA plays an important role in activated sludge processes, especially in the anaerobic-aerobic activated sludge process widely used for the biological removal of phosphorus from wastewater or enhance biological phosphorus removal (EBPR) process. In EBPR process, the metabolism of bacteria in activated sludge is not yet completely elucidated; several attempts to explain the biochemical pathway in EBPR process have been proposed. It is known that microorganism under anaerobic conditions can uptake short chain fatty acids as carbon substrate. Energy for taking up carbon substrate comes from the consumption of intracellular polyphosphate; the required reducing equivalents are originated from either glycogen consumption (Satoh et al., 1992; Smolders et al., 1994a; 1994b; Mino et al., 1998) or partial oxidation of acetyl-CoA through TCA cycle (Comeau et al., 1986) or maybe in both ways, which Pereira et al. (1996) estimated that 30% of reducing power obtained from TCA cycle while another 70% is from the glycolysis of glycogen. Stephens and Stensel (1998) compared the anaerobic metabolism from two different models as shown in Figure 2.5. The idea of the reducing power obtained from both TCA cycle and glycogen was more described by Louie et al. (2000). The authors reported that PHA accumulation decreased when the conversion of succinate into fumarate or citrate

into isocitrate, which are the reactions in TCA cycle, was inhibited. The authors stated that the model from Mino's group could not explain this occurrence and suggested that both of TCA cycle and glyoxylate pathway play important roles in PHA production in EBPR sludge. Satoh et al. (1998b), collaborators in Mino's group, also accepted the possibility that reducing power partially derived from TCA cycle.

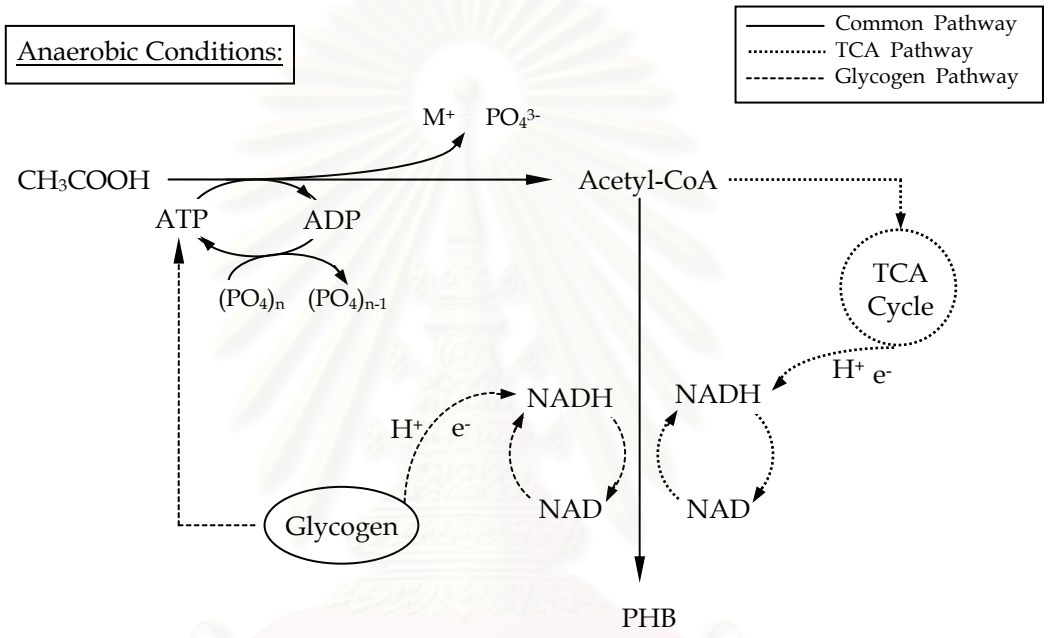


Figure 2.5: Compare the reducing power between derived from TCA cycle by Comeau model and from glycogen by Mino/Smolders (Stephens and Stensel, 1998)

During the turn of aerobic condition, PHA previously accumulated during anaerobic stage is metabolized for microorganism growth and anabolic precursors such as the precursor for glycogen formation. Energy produced is used for regeneration of polyphosphate with phosphate uptake from the external medium (Figure 2.6). Therefore, EBPR microorganisms possess the feature of phosphate removal and PHA accumulation. For that reason, most of studies with PHA production in activated sludge biomass were performed by using anaerobic-aerobic activated sludge process or similarly.

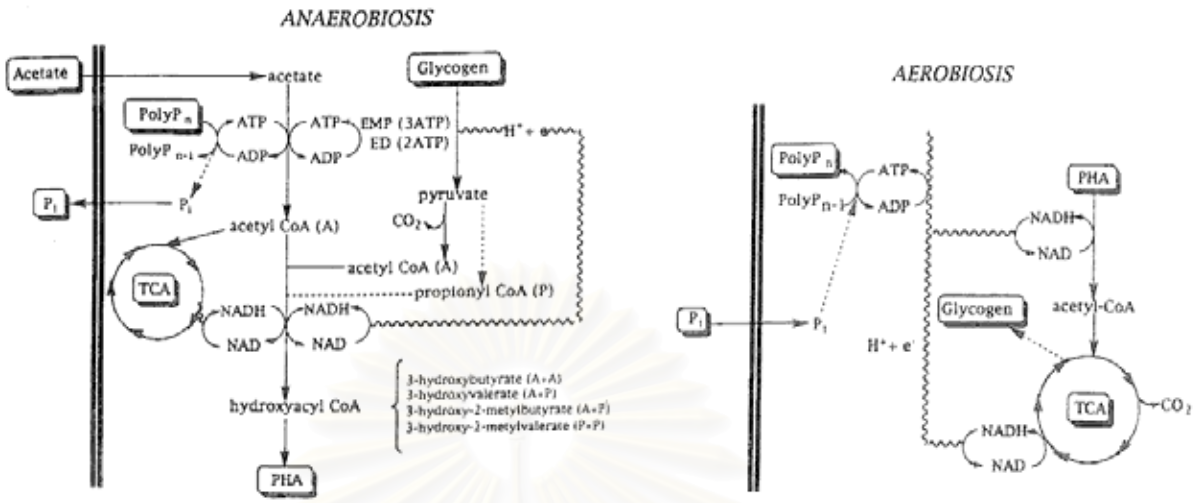


Figure 2.6: Carbon (acetate) and phosphorus metabolism for polyphosphate bacteria during anaerobic and aerobic conditions (Lemos et al, 1998)

Occasionally, anaerobic-aerobic activated sludge process does not function as EBPR process. Whether there is phosphate removal occurred or not, anaerobic-aerobic condition is still able to induce intracellular PHA synthesis due to the unfavorable growth condition by oxygen limitation. Satoh et al. (1994) reported the increase of intracellular PHA while there was no phosphate released during anaerobic stage. Liu et al. (1996) demonstrated the anaerobic-aerobic cultivation for non-EBPR process. The authors reported that acetate, propionate, butyrate, valerate, pyruvate, lactate and gluconate were taken up and stored as intracellular PHA, whereas glucose, fructose, maltose sucrose trehalose and raffinose were taken up and converted into both of glycogen and PHA. Satoh et al. (1999) also noted that anaerobic-aerobic process did not always promote the enrichment of polyphosphate accumulating organisms (PAOs). The authors explained the 3HV fermentation into PHA that it is a combination of glycolysis, propionate fermentation and PHA production. The authors also referred 3HV fermenters as glycogen accumulating organisms (GAOs) as they supposed to accumulate glycogen more than PAOs. For the polymer engineering aspect, the conversion of glucose into 3HV by GAOs does not require input of energy therefore the authors recommended that yield of PHA on substrate utilized by GAOs should be increased.

PHA production by activated sludge biomass

The excellence of PHA production using activated sludge biomass should be the cost reduction in cultivating PHA producing biomass, facility construction and gaining the valuable material from wastes. Satoh et al. (1998a) has investigated the possibility of PHA production in anaerobic-aerobic activated sludge process. Accumulated PHA obtained under anaerobic stage was around 20 % of cell dry weight and up to 33 % of cell dry weight under aerobic stage. The authors further increased the PHA content by introducing so-called microaerophilic-aerobic process, in which a limited amount of oxygen was supplied into the anaerobic phase. High PHA content of 62% of cell dry weight was obtained when supplying oxygen rate of 1.2 ml/min. They explained that the limited oxygen will support enough energy for only taking up substrate and accumulating PHA, without wasting to synthesize other cell materials such as protein or glycogen. The interesting discoveries from this work are as the followings;

- Microaerophilic-aerobic system has the potential in producing a large amount of PHA from activated sludge biomass cultivated on waste or wastewater as substrates.
- This is the cultivation of mixed culture that should reduce the cost of operation when compared to the cultivation of pure cultures.
- Microaerophilic-aerobic system will help select only PHA accumulators to be dominant.

The collaborators of Satoh, Takabatake et al. (1999), has demonstrated PHA production using activated sludge biomass by employing two steps strategy. The first step is called in abbreviation as PABER (PHA Accumulating Bacteria Enrichment Reactor), which was operated continuously and accumulating low amount of PHA. The second step is called PPR (PHA Production Reactor), sludge from the first stage was transferred to this reactor, which was operated in batch system. High concentration of volatile fatty acids was fed as substrates for promoting the production of PHA. The authors did the comparison among three different operating conditions of PABER, i.e., anaerobic-aerobic, microaerophilic-aerobic and fully aerobic. They

obtained 17-57 % of cell dry weight PHA from anaerobic-aerobic PABER, 33-50 % of cell dry weight PHA from microaerophilic-aerobic PABER and only 20-30 % of cell dry weight PHA from aerobic PABER. The authors concluded that microaerophilic-aerobic PABER provided the better sludge for more stable and higher PHA production.

PHA production by controlling carbon/nitrogen (C/N) ratio has been carried out by Chua et al. (1997). When the C/N ratio was raised from 24 to 144, the maximum PHA content of 37.4 % of cell dry weight was obtained while the specific growth rate decreased. The optimum C/N ratio of 96 maximized PHA yield up to 0.093 g of polymer /g of carbonaceous substrate consumed. Furthermore, Chua et al. (1999) reported that the intermittent addition of nitrogen into the system could enhance the yield of polymer up to 0.11 g polymer /g COD consumed. The authors explained that fed nitrogen helped maintain the normal condition of cells and suggested that nitrogen with C/N ratio of 96 should be fed in every 3 cycles of operating.

Hu et al. (1997) has demonstrated the synthesis of P(3HB-co-3HV) from activated sludge using synthetic food-processing wastewater containing reconstituted milk of 1.92 g/l. A sequencing batch reactor (SBR) with 1.2 l active volume and 1.5 day HRT was used during the stage of nutrient-rich feeding. The polymer-free sludge from the SBR reactor was transferred to the jar fermenter with nitrogen-free medium to induce PHA synthesis. The authors investigated PHA production from various fatty acids; the maximum PHA content of 40% was obtained. By varying the ratio of butyric and valeric acids in fed carbon substrates, the melting temperatures of the copolymers accumulated by activated sludge was in the range from 99 to 178^{o}C, which is a similar range from 96 to 160^{o}C of PHBV copolymers. The author noted that this technique of PHA production can reduce the quantity of excess sludge by about 37%.

Brdjanovic et al. (1998) stated that the ability of accumulating PHA depended on the intracellular area of bacterial cells. Particularly in EBPR process, the cell inclusions included of polyphosphate and glycogen in addition to PHA. Moreover, the

accumulating ability also depended on operating conditions such as time length of anaerobic and aerobic phases, temperature, SRT and the number of operating cycle. Kuba et al (1997) reported that at the same SRT, the fraction of PHA in cells increased as the number of operating cycle increased. Chuang et al. (1998a) also investigated the effect of different SRTs on PHA accumulation with the anaerobic-anoxic-aerobic system. They reported that lower SRT of 5 days resulted higher accumulated PHA than SRT of 10 days. The authors also found the closed relationship between PHA content and phosphorus release/uptake. Furthermore, when high organic loading of substrate was supplied, PHA accumulation in anoxic zone largely increased. Chuang et al. (1998b) further reported that the fraction of 3HV in PHA increased when F/M ratio was raised, which the authors explained that in the condition of high COD-SS loading, activated sludge accumulated 3HV monomer more than 3HB.

Two-step approach, growth phase following by nutrient limitation phase was investigated with activated sludge by Punrattanasin (2001). The author cultivated the activated sludge biomass with the same operation as treating wastewater until it was in steady state, then; nutrients were eliminated from the feed to stimulate PHA synthesis. Comparison among three operating conditions, i.e. microaerophilic-aerobic, anaerobic-aerobic and fully aerobic was carried out with nutrients limitation. While the microaerophilic-aerobic cultivation could produce PHA content as high as 45 % of cell dry weight, the fully aerobic was found to produce higher PHA content of 70 % of cell dry weight. After reaching the peak of PHA content, biomass was unstable and its concentration decreased and the ability of COD removal was lost due to the effect of lacking essential nutrients for growth. In this work, the author also performed the PHA production with industrial wastewater from the Celanese Acetate plant and received the maximum PHA content of 40 % of TSS and PHA productivity of 577 mg/l-d. However, the author recommended that PHA content should be increased more in order to optimize the economics of PHA production using this wastewater.

Chua et al. (2003) has investigated the production of PHA by activated sludge treating municipal wastewater. They studied on three operational factors, i.e. the acetate

concentration contained in the feed, pH and SRT in order to find the optimum operating condition for promoting the PHA production capability of activated sludge. The highest PHA content achieved only 30 % of cell dry weight in this study. However, this is the first study that used real municipal wastewater as substrate although that highest PHA content was obtained when wastewater supplemented with acetate was fed. The authors noted that at higher pH of 8 or 9, the PHA production was substantial and sludge with a shorter SRT of 3 days achieve 10% higher of PHA content than sludge with SRT of 10 days.

CHAPTER 3

MATERIAL AND METHODS

Part I Experiments with 660 mg/l COD (acetate and propionate) synthetic wastewater

This experimental part was conducted in the laboratory of Civil and Environmental Engineering Department, Virginia Polytechnic Institute and State University in United States of America.

3.1 Experimental design

A fully aerobic SBR system with a working volume of 4 liters was used in all experiments as Punrattanasin (2001) had shown that it had higher PHA productivity than the Anaerobic/Aerobic (An/Ae) and Microaerophilic/Aerobic (MA/A) cycling systems. Three different temperatures were selected for this study, i.e., 10, 20 and 30^{o}C. Three types of nutrient limitation, i.e., limitation of either N or P and limitation of both N and P, were investigated for each temperature. Therefore, there were totally 9 experiments planned for the study as tabulated in Table 3.1.

The SBR systems as illustrated in Figure 3.1 were operated with a hydraulic retention time (HRT) of 10 hours, a sludge age of 10 days and a 6-hour operating cycle consisting of 15 min influent feeding time, 4 hr aeration period, 1.5 hr settling and the last 15 min for effluent withdrawal mode. The volume in the reactor after effluent discharge was 1.6 l, resulting in a discharge volume of 2.4 l per cycle, or the ratio of V_F :V_O was 1.5:1 and the ratio of V_F :V_T was 3:5, where V_F was volume of the feed, V_O was remaining volume after supernatant withdrawal and V_T was total active volume of the reactor.

Table 3.1: Experimental planning.

Exp. No.	Nitrogen limitation			Phosphorus limitation			Nitrogen and phosphorus limitation		
	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C
1	●								
2		●							
3			●						
4				●					
5					●				
6						●			
7							●		
8								●	
9									●

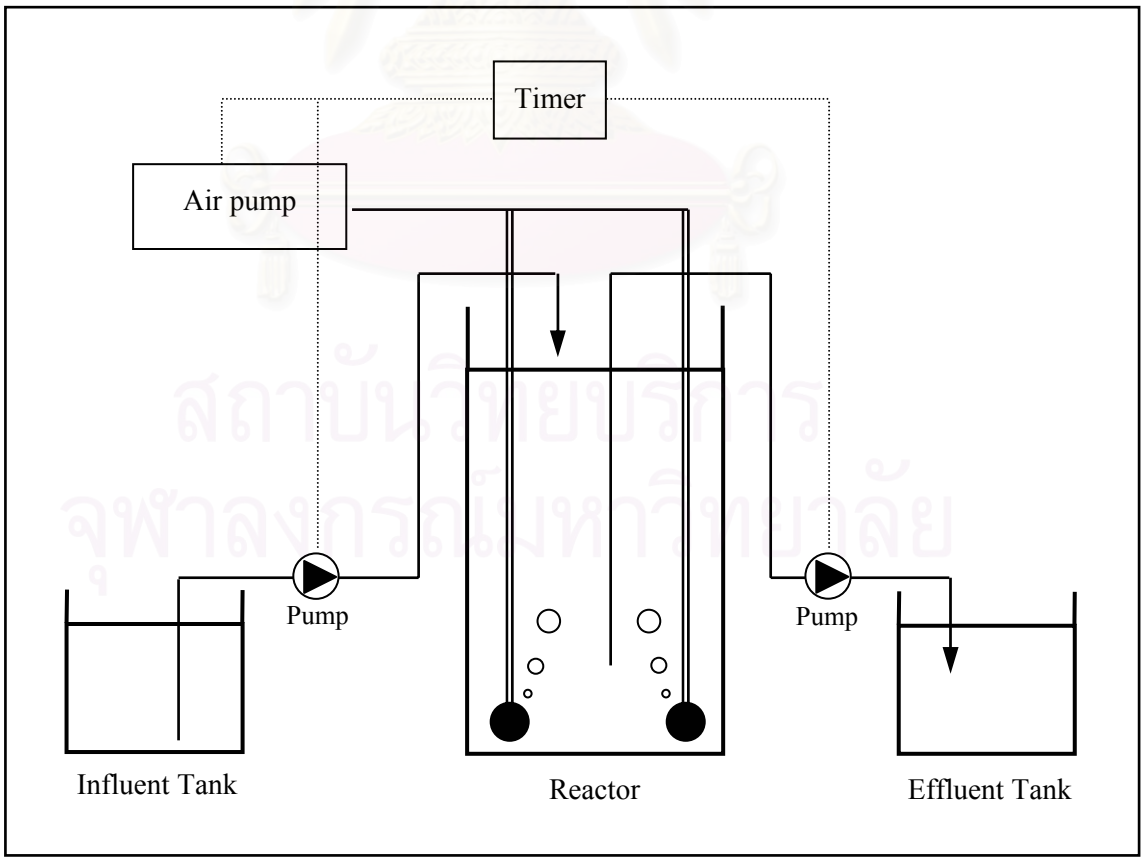


Figure 3.1: Experimental setup.

The experimental systems used were fully aerobic sequencing batch reactors (SBRs) and were cultivated using a two-stage approach, i.e. growth phase and then nutrient limitation phase, that had been earlier utilized for PHA production with pure cultures (Du et al., 2001; Wendlandt et al., 2001) and adapted by Punrattanasin (2001) for activated sludge. During the growth phase, nutrients were optimized to develop the biomass concentration and cell nutrition. The 10-d SRT was maintained by daily excess sludge withdrawal, for about 2-3 SRTs in order to allow the biomass in each experiment to adjust and increase similarly on the same influent components. Then, nutrient limitation phase was introduced by eliminating one or more nutrients from the feed in order to stimulate PHA accumulation in cells. Sludge wasting was terminated during this PHA accumulation phase to maximize biomass concentration in the reactors, except that wasted as SS discharged along with the effluent withdrawal. Each experiment was operated through two consecutive growth & nutrient limitation cycles, in accordance with the conclusion stated by Punrattanasin (2001) that the two consecutive cycles would enrich the PHA accumulating bacteria and reduce the production time, also produce higher PHA content. PHA accumulation in sludge was monitored so that the maximum PHA accumulation and the time to obtain it could be determined. The systems were inoculated with excessive activated sludge from the Integrated Fixed Film Activated Sludge (IFAS) pilot plant system operated as UCT/VIP configuration by Sriwiriyarat (2002).

3.2 Synthetic wastewater

The synthetic wastewater was composed of sodium acetate (330 mg/l as COD) and sodium propionate (330 mg/l as COD) as the sole carbon sources. When the system was operated during the growth phase, excess nitrogen and phosphorus [$(NH_4)_2SO_4$ of 33 mg N/l and KH_2PO_4 of 17 mg P/l] were added, resulting in the feed ratio of COD:N:P of 100:5:2.5. Other nutrients and micronutrients used in this experiment are shown in Table 3.2. The synthetic wastewater was prepared daily with tap water.

Table 3.2: Composition of synthetic wastewater.

Substances	Concentration (mg/l)
Sodium acetate	330 as COD
Sodium propionate	330 as COD
NH_4SO_4	33 as N
KH_2PO_4	17 as PO_4-P
$CaCl_2$	28.3 (10.2 as Ca)
$MgCl_2.6H_2O$	250 (29.9 as Mg)
Micronutrients	Concentration (μg/L)
$FeCl_3$	107 (36.8 as Fe)
HBO_3	18.3 (3.2 as B)
$CuSO_4$	3.5 (1.38 as Cu)
KI	22 (5.2 as K)
$MnSO_4.H_2O$	14.7 (4.8 as Mn)
$NaMoO_4.2H_2O$	7.3 (2.9 as Mo)
$ZnSO_4.7H_2O$	31 (7.1 as Zn)
$CoCl_2.6H_2O$	18.3 (4.5 as Co)

3.3 Analytical methods

The following parameters were measured daily throughout the growth and accumulation cycles: MLSS, MLVSS, PHA, SVI and supernatant filtered (with 0.45-micron membrane filter) COD. The PHB and PHV fractions of the PHA were separately determined. MLSS, MLVSS, SVI and COD analyses were performed according to Standard Methods (APHA et al., 1999).

PHA analysis

PHA, PHB and PHV contents were determined using the methanolysis-GC method developed by Hart (1994), with modifications described by Punrattanasin (2001). That is, biomass collected from the systems was centrifuged and dried at 100^{o}C for at least 24 hours. Weighed biomass was put into a 5 ml high pressure Wheaton vial and 2 ml of the benzoic acid solution (50 mg of benzoic acid dissolved in 100 ml of 3% sulfuric acid in methanol (v/v)) was added into each vial, then, followed by another 2 ml of chloroform. The vials were sealed by Teflon caps and incubated at 100^{o}C for 3.5 hours. After the vials were cool down, 1 ml of distilled water was added into every vial and the vials were then shaken for about 10 minutes. The layers of chloroform and sulfuric-methanol solution were separated from each other and 1 µl from the chloroform (bottom) layer of each sample was injected into a Hewlett-Packard Model 5890 GC equipped with a Stabilwax capillary column (0.25 x 3 mm inner diameter) attached to an FID detector. The oven temperature was programmed to increase from 90 to 130^{o}C with rate of 20^{o}C/min, while the temperatures of injector and detector were 160 and 200^{o}C, respectively. Pure substance of P(3HB-co-3HV) copolymer (contained 12 % of 3HV) purchased from Sigma-Aldrich Company in USA was used as an external standard. PHA content was defined as %TSS, i.e., the mass of PHA in the total dry weight biomass, expressed as percent. PHA concentration and residual biomass were calculated using equations (1) and (2), respectively.

$$\text{PHA concentration} = \text{PHA content} \times \text{MLSS} \quad \text{(mg/l)}. \qquad (1)$$

$$\text{Residual biomass} = \text{MLSS} - \text{PHA concentration} \quad \text{(mg/l)}. \qquad (2)$$

Part II Experiments with industrial (candy) wastewater

This part of the study was done in the laboratory of the Sanitary Engineering Department, Faculty of Public Health, Mahidol University in Thailand. The purpose of this experimental part was to investigate PHA production with industrial (candy, 10000 mg/l COD of glucose and sucrose) wastewater as a substrate. The water temperature of 20°C and room temperature (28-31°C) were selected although the results with synthetic wastewater illustrated that the 10°C setup provided the higher PHA production, as the 10°C temperature is rarely found in Thailand. In addition, the combined nitrogen and phosphorus limitation was selected to stimulate PHA accumulation in this step.

3.4 Experimental setup for experiments at 20°C

Two fully aerobic sequencing batch reactors (SBR) with working volume of 16 liters were set up for cultivating at 20°C. Each SBR was controlled within $\pm$0.5°C of the 20°C temperature. The SBR systems as also illustrated in Figure 3.1 were operated with a hydraulic retention time (HRT) of 8 days, a sludge age of 20 days and a 24-hour operating cycle consisting of 25 min influent feeding time, 21.5 hr aeration period, 2.0 hr settling and the last 5 min for supernatant withdrawal step. The volume in the reactor after effluent discharge was 14 l, resulting in a discharge volume of 2 l per cycle, or the ratio of V_F :V_O was 1:7 and the ratio of V_F :V_T was 1:8, where V_F was volume of the feed, V_O was remaining volume after withdrawal and V_T was total active volume of the reactor.

The experimental systems were cultivated by using a two-stage approach, i.e. growth phase and then nutrients limitation phase that had been earlier utilized for PHA production in the first part of this study. During the growth phase, nutrients were optimized to develop the biomass concentration and cell nutrition. The 20-d SRT was maintained by daily excess sludge withdrawal. Firstly, 10-d SRT was exploited for this part of experiments, but the systems failed due to overloading problem. The

biomass in both reactors was adjusted and increased similarly on the same COD loading of 1.25 g/l-d. Then, the nutrients limitation phase was introduced by stopping the addition of both nitrogen and phosphorus to the feed. Sludge wasting was terminated during this PHA accumulation phase to maximize biomass concentration, except that wasted as SS discharged along with the effluent withdrawal. Once the nutrients were eliminated from the feed, one reactor was fed with the same COD loading as the growth phase and operated through two consecutive growth & nutrients limitation cycles; another reactor was on the other hand fed with double COD loading (or 2.5 g/l-d) to provide more excess carbon condition and operated through only one cycle of growth & nutrients limitation.

3.5 Experimental setup for experiments at room temperature

Five fully aerobic sequencing batch reactors (SBR) with working volume of 4 liters were set up for the investigation at Bangkok room temperature (28-31^{o}C). The schematic of the systems was illustrated also in Figure 3.1. The operating condition was the same as that in the experiments at 20^{o}C (see section 3.4). All five of them were fed with the COD loading of 1.25 g/l-d during the growth phase. On the other hand, when the nutrients limitation phase started, five different COD loadings of 1x, 2x, 3x, 4x and 6x or 1.25, 2.5, 3.75, 5 and 7.5 g/l-d, respectively, were fed to each of those five reactors. The list and details of all experiments in this part are shown in Table 3.3.

Table 3.3: The list and operating details of the experiments with candy wastewater.

Exp. No.	Operating Temp.	Limited nutrients	COD loading (g/l-d)		2^{nd} growth and nutrients limitation.
			Growth phase	Nutrients limitation phase	
20C-1x	20°C	N&P	1.25	1x*	Yes
20C-2x	20°C	N&P	1.25	2x	No
RT**-1x	28-31°C	N&P	1.25	1x	No
RT-2x	28-31°C	N&P	1.25	2x	No
RT-3x	28-31°C	N&P	1.25	3x	No
RT-4x	28-31°C	N&P	1.25	4x	No
RT-6x	28-31°C	N&P	1.25	6x	No

Note: * 1x = 1.25 g/l-d.

**RT = room temperature

3.6 Industrial (candy) wastewater and inoculated sludge

The candy factory, which is the source of wastewater and seed sludge, was located in Samut Prakarn Province of Thailand. The seed sludge was obtained from the sludge holding tank of the wastewater treatment plant of this factory. The treatment plant used an SBR activated sludge system. This sludge holding tank contained excess activated sludge wasted from their plant.

The wastewater was collected from a sump that received wastewater from the candies production line. This sump is to retain this wastewater, which will be subsequently pumped into the equalization tank for nutrients addition. The reason for choosing this wastewater was that it contained high sucrose and glucose based COD (which was expected to be relatively readily biodegradable) and low in both nitrogen and phosphorus. The occasionally collected wastewater (since March to July, 2003) had characteristics as the followings;

- COD : 10000 to 20000 mg/l
- TKN : 10 to 80 mg/l
- TP : undetectable (by Vanadomolibdophosphric acid methods).
- Ca : 110 mg/l as $CaCO_3$
- Mg : 50-55 mg/l as $CaCO_3$
- Na : 80 mg/l as Na
- K : 7.15-7.26 mg/l as K

This wastewater was diluted with tap water to obtain approximately COD concentration of 10000 mg/l before fed to the system.

3.7 Analytical methods

Most parameters were performed according to Standard Methods (APHA et al., 1999). PHA, PHB and PHV were determined with the method described in section 3.3. After the extraction step, 1 μl from the chloroform layer was injected into a Shimadzu Model GC-17A equipped with a DB-wax capillary column (0.25 x 3 mm inner diameter) attached to an FID detector. The operating temperatures of oven, injector and detector were similar to those described in section 3.3.

CHAPTER 4

RESULTS AND DISCUSSION

Part I Experiments with 660 mg/l COD (acetate and propionate) synthetic wastewater

4.1 PHA production with nitrogen limitation

This section contains the results of experiments 1 to 3 as listed in chapter 3. The systems were cultivated at the temperatures of 10°C, 20°C and 30°C to investigate the effects of temperature as well as the limitation of nitrogen on PHA production of activated sludge biomass. For the record, these three experiments were not operated at the same period because of temperature control room problem. Hence, the duration times of growth phase of these three systems were not exactly the same. The 20°C and 30°C systems were operated for about 3 SRTs for the growth phase as these two systems were seeded by sludge directly wasted from the IFAS system; they therefore needed a period of acclimatization. The 10°C system was operated for only 1 SRT for the growth phase because the inoculated seed was the sludge wasted from the growth phase of other 10°C systems.

4.1.1 PHA production

During the nutrient limitation phase, nitrogen was eliminated from the influent, and only phosphorus was added. Figures 4.1 to 4.3 show the results from the experiments at 10°C, 20°C and 30°C, respectively. The maximum PHA contents at 10°C, 20°C and 30°C were 38, 40 and 32 % of TSS, which were obtained at 6, 12 and 15 days after the N limitation phase, respectively. The maximum PHA contents obtained during the second N limitation were 36, 32 and 27 % of TSS for 10°C, 20°C and 30°C experiments, respectively. The operating periods required to achieve the maximum

%PHA in the second N limitation were shorter than in the first one, except for the 10°C experiment.

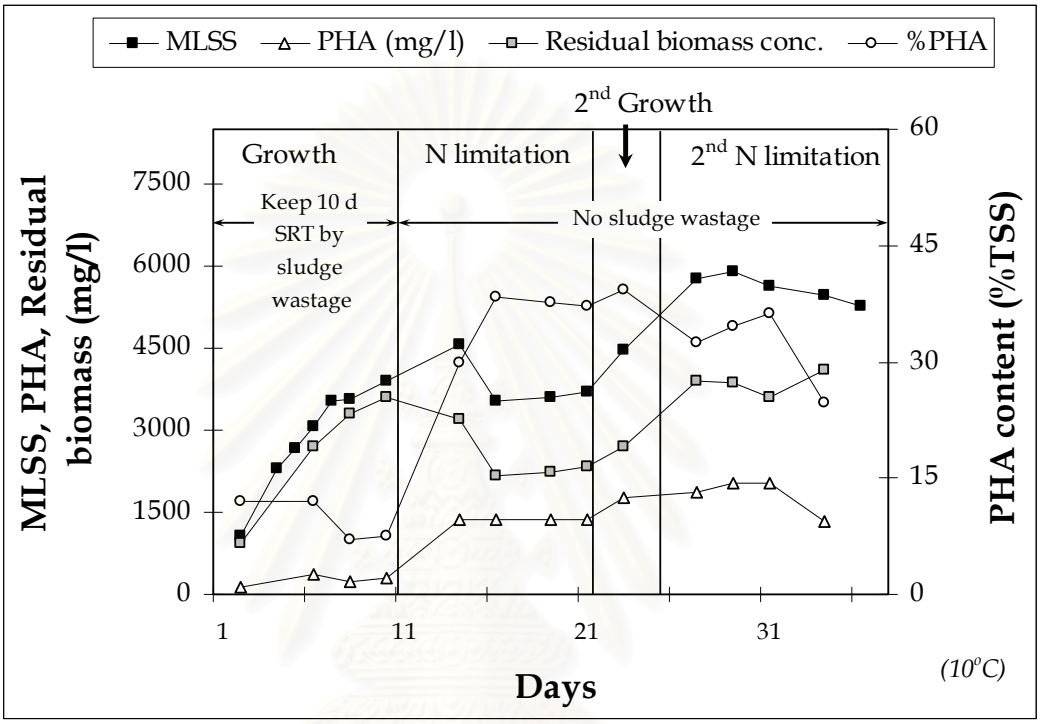


Figure 4.1: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 10°C experiment with N limitation.

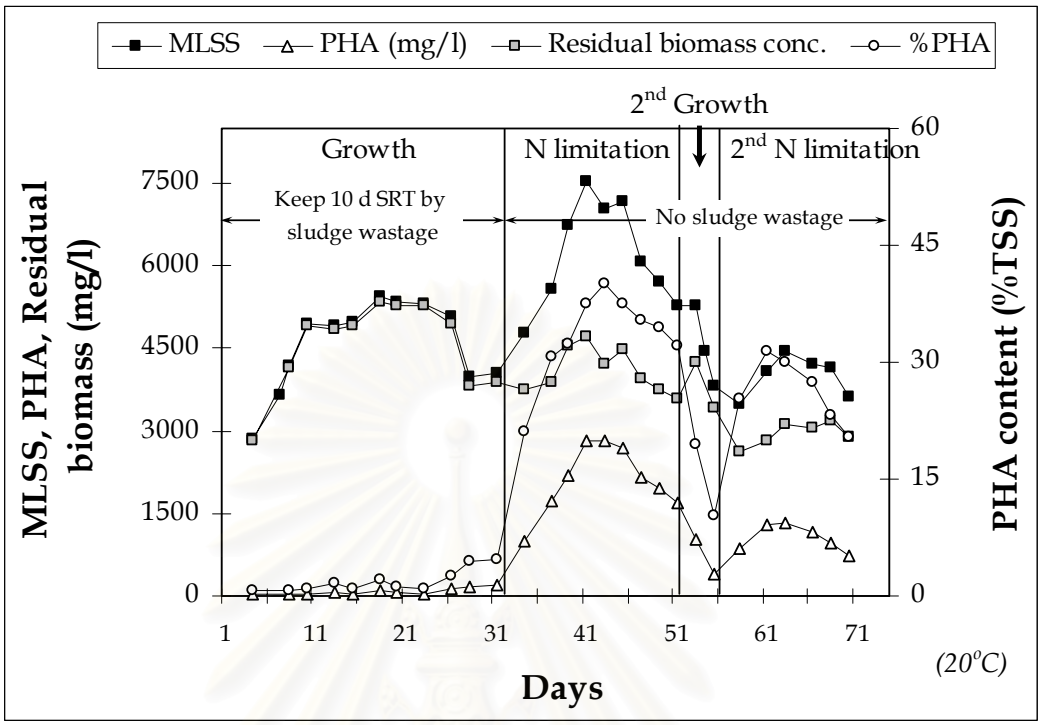


Figure 4.2: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 20°C experiment with N limitation.

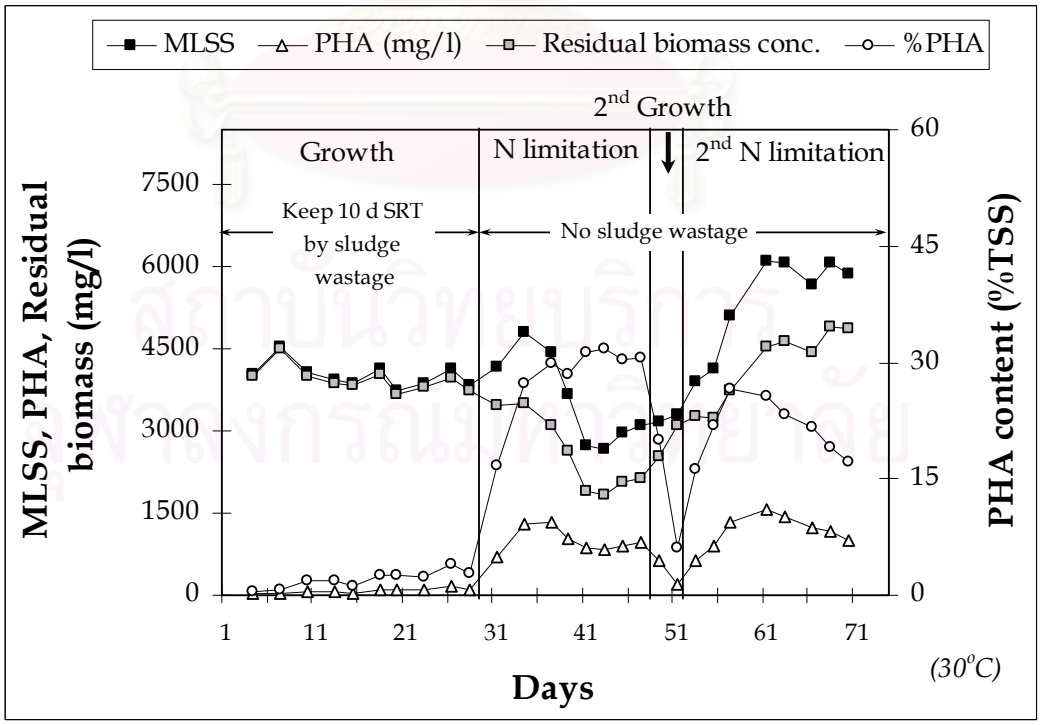


Figure 4.3: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 30°C experiment with N limitation.

Table 4.1: Summary of PHA production and biomass concentration of experiments with N limitation

	N limitation					
	10°C		**20°C**		**30°C**	
	1st	2nd	1st	2nd	1st	2nd
Max. PHA (% of TSS)	38	36	40	32	32	27
Days to Accumulate Max. %PHA (days)	6	7	12	6	15	6
Max. PHA concentration (mg/l)	1367	2043	2830	1292	1326	1348
Corresponding PHA (% of TSS)*	30	36	38	32	30	27
Corresponding MLSS (mg/l)*	4558	5627	7548	4100	4421	5087
Days to Accumulate Max. PHA concentration (days)	4	7	10	6	9	6
PHA Productivity (mg/l-d)	342	292	283	215	147	225
PHV/PHA on day of Max. PHA concentration (%)	43	35	25	37	20	24
PHA Yield/Substrate Utilized (mg PHA/mg COD_u)	0.22	0.24	0.20	0.14	0.10	0.15

Remark: *Corresponding PHA (% of TSS) is PHA content on the day that PHA concentration is maximum, not always the same day of maximum PHA content

*Corresponding MLSS (mg/l) is MLSS on the day of maximum PHA concentration.

As shown in Table 4.1 and Figure 4.4, the maximum PHA concentration of the 20°C system, 2830 mg/l, was clearly higher than those 1367 and 1326 mg/l of the 10°C and 30°C system, respectively. This was because high biomass concentration remained in the reactor at 20°C. The PHA productivities (Figure 4.5) clearly decreased as the temperature increased, i.e., 342, 283 and 147 mg/l-d at temperature of 10°C, 20°C and 30°C, respectively. Although the maximum PHA concentration of the 10°C system was significant lower than that of the 20°C, its PHA productivity was still the highest because the 10°C sludge accumulated the maximum PHA concentration two times

faster than the 20°C and 30°C sludge. In addition, PHA yields of 10°C, 20°C and 30°C were 0.22, 0.20 and 0.10 mg PHA/mg COD_u, respectively, as shown in Figure 4.6. The copolymer was also affected by temperature, i.e., the PHV/PHA ratios decreased from 43 and 25 to 20 (Table 4.1) as the temperature increased from 10°C and 20°C to 30°C, respectively. Moreover, the ratios of PHV/PHA in the second limitation were different from those of the first limitation in every temperature. This illustrated that **the operating procedure and temperature could affect the type of copolymer, not only because of the type of substrate** as reported by Shimizu et al., (1999), who used the ratios of C:N and butyric acid to valeric acid to control the copolymer composition.

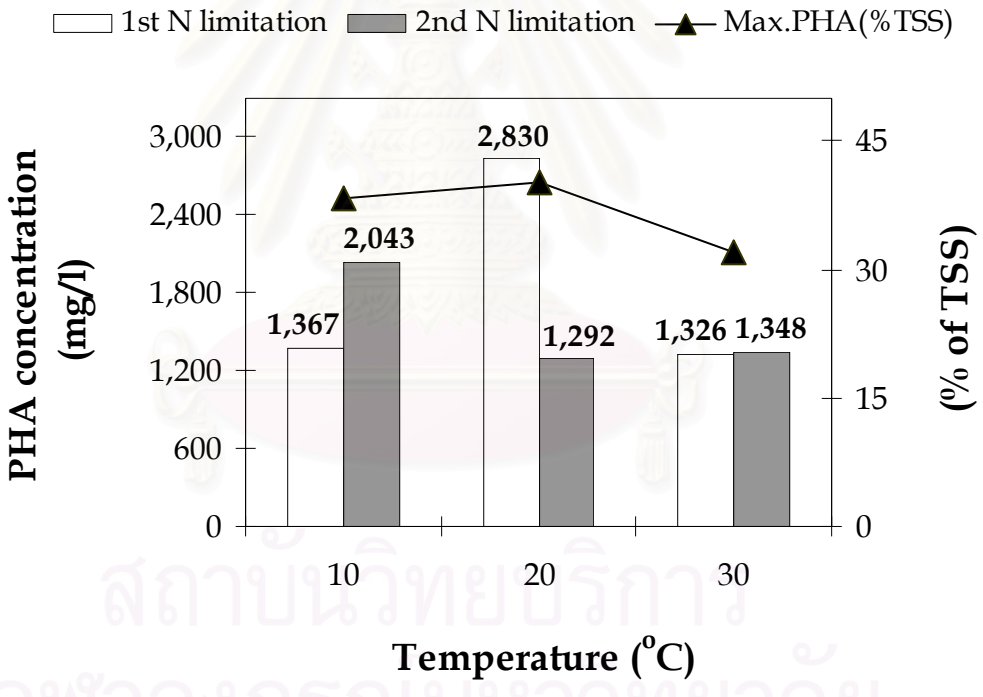


Figure 4.4: The maximum PHA concentrations and PHA contents with N limitation.

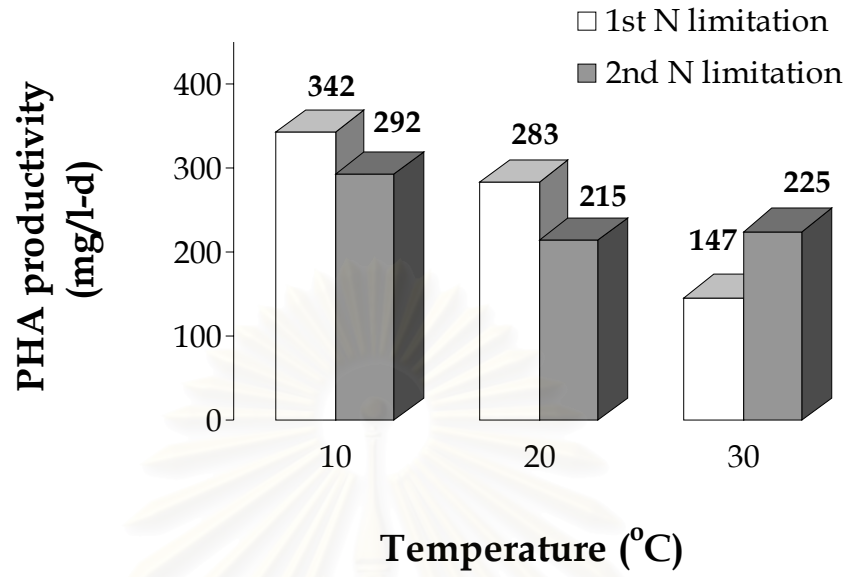


Figure 4.5: The corresponding PHA productivity with N limitation.

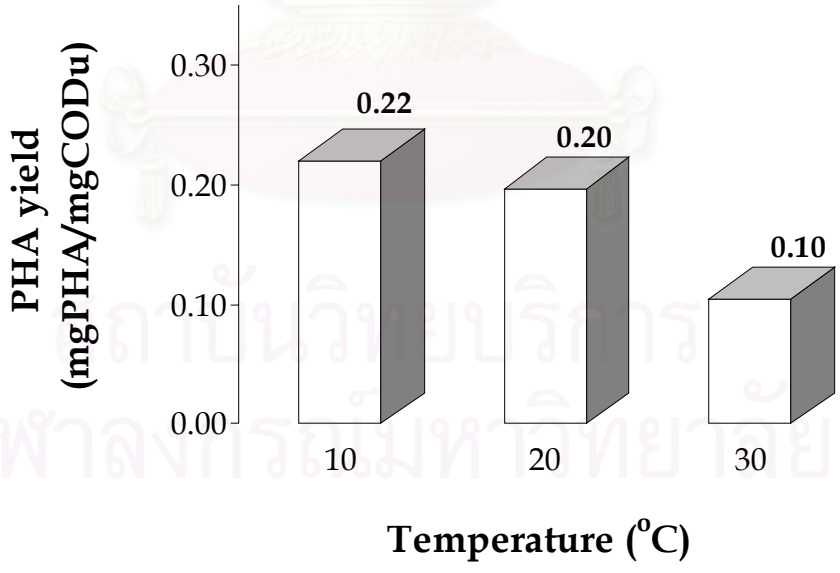


Figure 4.6: The yield of PHA on substrate utilized with N limitation.

(Note: for the first limitation scenario only.)

4.1.2 Biomass concentration and sludge quality

Figure 4.7 shows that the 10°C MLSS increased from 3892 mg/l to 4558 mg/l after 4 days of first N limitation. Although the SVI curve shows that the sludge had fine settleability during nitrogen limitation (SVI values were around 80-90 ml/g MLSS), the MLSS surprisingly decreased. This could only be reasoned that there still was biomass loss with the supernatant withdrawal. On the day 11 of first limitation phase, the SVI increased to a little higher than 150 ml/g MLSS, which illustrated that biomass began to not settle well. However, during the second cycle of growth and nitrogen limitation, the settleability of biomass became better and the MLSS could increase to the peak of 5898 mg/l. For 20°C system, the MLSS increased from 4059 to 7548 mg/l within 10 days of the first PHA accumulation phase. As shown in Figure 4.8, the SVI course of some 40-70 ml/g MLSS shows the good settleability of sludge as well. Consequently, the MLSS increased to as high as 7548 mg/l as mentioned above. Similarly to 10°C, the MLSS (after reaching the maximum value) decreased though the SVI still showed its good settleability. During the second growth phase, sludge bulking problem occurred and some biomass was lost with the effluent while the settleability of sludge seemed to recover after the second limitation of nitrogen. The 30°C system had similar pattern to 10°C, that is, the MLSS increased from 3826 to the peak of 4807 mg/l during the first PHA accumulation phase, and further increased to the maximum of 6090 mg/l during the second limitation as shown in Figure 4.9. From the SVI courses of all three experimental temperatures, the 30°C system is likely to have the serious problem of sludge bulking, which is consistent to the study by Krishna and Van Loosdrecht (1999) who concluded that activated sludge had less settleability when the temperature reached 30°C.

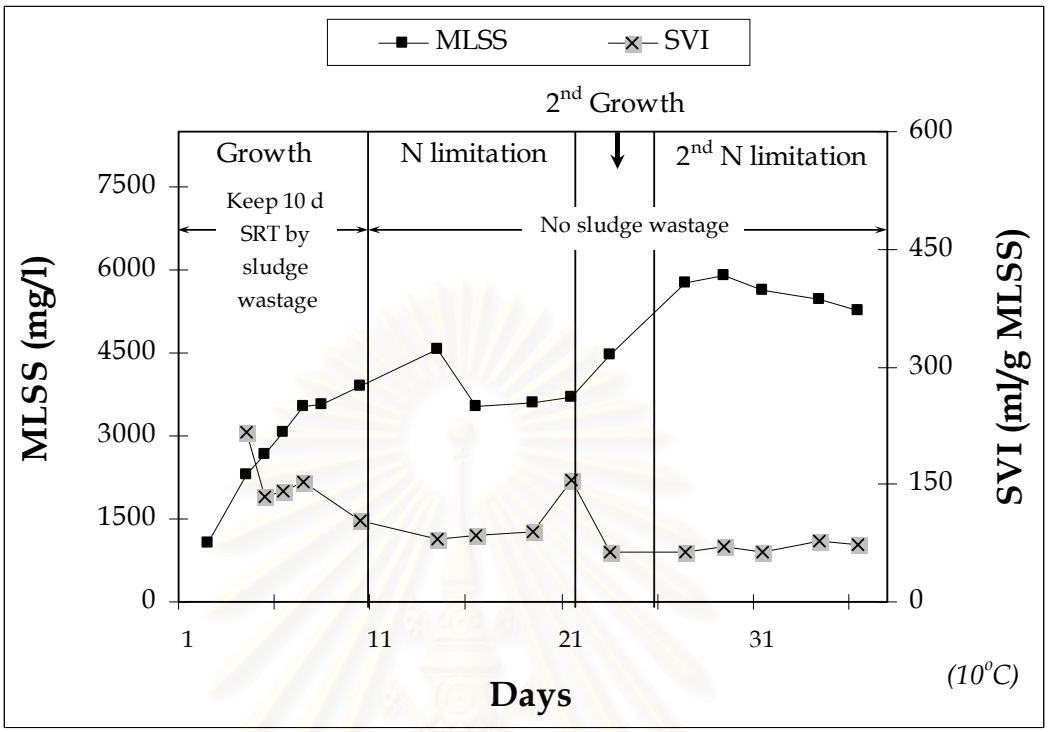


Figure 4.7: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 10°C experiment with N limitation.

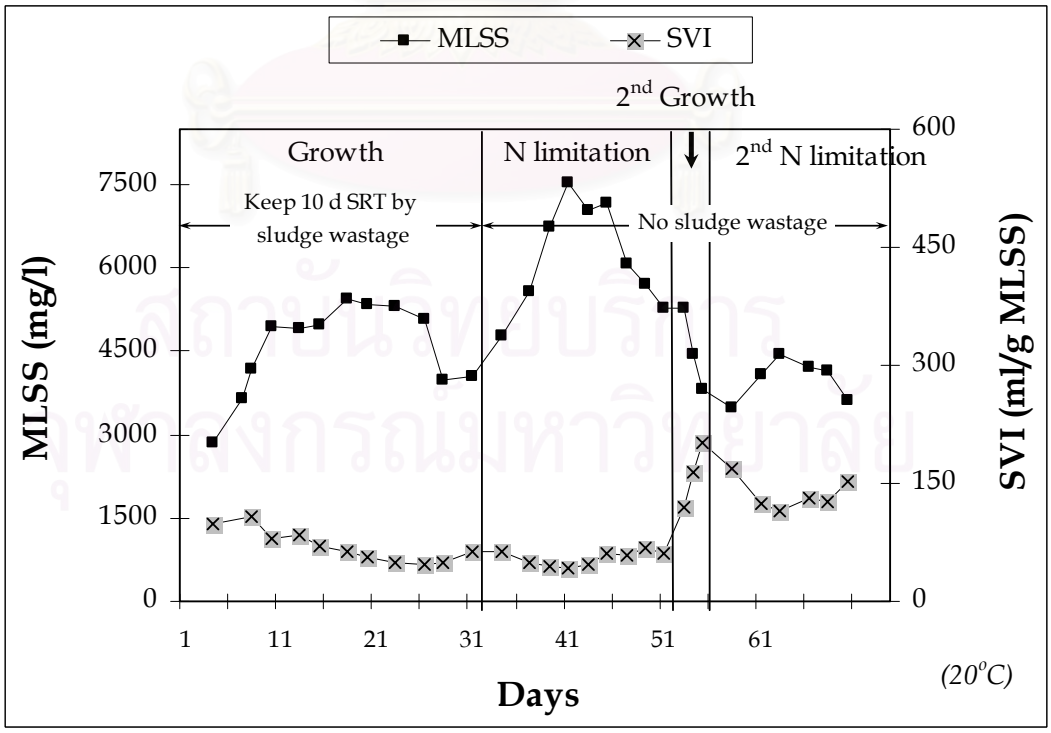


Figure 4.8: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 20°C experiment with N limitation.

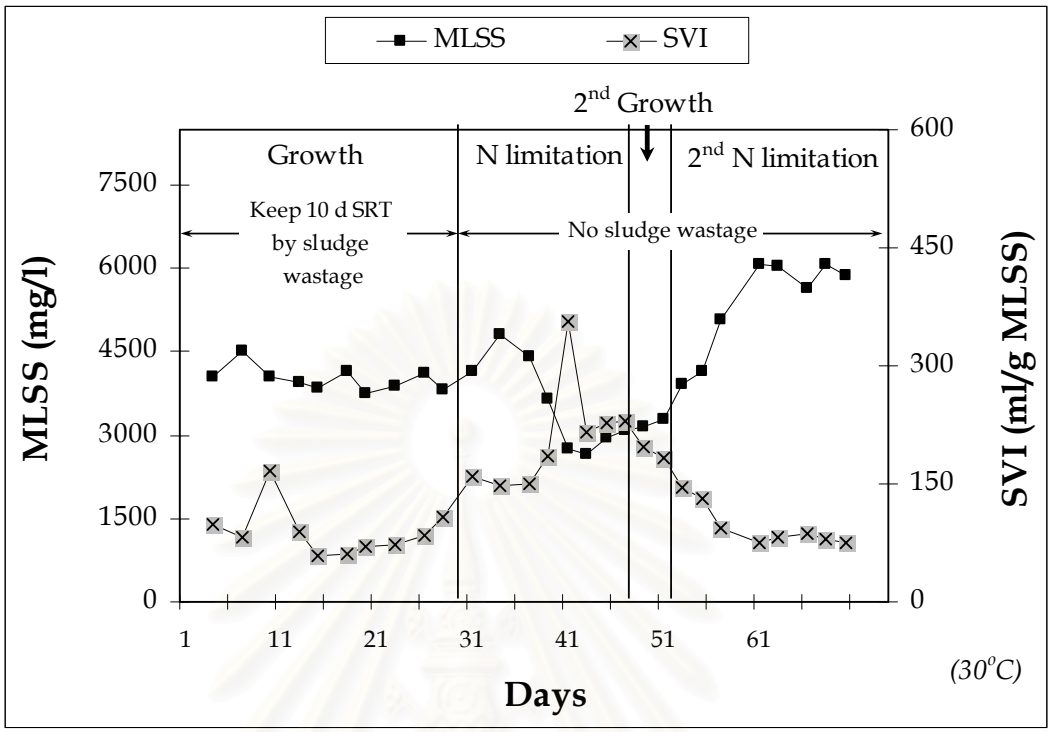


Figure 4.9: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 30°C experiment with N limitation.

Seemingly, the 30°C sludge had the least ability to accumulate PHA. However, it is possible that biomass cultured at 30°C might synthesize PHA nearly the same amount as those at 10°C and 20°C, but it would need more energy for its metabolism and cell maintenance than its counterparts at lower temperature biomass. Then, they catabolize some of intracellular PHA to obtain carbon and energy for cell living and maintenance. In addition, as we have known that phosphorus-accumulating organisms (PAOs) in the enhanced biological phosphorus removal (EBPR) system has a capacity to accumulate intracellular PHA, and Panswad et al. (2003) have stated that the PAOs should be lower-range mesophiles, it possibly implied that PHA-accumulating activated sludge would also be lower-range mesophiles. Therefore, the sum of accumulated PHA in 30°C sludge was less. Moreover, there was a sludge bulking problem in the 30°C system during the first nitrogen limitation phase, when biomass was lost in the effluent, resulting in low overall biomass concentration.

4.1.3 The residual biomass

The residual biomass value was used to express cell growth during PHA accumulation. As shown in Figure 4.1, cell growth (residual biomass) in the 10^{o}C system decreased after nitrogen was limited. This should be theoretically impossible because there was still substrate utilization and no sludge wasting during the PHA accumulation phase, thus, the value should remain at least steady or otherwise increase. The only reasonable explanation was that biomass was lost with the effluent. However, as shown in Figure 4.2, the cell growth in the 20^{o}C system gradually increased concurrently with PHA content and started to decrease a little prior to the PHA content reaching its maximum value. Liu et al. (1998) explained that when cell contains high PHA, it might lose the ability to divide itself further, consequently, lowering its growth. Similarly to the 10^{o}C system, cell growth in the 30^{o}C system (Figure 4.3) decreased immediately after the absence of nitrogen in the influent and more drastically due to sludge bulking as mentioned above. Therefore, maintaining biomass in the system was important for obtaining high PHA concentration and productivity.

4.1.4 Substrate utilization

The profiles of COD utilization (substrate utilization) and PHA content in Figures 4.10 to 4.12 show that there is a relationship between these two parameters. When the COD utilization started to decrease, the PHA content concurrently increased almost to the maximum value. As mentioned before, the cell growth was reduced when there was high intracellular PHA content and biomass was lacked of essential nutrients for quite a time such that the cell consequently lost their substrate utilization. From this point of view, we may be able to use this parameter (COD utilization) for operating the PHA production system, as COD analysis is faster and easier than PHA determination.

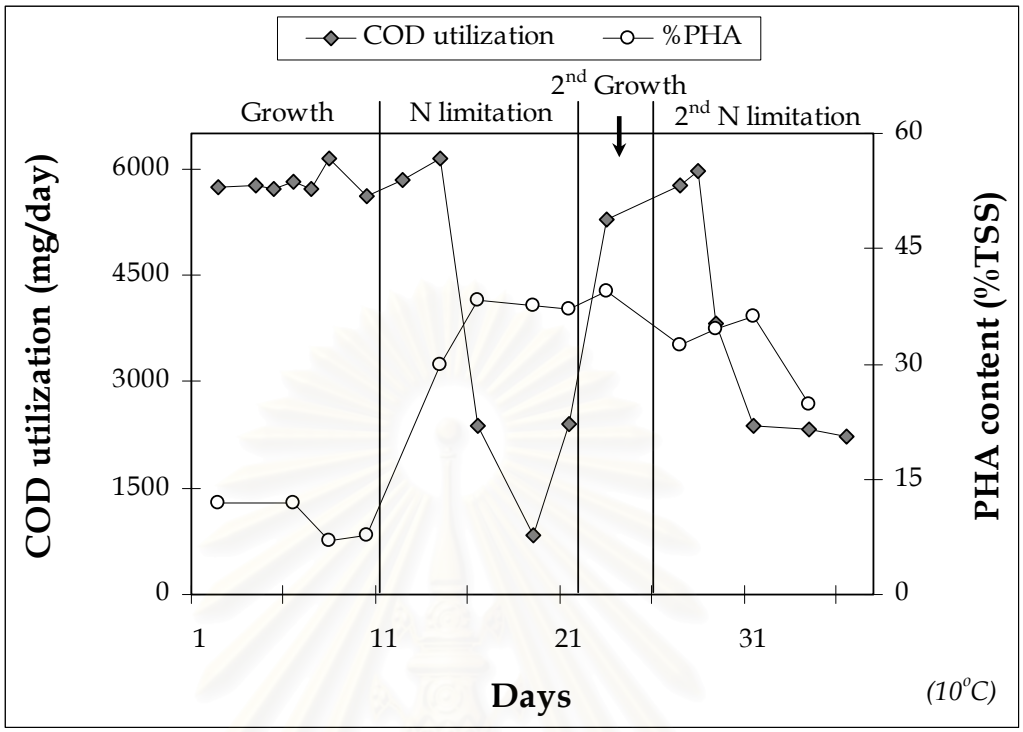


Figure 4.10: Profiles of COD utilization and PHA content (% of TSS) for the 10°C experiment with N limitation.

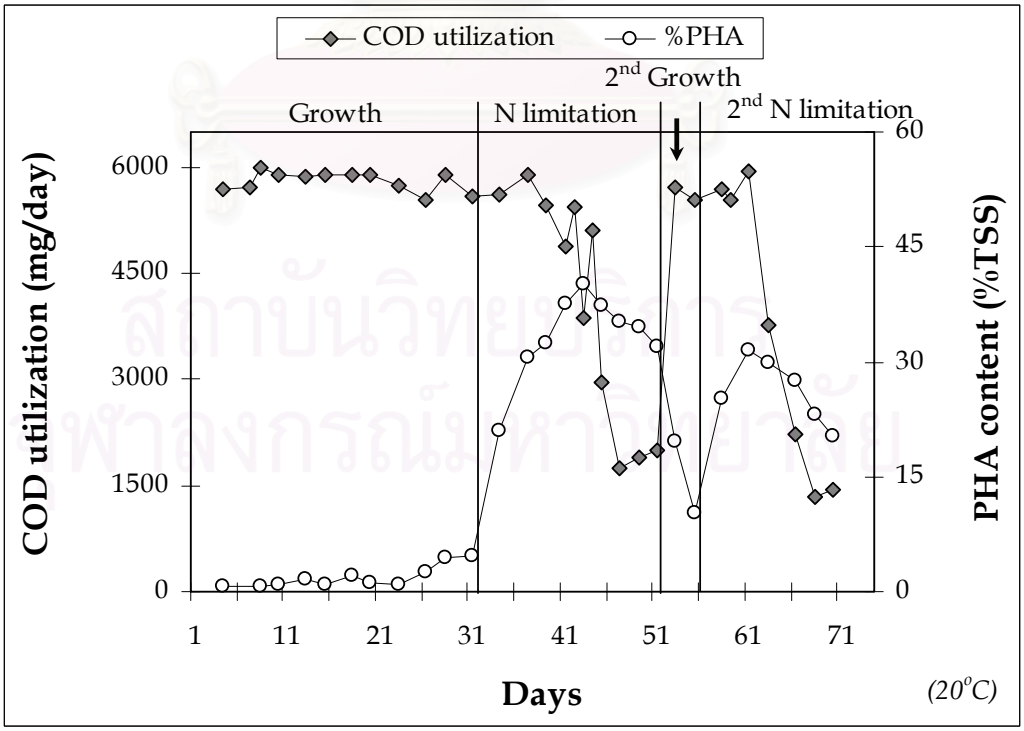


Figure 4.11: Profiles of COD utilization and PHA content (% of TSS) for the 20°C experiment with N limitation.

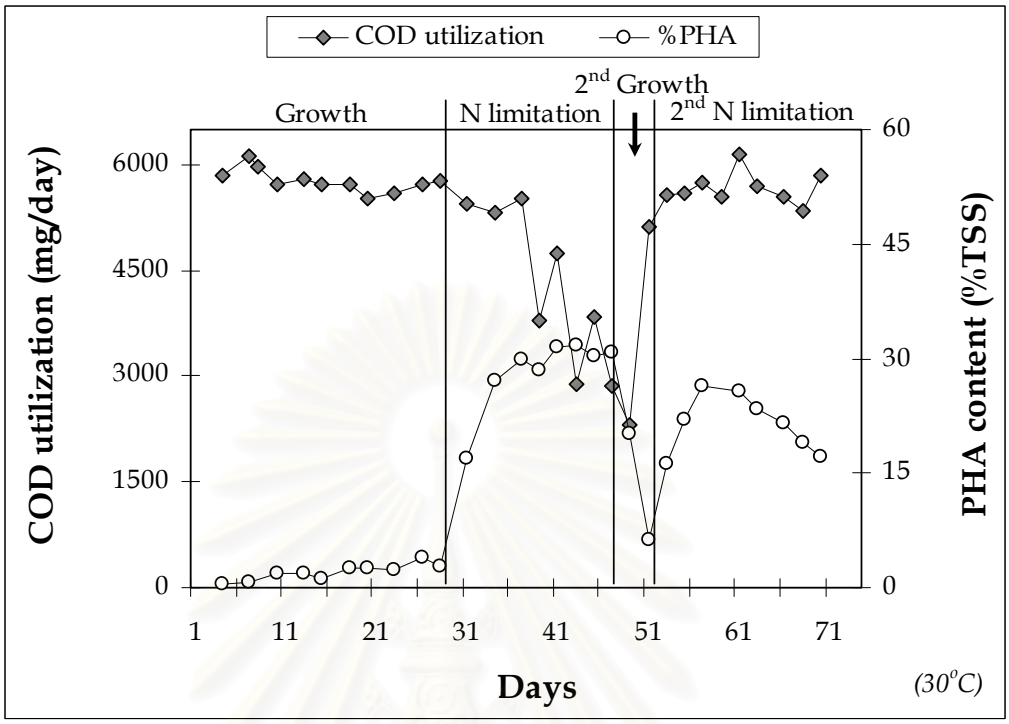


Figure 4.12: Profiles of COD utilization and PHA content (% of TSS) for the 30°C experiment with N limitation.

4.1.5 Conclusions

The experimental results illustrated that limiting nitrogen could induce PHA accumulation. In addition, temperature can also have a significant effect on PHA production. The 10°C system evidently stimulates PHA accumulation faster than the systems operated at higher temperatures at 20 and 30°C. The maximum PHA contents of the 10°C and the 20°C systems were nearly identical while that of the 30°C setup was the lowest. Unfortunately for tropical region, the results indicated that high temperature (30°C) would not be appropriate for commercial PHA production in ambient temperature. However, if the biomass could be totally maintained in the reactor, the PHA concentration of the 30°C system should be more promising than the results received from this study.

4.2 PHA production with phosphorus limitation

4.2.1 PHA production

The 10°C and 20°C system were operated under normal growth conditions for 2 and 3 sludge ages, respectively, and then phosphorus, only, was eliminated from the influent feed for the nutrient limitation period or PHA accumulation phase. For the 30°C system, the growth phase was operated for 4 sludge ages because sludge bulking occurred during the early stage of this period. Therefore, this growth phase had to be prolonged. The profiles determined during the experiments at the three temperatures are shown in Figures 4.13 to 4.15. Figure 4.13 shows that the PHA content in the 10°C reactor barely increased during the first two weeks of P limitation, then rapidly increased and reached the maximum content of 52 % of TSS 22 days after P limitation in the influent was initiated. After a second short growth phase, the PHA content of the sludge increased to the maximum value of 48 % of TSS within 6 days during the second phase, which was much faster than during the first P limitation phase.

Figure 4.14 shows that during the 20°C experiment, the PHA content increased gradually and did not reach the maximum content of 45 % of TSS until 48 days after P limitation was initiated. The PHA content during the second P limitation period at 20°C required 27 days to reach the maximum value of 35 % of TSS, which was lower than that obtained during the first limitation period. The profile of PHA content during the 30°C experiment as shown in Figure 4.15 was similar to the results of the other two experiments. The maximum PHA content of 47 % of TSS was obtained 35 days after initiation of P limitation, and a peak of 41 % of TSS was obtained 26 days after the second P limitation period.

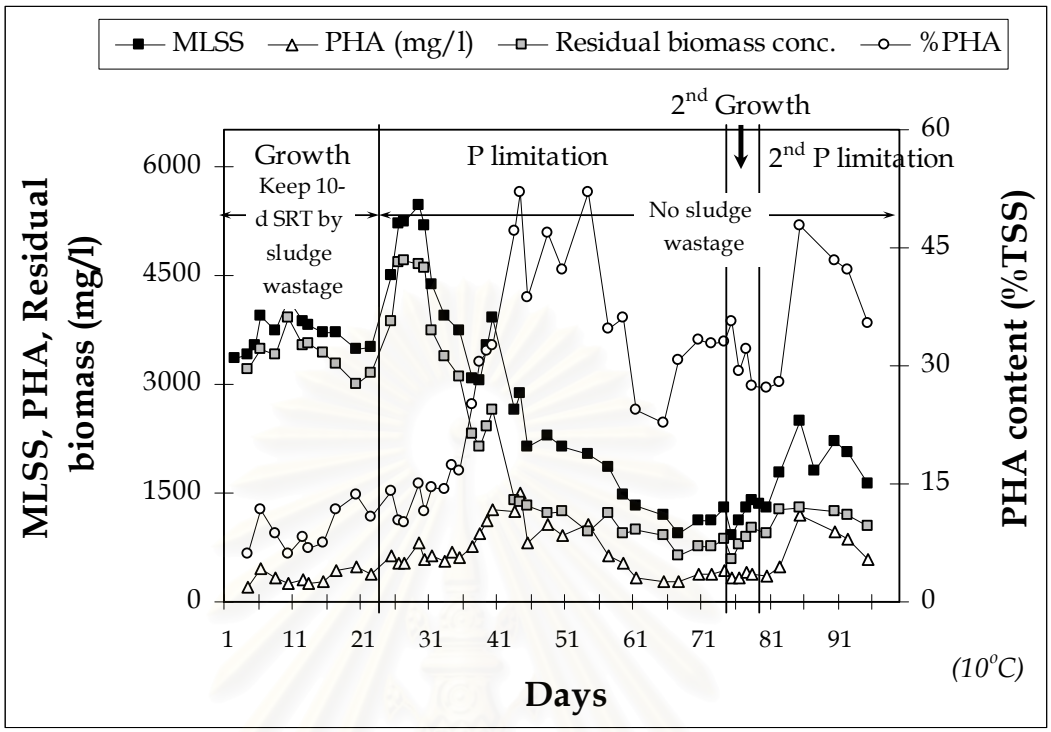


Figure 4.13: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 10°C experiment with P limitation.

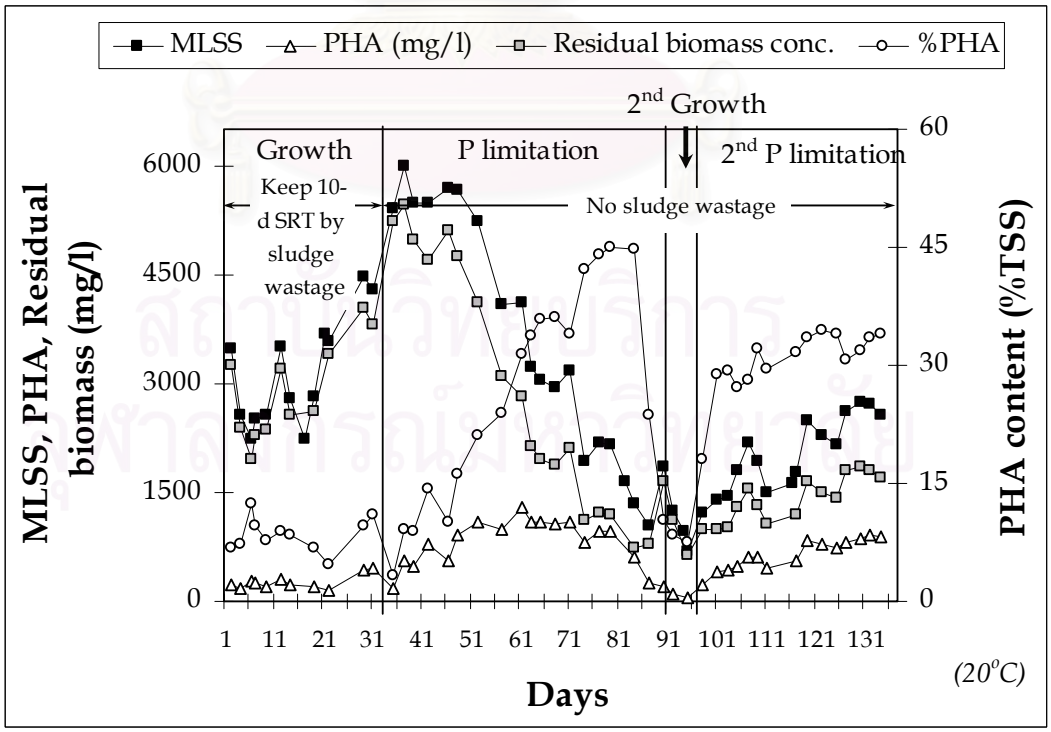


Figure 4.14: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 20°C experiment with P limitation.

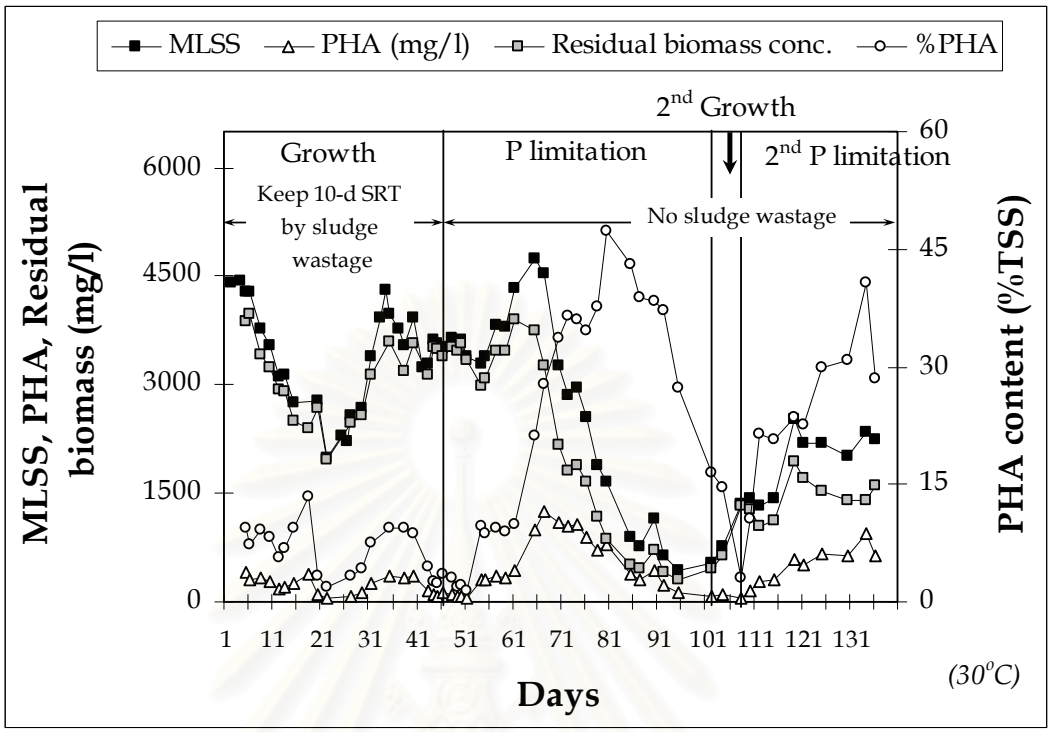


Figure 4.15: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 30°C experiment with P limitation.

The results indicated that peak PHA content can typically be obtained faster during the second phosphorus limitation period, but the maximum PHA content obtained will probably be less than those during the first accumulation phase. It was noted that the P limitation experiments of this study required significantly longer times to obtain maximum PHA content than was required during nitrogen limitation experiments as reported in section 4.1. Based on the results obtained by Punrattanasin (2001), it is probable that, even though the influent contained no phosphorus, the biomass still had phosphorus stored within it and this had to be depleted before PHA accumulation could begin.

Information regarding the PHA accumulation is tabulated in Table 4.2. The primary conclusion from the data is that PHA accumulation decreased with an increase in temperature. The maximum PHA concentrations were 1491, 1294 and 1260 mg/l for the 10°C, 20°C and 30°C experiments (Figure 4.16), respectively, and the corresponding PHA productivity rates as shown in Figure 4.17 were 68/197, 43/35 and

57/37 mg/l-d for the two runs at each temperature. The PHA accumulation at the temperature of 10^{o}C was significantly higher than the amounts and rates observed at 20 and 30^{o}C, but the difference was small between 20 and 30^{o}C. The actual difference between PHA accumulations at 10^{o}C was even greater than the PHA numbers indicate because the MLSS concentration maintained at the lowest temperature was substantially less than those at the other temperatures were. Table 4.2 shows that the respective MLSS concentrations were 2867, 4108 and 4533 mg/l at the 10, 20 and 30^{o}C temperatures when the first PHA concentration peak was observed. The lowest temperature would have been considerably better for PHA accumulation if the biomass solids had been retained better in the reactor. Note, however, that all three reactors had lost a lot of biomass by the time of the second % PHA peak.

Table 4.2: Summary of PHA production and biomass concentration of experiments with P limitation.

	P limitation					
	10°C		20°C		30°C	
	1st	2nd	1st	2nd	1st	2nd
Max. PHA (% of TSS)	52	48	45	35	47	41
Days to Accumulate Max. %PHA (days)	22	6	48	27	35	26
Max. PHA concentration (mg/l)	1491	1184	1294	832	1260	954
Corresponding PHA (% of TSS)*	52	48	32	34	28	41
Corresponding MLSS (mg/l)**	2867	2477	4108	2477	4533	2343
Days to Accumulate Max. PHA concentration (days)	22	6	30	24	22	26
PHA Productivity (mg/l-d)	68	197	43	35	57	37
PHV/PHA on day of Max. PHA concentration (%)	45	56	14	26	37	46
PHA Yield/Substrate Utilized (mg PHA/mg COD_u)	0.05	0.13	0.03	0.03	0.04	0.03

Remark: *Corresponding PHA (% of TSS) is PHA content on the day that PHA concentration is maximum, not always the same day of maximum PHA content

**Corresponding MLSS (mg/l) is MLSS on the day of maximum PHA concentration.

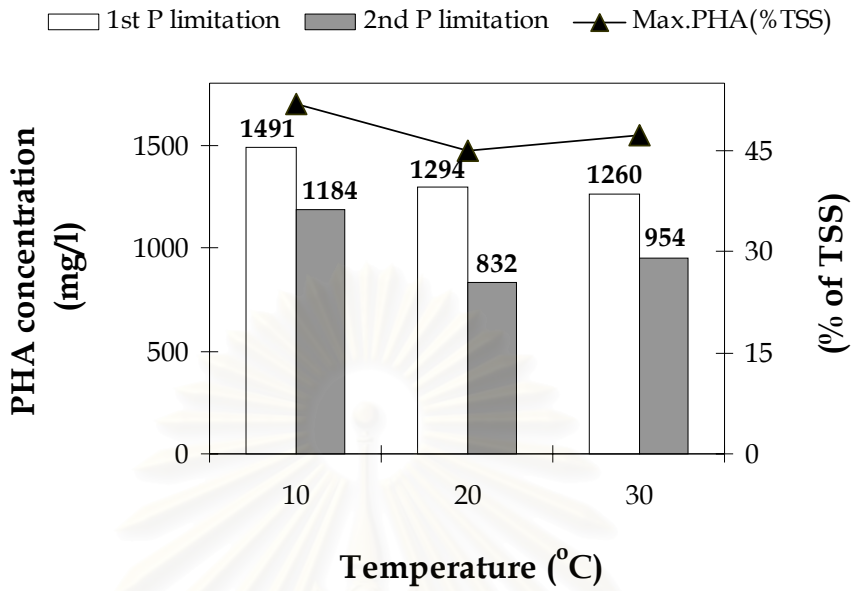


Figure 4.16: The maximum PHA concentrations and PHA contents with P limitation.

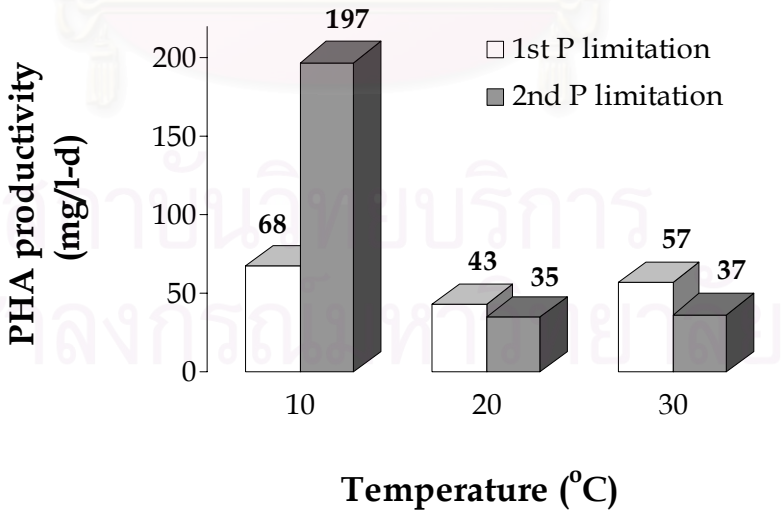


Figure 4.17: The corresponding PHA productivity with P limitation

The PHA yields per unit substrate at 10°C, 20°C and 30°C were 0.05, 0.03 and 0.04 mg PHA/mg COD_u, respectively (Figure 4.18). These PHA yields were significantly lower than those happened in the experiment with nitrogen limitation (Figure 4.6) as these experiments were required longer PHA accumulation periods (P limitation phase) before accomplishing the maximum PHA concentration. In addition, the utilization of COD still functioned quite well till the maximum PHA content was reached (Figures 4.19 to 4.21), therefore the COD utilization was totally high and consequently gave the low values of PHA yield. In circumstances, it can be therefore concluded that the P limitation step shall not be employed in the PHA production process using activated sludge biomass.

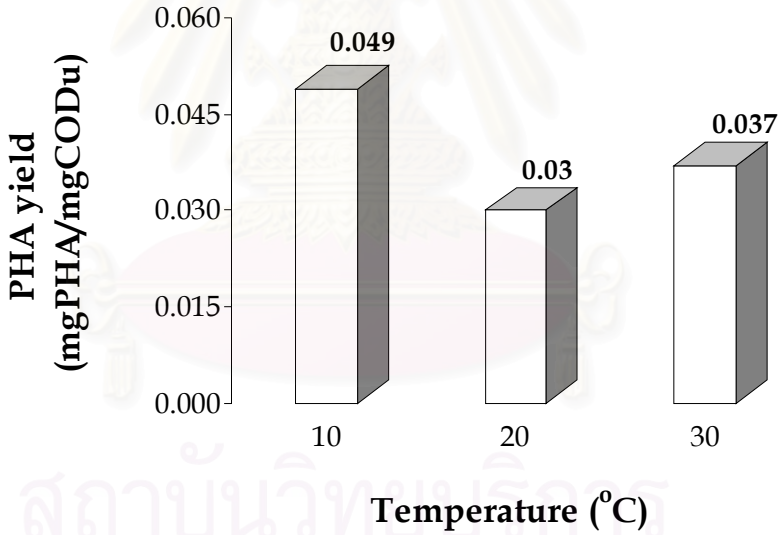


Figure 4.18: The yield of PHA on substrate utilized with P limitation.
(Note: for the first limitation scenario only.)

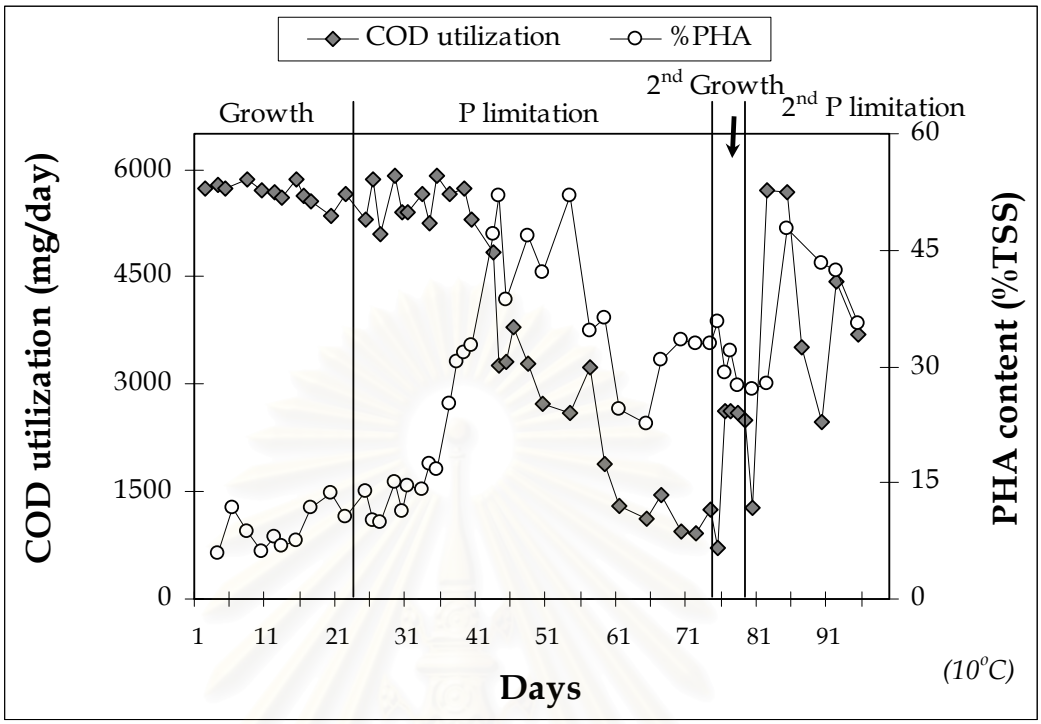


Figure 4.19: Profiles of COD utilization (mg/day) and PHA content (% of TSS) for the 10°C experiment with P limitation.

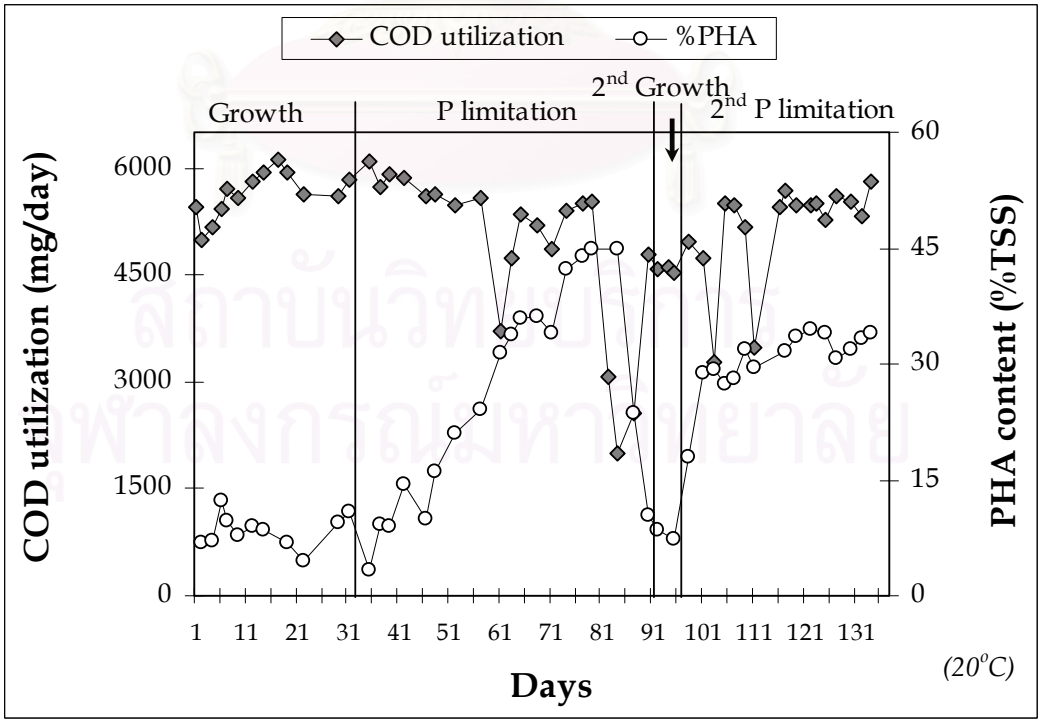


Figure 4.20: Profiles of COD utilization (mg/day) and PHA content (% of TSS) for the 20°C experiment with P limitation.

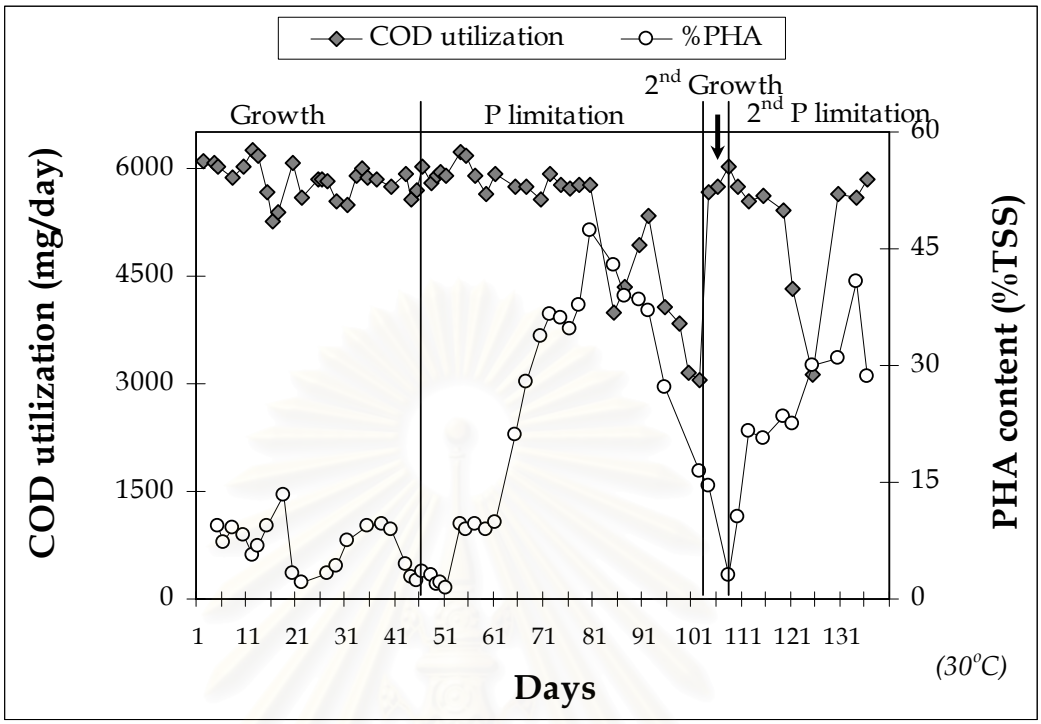


Figure 4.21: Profiles of COD utilization (mg/day) and PHA content (% of TSS) for the 30°C experiment with P limitation.

4.2.2 Biomass concentration and sludge quality

Sludge bulking problems caused considerable variation in the MLSS concentrations in the reactors. The MLSS concentration in the 10°C system was 3514 mg/l when the P limitation phase began, then increased to 5433 mg/l when sludge wasting was discontinued to achieve PHA accumulation. Thereafter, it decreased drastically throughout the first P limitation phase to a value of 930 mg/l because of sludge bulking problems. The biomass concentration changes in the 20 and 30°C systems had similar patterns to the 10°C system, and the 30°C MLSS decreased from 3573 to 422 mg/l because of sludge bulking. Figures 4.22 – 4.24 show that the SVI changes correlated with the MLSS changes. Krishna et al. (1999) have stated that sludge bulking is likely to happen when the temperature exceeds 30°C. It is of interest that the sludge bulking problems were worse during the phosphorus limitation experiments than during the preceding nitrogen limitation experiments (see section 4.1)

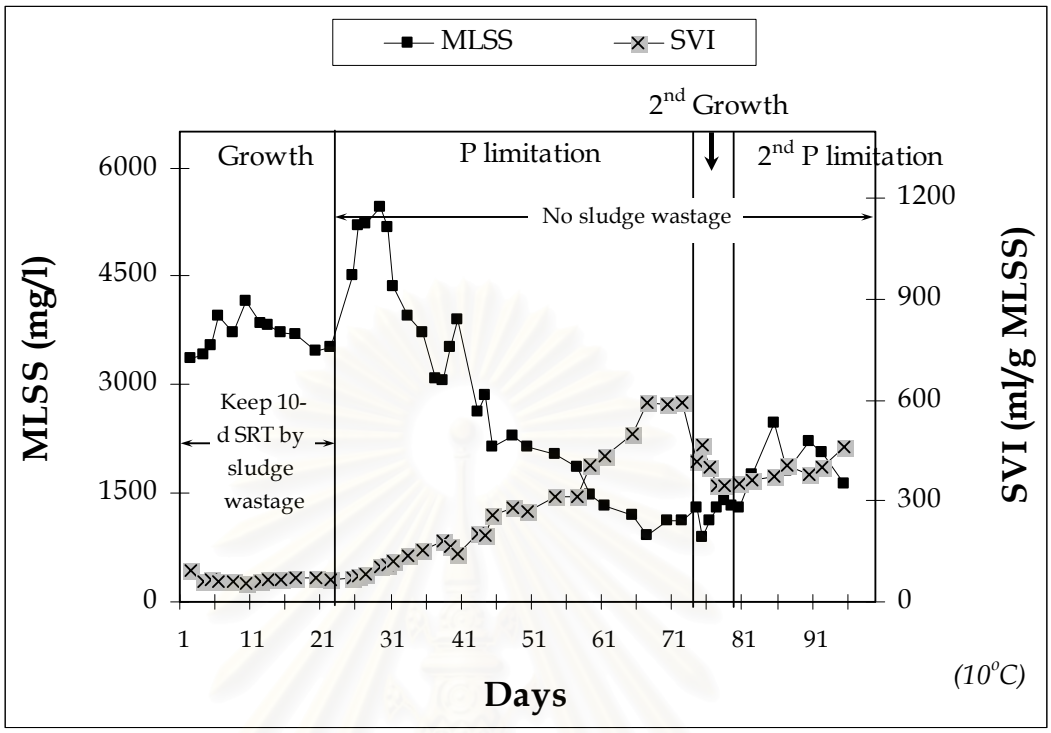


Figure 4.22: Profiles of MLSS (mg/l) and SVI (ml/g MLSSS) for the 10°C experiment with P limitation.

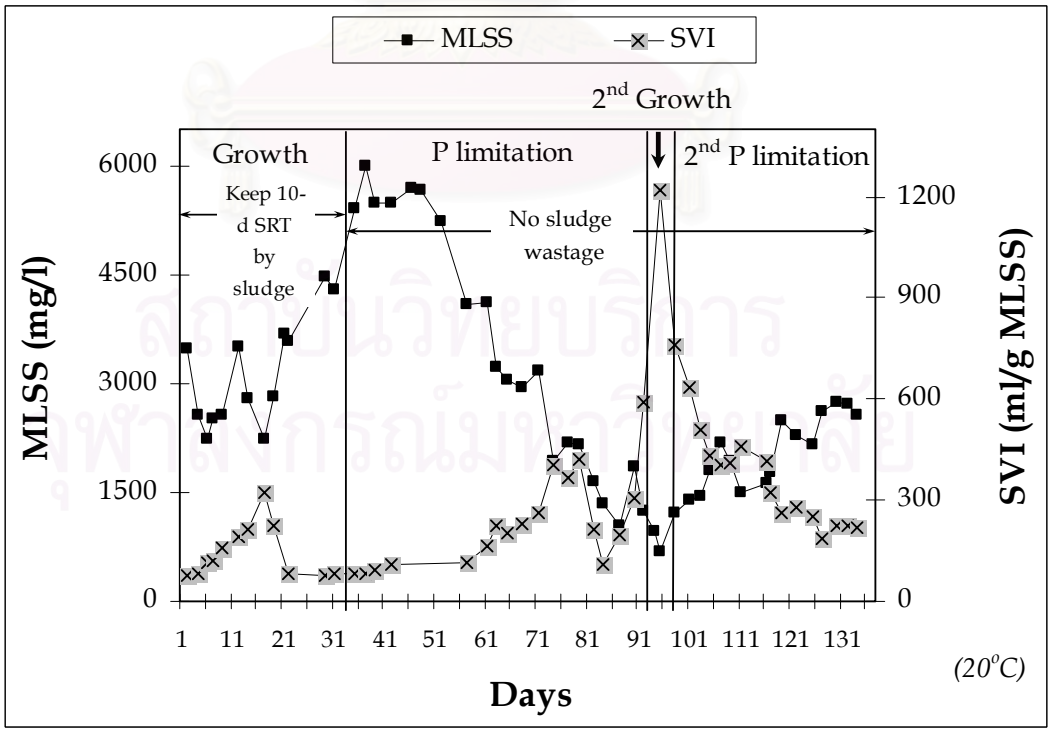


Figure 4.23: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 20°C experiment with P limitation.

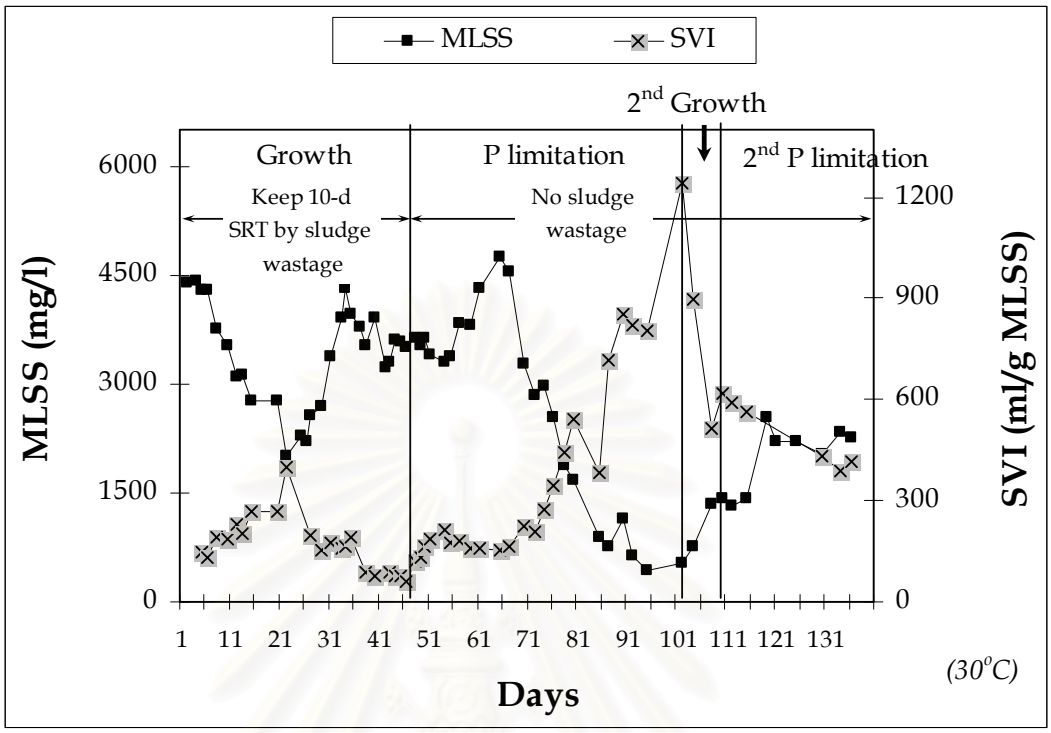


Figure 4.24: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 30°C experiment with P limitation.

Although all experiments with P limitation resulted in large accumulations of PHA, expressed as % of TSS in the biomass, the PHA yields (mg PHA/mg COD_u) and productivities (mg/l-d) were significantly lower than those obtained during the nitrogen limitation experiments. Based on results previously reported by Punrattanasin (2001), it is probable that the biomass cells contained substantial amounts of stored P when P limitation was initiated, and PHA accumulation was delayed until the stored P had been depleted. In addition, the feed contained nitrogen throughout the P limitation experiments, which enabled the biomass to continue cell growth and anabolic metabolism. Several researchers (Steinbuchel and Schlegel, 1991; Kessler and Witholt, 2001; Du et al., 2001) have explained that under growth conditions, intracellular concentrations of acetyl-CoA (precursor for PHB synthesis) are presumably low as it mostly enters the TCA cycle. This liberates large amounts of free coenzyme A (CoASH), which could inhibit the condensation of acetyl-CoA to acetoacetyl-CoA by the enzyme 3-ketothiolase and, thus, inhibit PHB synthesis. Some researchers (Ryu et al., 1997; Wendlandt et al., 2001) have reported that they could

accumulate PHB under P limitation better than limitation of nitrogen or other essential nutrients. Similar results might have been obtained during these studies if much of the biomass had not been lost from the systems because of sludge bulking. It is recommended that future P limitation experiments utilize a method to reduce the loss of biomass, such as the use of membrane separation or the use of batch or fed-batch systems applied to pure culture instead of a continuously fed (like wastewater treatment) system.

4.2.3 The residual biomass

As shown earlier in Figures 4.13 - 4.15, the residual biomass of all three experimental temperatures increased for some time during the early period of P limitation. That means biomass in that period still had the ability to utilize substrate for cell growth although phosphorus was not added to the feed anymore. Then, the residual biomass decreased drastically before the PHA fractions reach the maximum. This decreasing of residual biomass should not be interpreted that cell growth was terminated, but the major reason should be granted to the occurrence of sludge bulking problem that caused severe loss of biomass from the reactor with the effluent withdrawal.

4.2.4 Conclusions

From the experimental results, P limitation is not a good strategy for PHA production by activated sludge biomass because they produced low PHA productivities and yields, although the maximum PHA contents (% of TSS) were higher than N limitation in every experimental temperature. Nonetheless, the results strongly indicate that PHA accumulation in activated sludge under P limitation conditions requires less time and increases to a higher fraction of the TSS when the mixed liquor temperature is 10^{o}C, as compared to 20 or 30^{o}C. They also indicate that PHA accumulation will peak in a much shorter time period during the second P limitation period than the first, but probably not to as high a fraction of the TSS. The probable cause of the prolonged period of PHA accumulation during the first limitation period is the presence of stored

P in the cells when the feed without P is initiated. However, P limitation might be a considerable strategy for PHA production if the local wastewater already contained nitrogen source.

4.3 PHA production with combined nitrogen and phosphorus limitation

4.3.1 PHA production

In this section, the duration of growth phases of these three systems were not the same, depending on how fast the steady-state was achieved, e.g. the 10^{o}C and 20^{o}C systems required 2 and 3 sludge ages, respectively, and the 30^{o}C was operated for 4 sludge ages before starting nutrient limitation conditions because of a sludge bulking problem. Figure 4.25 shows the profiles of MLSS (mg/l), PHA content (% of TSS) and PHA concentration (mg/l) during the experiments of 10^{o}C with combined N and P limitation. The system was operated for 22 days with excess N and P added to the influent for biomass growth. The SRT was maintained at 10 days over the 22 days of the growth period by wasting a calculated amount of mixed liquor after accounting for the SS wasted during supernatant withdrawal, which occurred at the end of the aeration period. Once N&P limitation was started, mixed liquor wastage was terminated in order to maximize biomass concentration, and therefore PHA accumulation, in the system.

After N&P was eliminated from the influent, PHA accumulation clearly happened as PHA content increased rapidly and reached the maximum content of 45 % of TSS on day 5 after N&P limitation was started. Surprisingly, the PHA contents could remain nearly constant above 40 % of TSS for almost a month after reaching the maximum percentage, before decreasing simultaneously with the biomass concentration.

Next, nutrients addition was resumed and the system was operated under growth phase conditions again for approximately 5 days, and then both N and P were limited for a second time. During the second N&P limitation period, both the PHA content and the

MLSS concentration were nearly the same as during the first growth period. That was inconsistent with the study by Punrattanasin (2001), in which she reported that two consecutive periods of growth and nutrient limitation would typically result in higher PHA content during the second limitation period. However, the operating days during the first N&P limitation in this study totaled 43 days. It is possible the biomass completely lost their capability to accumulate PHA because they lacked both nitrogen and phosphorus over this long period of time.

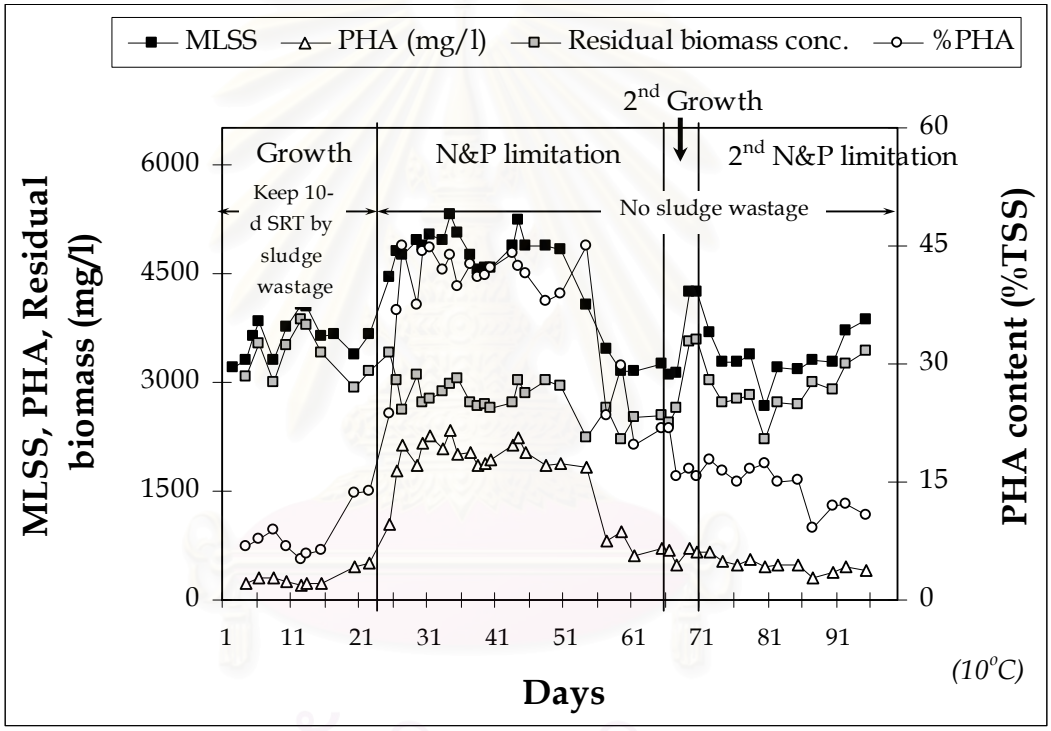


Figure 4.25: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 10°C experiment with N&P limitation.

Figures 4.26 and 4.27 show the time course of MLSS (mg/l), PHA content (% of TSS) and PHA concentration (mg/l) during the experiments of 20°C and 30°C, respectively, with combined N&P limitations. Similarly to the experiment of 10°C, the systems were operated for 2 SRTs for normal growth phase. The PHA contents during both experiments increased slower during the experiment of 10°C. The maximum PHA content of 43 % of TSS in the experiment of 20°C and 33 % of TSS in the experiment of 30°C were obtained 11 and 10 days after N&P limitations, respectively.

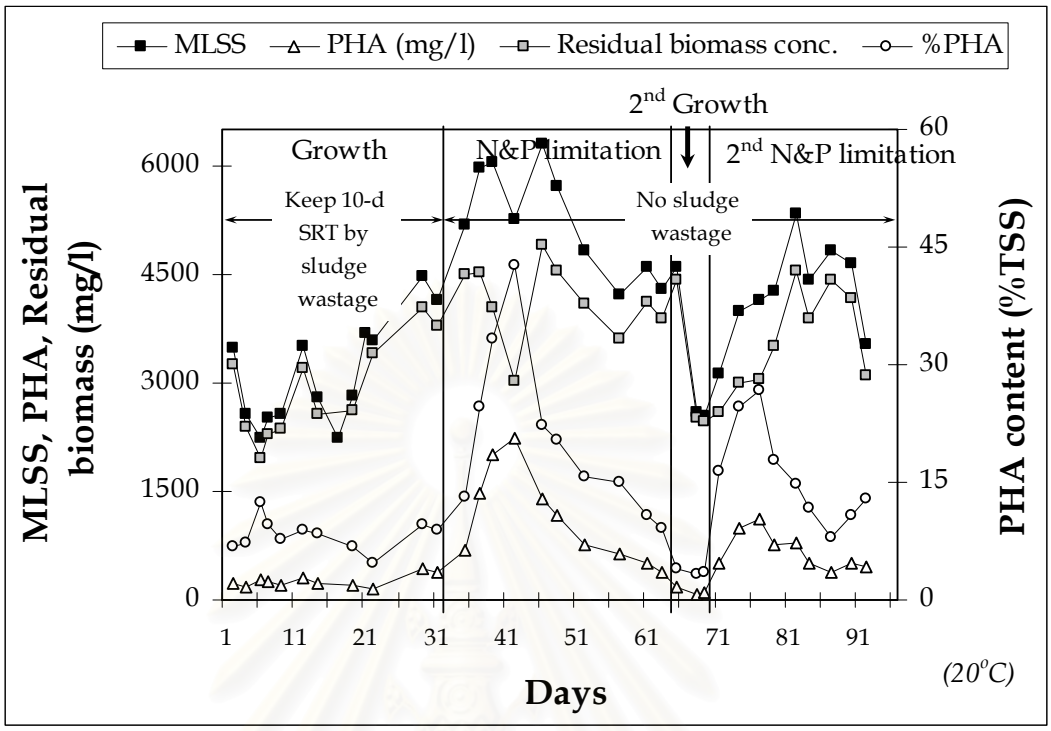


Figure 4.26: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 20°C experiment with N&P limitation.

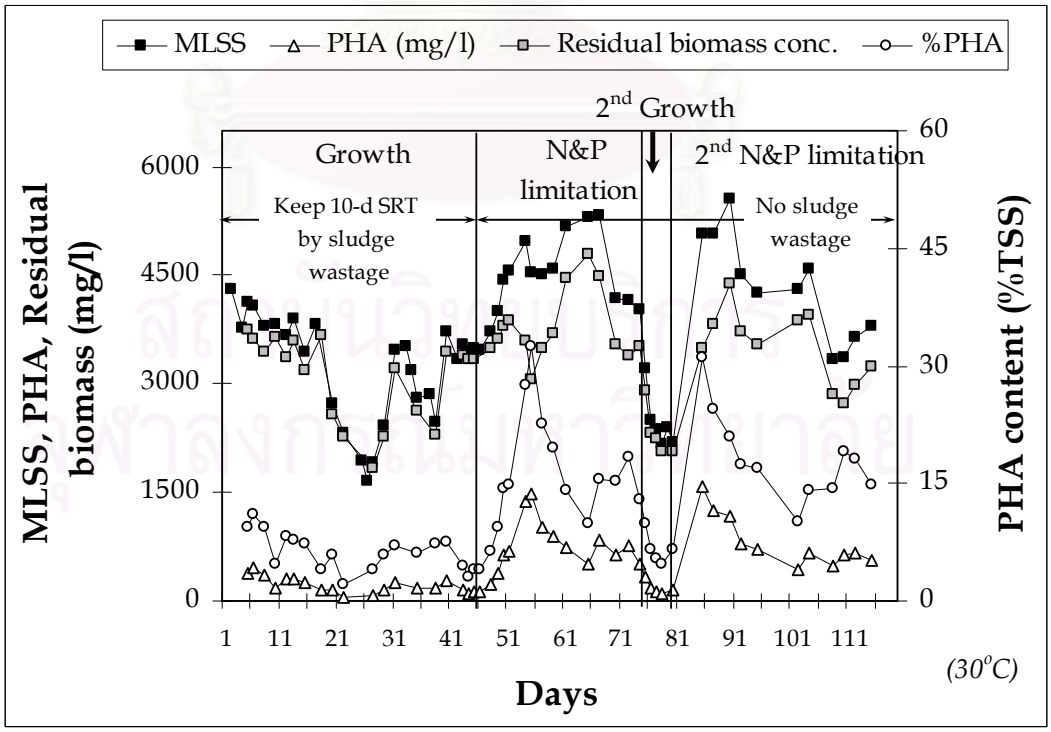


Figure 4.27: Profiles of PHA content (% of TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the 30°C experiment with N&P limitation.

Unlike the 10°C experiment results, the PHA contents in both the 20 and 30°C experiments decreased almost immediately after reaching the maximum value. After the second period of N&P limitation conditions were applied, the PHA contents in both experiments reached the maximum values faster than that during the first N&P limitations period. The values reached 27 % of TSS in the 20°C experiment and 31 % of TSS in the 30°C, after 8 and 6 days, respectively. Although, the peak PHA accumulations occurred during the second nutrients limitations period at a faster rate than the first limitations period, the maximum amount accumulated were less than the fractional contents obtained during the first limitations period. This result also was different from the results obtained by Punrattanasin (2001), who reported maximum PHA contents obtained during the second limitation were higher than those obtained during the first limitations period (Table 4.3).

Table 4.3: Comparison of PHA production from this study with Punrattanasin (2001).

	This study; 20°C w/ N&P limitation		Punrattanasin (2001); fully AE, 20°C w/ N&P limitation	
	1st limitn	2nd limitn	1st limitn	2nd limitn
Max. PHA (% of TSS)	43	27	46	70
Max. PHA concentration (mg/l)	2239	1107	1827	2265
Corresponding PHA (% of TSS)*	43	27	46	70
Corrresponding MLSS (mg/l)**	5255	4147	3934	3238
Days to Accumulate Max. PHA concentration (days)	11	8	7	5
PHA Productivity (mg/l-d)	204	138	261	453
Yield of PHA on Substrates (mg PHA/mg COD_u)	0.16	0.10	0.23	0.34

Remark: *Corresponding PHA (% of TSS) is PHA content on the day that PHA concentration is maximum, not always the same day of maximum PHA content

**Corresponding MLSS (mg/l) is MLSS on the day of maximum PHA concentration.

PHA concentration (mg/l), PHA productivity (mg/l-d) and PHA yield (mg PHA/mg COD_u) obtained during the N&P limitation periods are summarized in Table 4.4. As shown in the table, the maximum PHA content occurred on the same day the maximum PHA concentration were observed. The maximum PHA concentrations at 10°C and 20°C were not much different, i.e., 2133 and 2239 mg/l, while the 30°C concentration was only 1476 mg/l, much lower than those obtained at the other two temperatures (Figure 4.28).

Table 4.4: Summary of PHA production and biomass concentration of experiments with combined N&P limitation.

	N&P limitation					
	10°C		**20°C**		**30°C**	
	1st	2nd	1st	2nd	1st	2nd
Max. PHA (% of TSS)	45	18	43	27	33	31
Days to Accumulate Max. %PHA (days)	5	2	11	8	10	6
Max. PHA concentration (mg/l)	2133	654	2239	1107	1476	1577
Corresponding PHA (% of TSS)*	45	18	43	27	33	31
Corrresponding MLSS (mg/l)**	4740	3673	5255	4147	4542	5070
Days to Accumulate Max. PHA concentration (days)	5	2	11	8	10	6
PHA Productivity (mg/l-d)	427	327	204	138	148	263
PHV/PHA on day of Max. PHA concentration (%)	43	48	21	31	25	42
Yield of PHA on Substrates (mg PHA/mg COD_u)	0.38	0.22	0.16	0.10	0.11	0.17

Remark: *Corresponding PHA (% of TSS) is PHA content on the day that PHA concentration is maximum, not always the same day of maximum PHA content

**Corresponding MLSS (mg/l) is MLSS on the day of maximum PHA concentration.

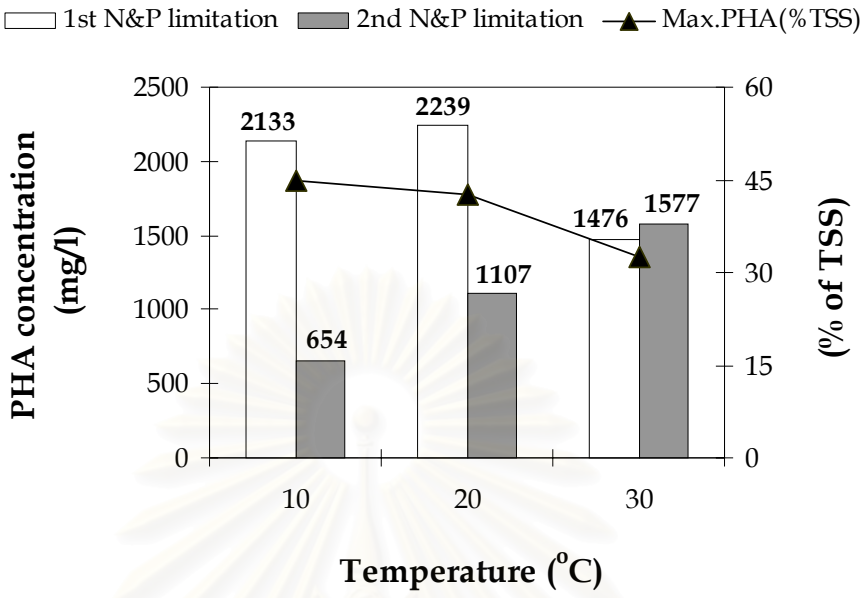


Figure 4.28: The maximum PHA concentrations and PHA contents with N&P limitation.

The PHA productivities and yields clearly decreased as temperature increased, i.e., declining from 427 to 204 and 148 mg/l-d (Figure 4.29) and from 0.38 to 0.16 and 0.11 mg PHA/mg COD_u (Figure 4.30) at the temperature of 10°C, 20°C and 30°C, respectively. The PHA productivities in this study were significantly less than those reported in several papers using pure cultures. This is because the concentrations of activated sludge biomass and substrate used in these experiments were very much lower. However, the above PHA yields were quite promising, especially at 10°C (0.38 mg PHA/mg COD_u) when compared to the theoretical yield of 0.48 mg PHA/mg acetic acid or equivalent to 0.45 mg PHA/mg COD_u (1 mg of acetic acid is equal to 1.07 mg of COD), calculated by Yamane (1993), or the yields obtained using pure cultures, e.g., a yield of 0.46 g PHB/g glucose with *Alcaligenes eutrophus* DSM 545 (Marchessault and Sheppard, 1997) and a yield of 0.36 g PHB/g glucose with *Ralstonia eutropha* (Du et al., 2001). The 10°C PHA yield obtained during this research using N&P limitation (0.38 mg PHA/mg COD_u) was higher than the yields obtained at 10°C yields with nitrogen limitation only (0.22 mg PHA/mg COD_u) and with phosphorus limitation only (0.05 mg PHA/mg COD_u).

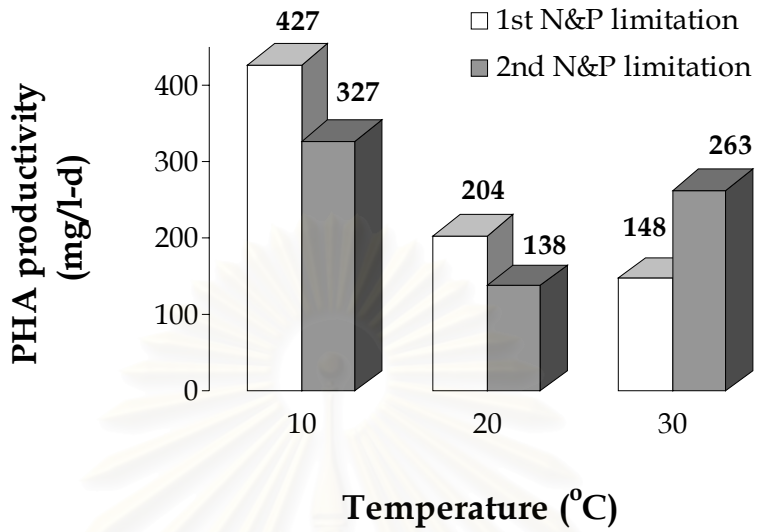


Figure 4.29: The corresponding PHA productivity with N&P limitation.

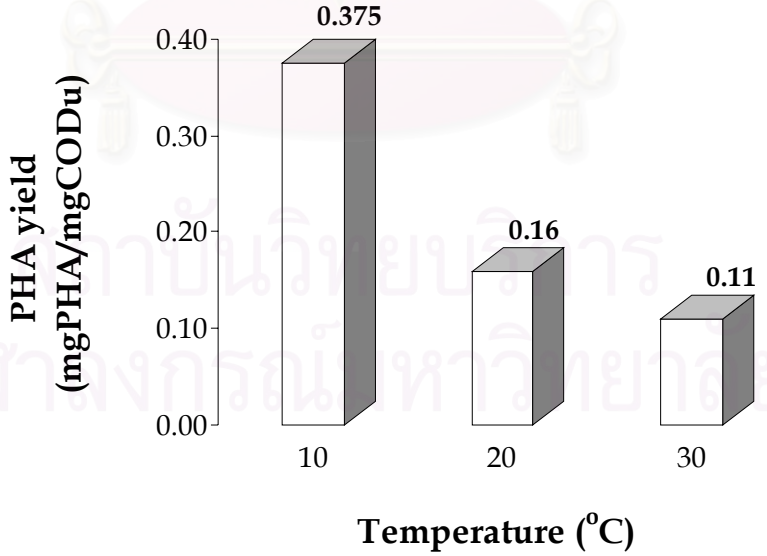


Figure 4.30: The yield of PHA on substrate utilized with N&P limitation.
(Note: for the first limitation scenario only.)

4.3.2 Biomass concentration and sludge quality

Figure 4.31 shows that the 10°C MLSS concentration before N&P limitation was 3647 mg/l and it gradually increased to the peak of 5300 mg/l after limitation was started. The 10°C MLSS concentration was maintained in the range of 4500 – 5300 mg/l for about a month after the addition of N&P stopped, primarily because sludge settleability was good for most of that period, as shown by SVI values of between 60-80 ml/g MLSS. Then, biomass settleability worsened to SVI values in excess of 150 ml/g MLSS as the lack of N&P began to affect metabolism. The MLSS barely increased during the second limitation period, and the increase did not occur until the last two observations.

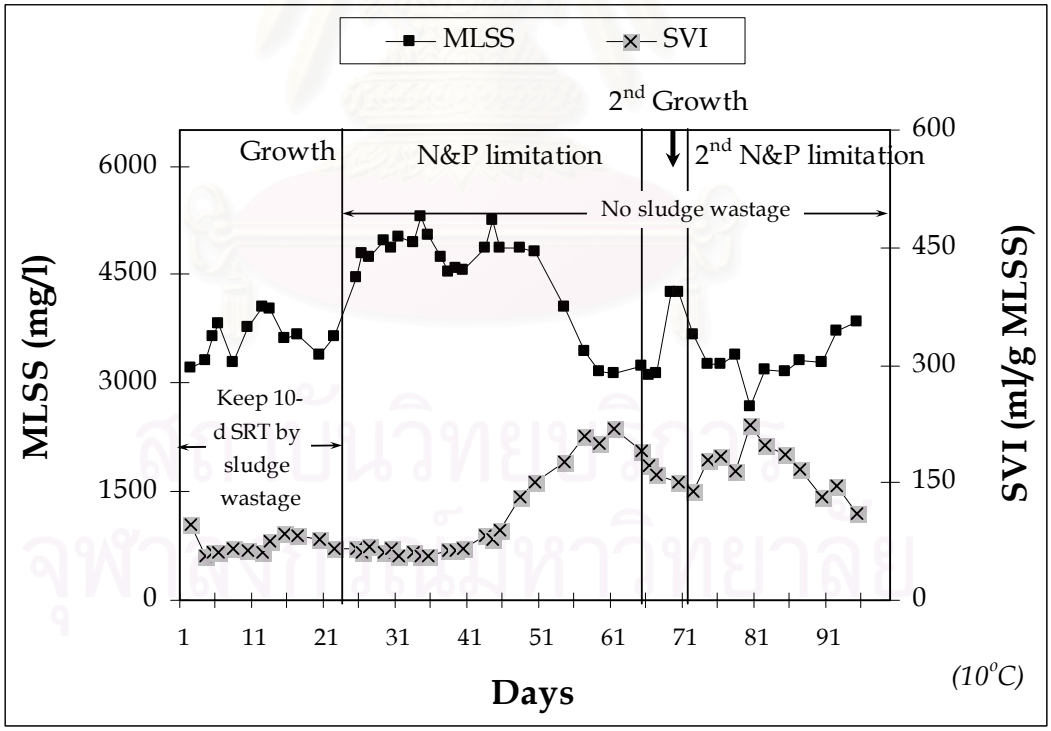


Figure 4.31: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 10°C experiment with N&P limitation.

The MLSS in the 20°C system increased from 4151 to 6302 mg/l after N&P addition was ceased, but then decreased sharply to 4286 mg/l before nutrients addition was resumed. Additional MLSS decrease occurred during the period of nutrients addition, but considerable growth occurred during most of the second limitation period (Figure 4.32). Apparently the 32-day long first period of nutrients limitation at 20°C did not cause a lag in growth similar to that observed in the 10°C system after a 42-day period of nutrients limitation.

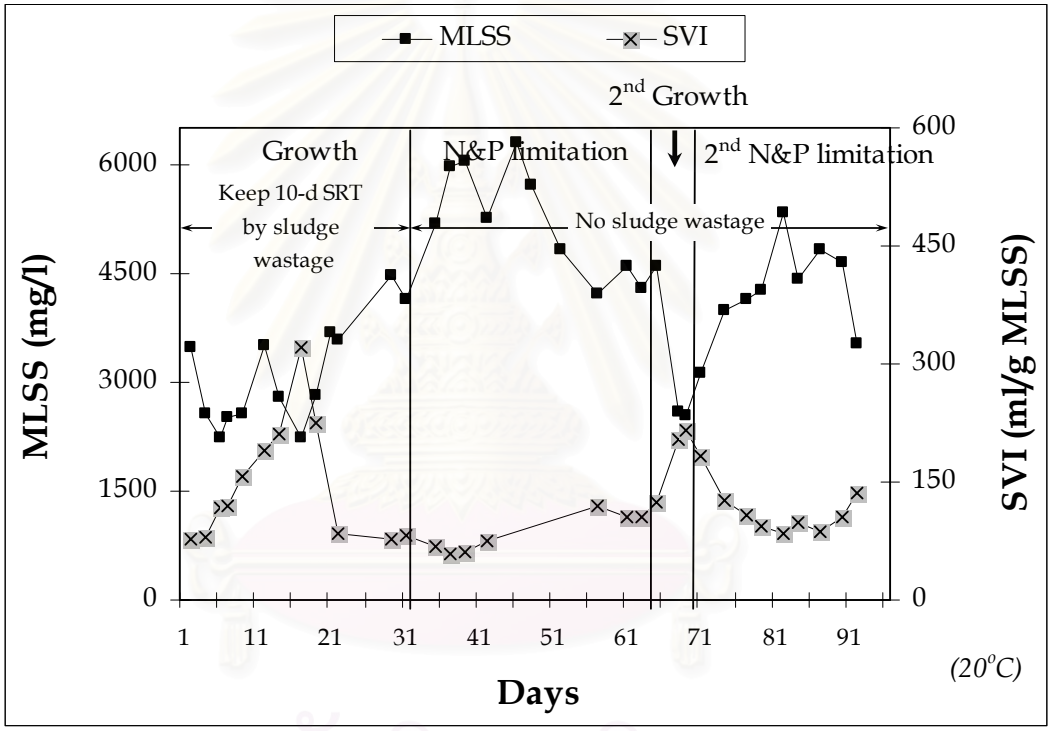


Figure 4.32: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 20°C experiment with N&P limitation.

Figure 4.33 shows changes in the MLSS concentration during the 30°C experiment. The concentration increased from 3484 mg/l at the end of the growth period to a maximum of 5327 mg/l during the PHA accumulation phase before it began decreasing. Subsequently, the SVI increased to nearly 300 ml/g MLSS because a sludge bulking problem occurred, resulting in decreases in the MLSS because of losses in during supernatant wasting. Similarly to the 20°C system, the 30°C MLSS increased almost immediately during the second N&P limitation period, which was

less than a month long. The SVI values indicate that sludge bulking problems are likely to occur during N&P limitation periods, and this will reduce the MLSS concentration, and therefore the PHA concentration.

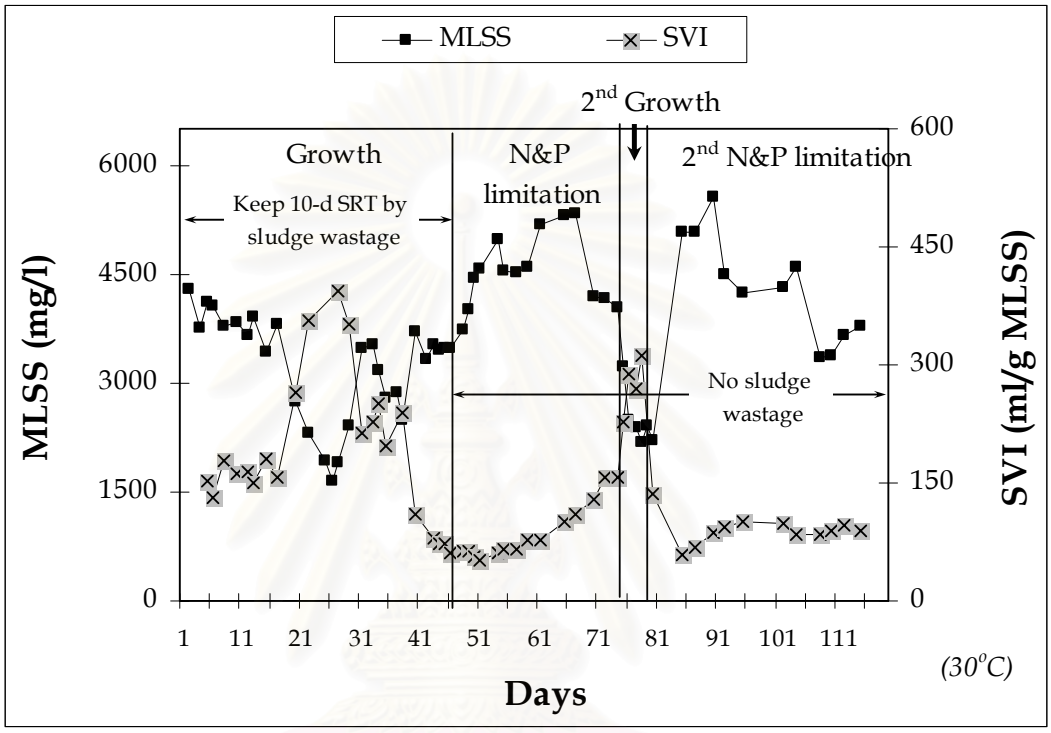


Figure 4.33: Profiles of MLSS (mg/l) and SVI (ml/g MLSS) for the 30°C experiment with N&P limitation.

4.3.3 The residual biomass

Changes in the residual biomass values are illustrated in Figures 4.25 – 4.27. The residual biomass denotes cell growth during PHA accumulation. The biomass growth at 10°C seemed to cease as soon as the nutrients were eliminated from the feed, while at 20 and 30°C there was a little increase in growth before the maximum PHA content was reached. It appears that the 10°C biomass utilized more of the organic carbon for PHA accumulation than for cell growth, while the reverse occurred at the higher temperatures of 20 and 30°C. Consequently, PHA accumulation was slower at the higher temperatures than at 10°C.

4.3.4 Substrate utilization

Figures 4.34 to 4.36 show the time courses of COD utilization (substrate utilization) and PHA content during the experiments. The results indicate that there is a relationship between these two parameters during N&P limitation conditions, and a similar relationship was observed when only nitrogen was limited (section 4.1). The data plots of the experiments at 10, 20 and 30°C show that COD utilization decreased rapidly while the PHA fraction increased to peak values. During the second N&P limitations periods, the decrease of COD utilization and increase of PHA content at 10°C and 20°C occurred concurrently, while COD utilization in the 30°C system was maintained at a surprisingly high level. Regarding the residual biomass curve of 30°C system in Figures 4.27, which shows there was still cell growth, it was observed that the 30°C biomass utilized COD for both PHA storage and cell growth, while the biomass maintained at the two lower temperatures utilized COD mostly for PHA accumulation.

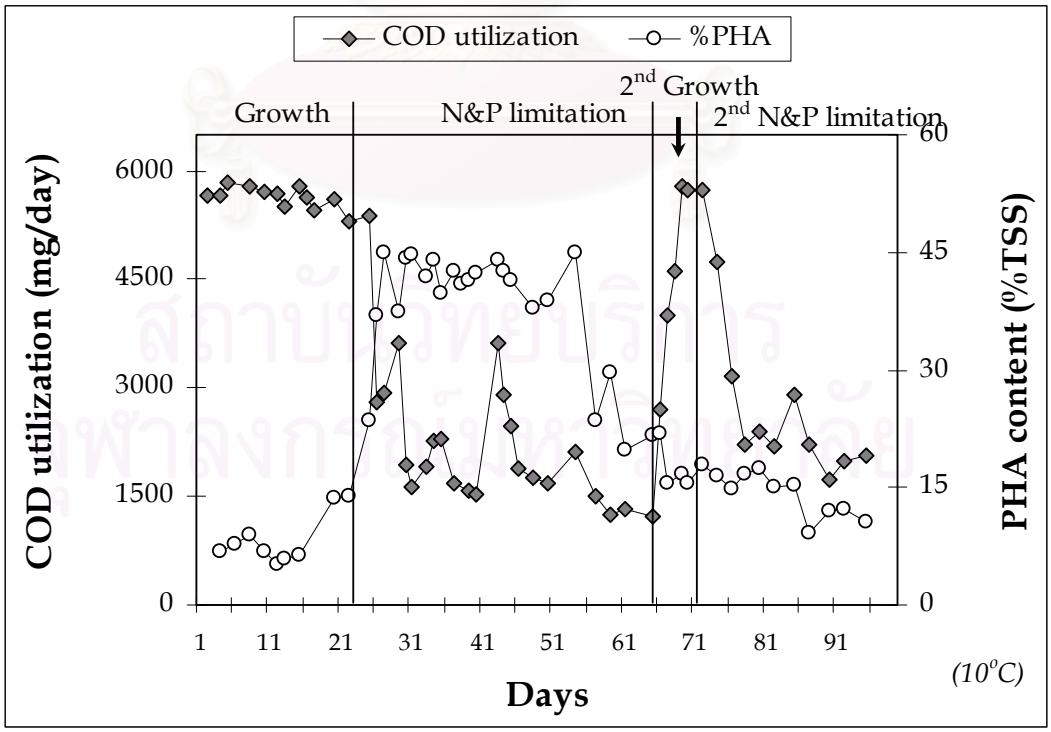


Figure 4.34: Profiles of COD utilization and PHA content (% of TSS) for the 10°C experiment with N&P limitation.

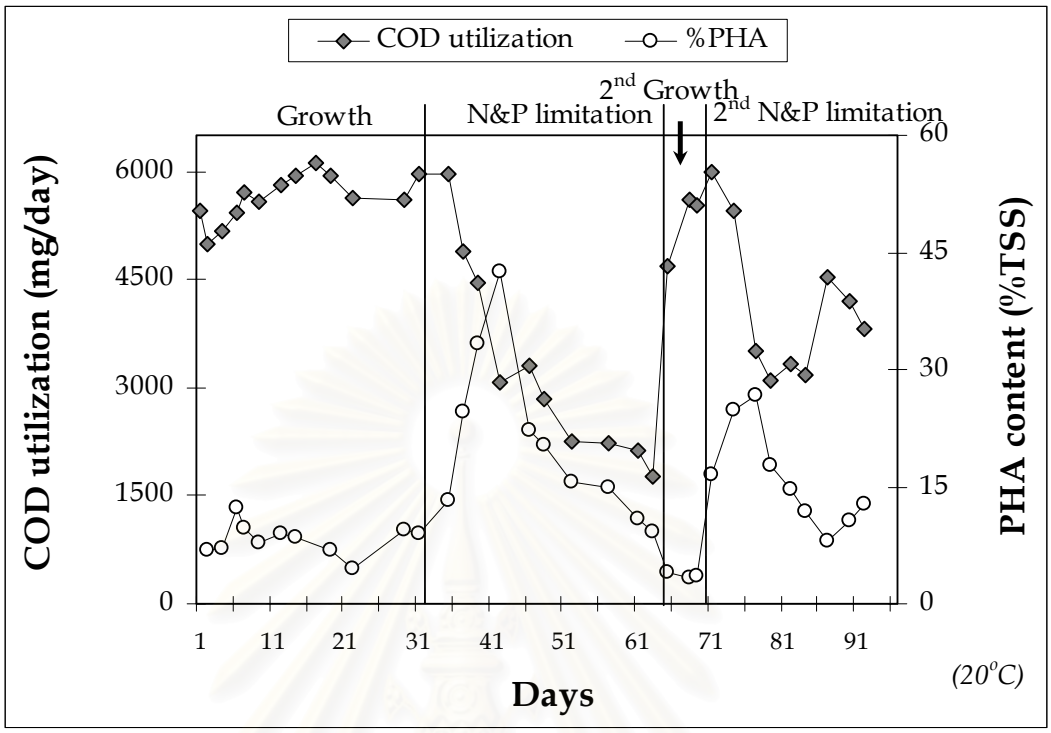


Figure 4.35: Profiles of COD utilization and PHA content (% of TSS) for the 20°C experiment with N&P limitation.

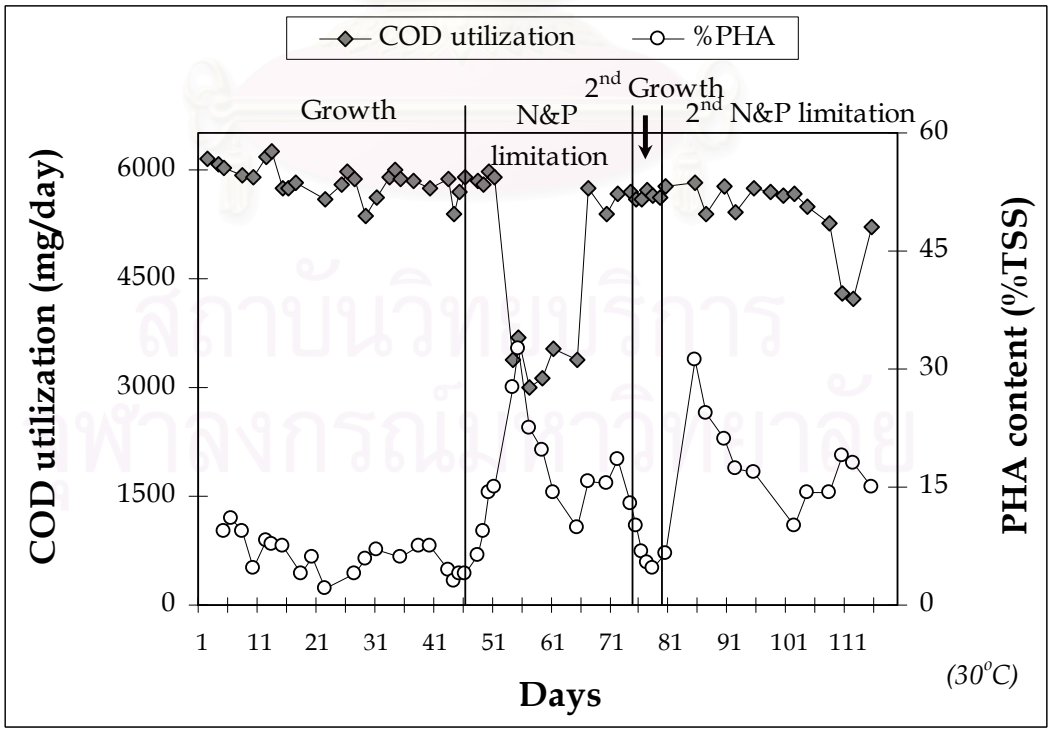


Figure 4.36: Profiles of COD utilization and PHA content (% of TSS) for the 30°C experiment with N&P limitation.

4.3.5 Conclusions

It is evident from the N&P limitation experimental data that PHA accumulation was better in the 10°C system compared to the two systems operated at the higher temperatures of 20 and 30°C. Although the 20°C system produced the highest MLSS concentration during the PHA accumulation, the 10°C biomass stored a higher fraction of PHA content with less production time, resulting in the highest PHA yield (mg PHA/mg COD_u) in the 10°C system. It was also observed that the PHA contents during the second N&P limitation reached the period maximum value in shorter times than required during the first limitation period, but greater productions were obtained during the first limitation periods. In addition, it was observed that sludge bulking was likely to happen faster in the 30°C system, and this resulted in lower biomass concentrations and, consequently, lower PHA production in this system compared to the other two. Based on the results of these experiments, it is recommended that low temperature, such as 10°C, be used for PHA production and harvesting for biodegradable plastics production.

4.4 Comparison of PHA productions using acetate-propionate synthetic wastewater

There are several factors affecting the economical production of the biodegradable polymer. These include PHA yield, PHA productivity, PHA content, PHA concentration, the price of substrate and the method of recovery process. The yield of PHA, defined as the amount of PHA produced by the amount of carbon substrate consumed, shows the efficiency of substrate conversion into PHA material. As the cost of carbon substrate has a significant effect on the overall production cost of PHA (Yamane, 1992; Van Wegen et al., 1998), this yield should be as high as possible so the substrate would not be wasted to non-PHA cell materials (Lee, 1996a).

The productivity is defined as the amount of PHA produced by unit volume in unit time. Choi and Lee (1999) reported that PHA productivity affects equipment-related costs, that is, for the same amount of PHA produced, the larger equipment would be required for the lower productivity. The highest productivity reported to date was 4.94 g P(3HB)/l-h, obtained by *Alcaligenes latus* (Wang and Lee, 1997). The lowest production cost of US$ 2.6/kg P(3HB) was evaluated by Lee and Choi (1998) due to this high productivity.

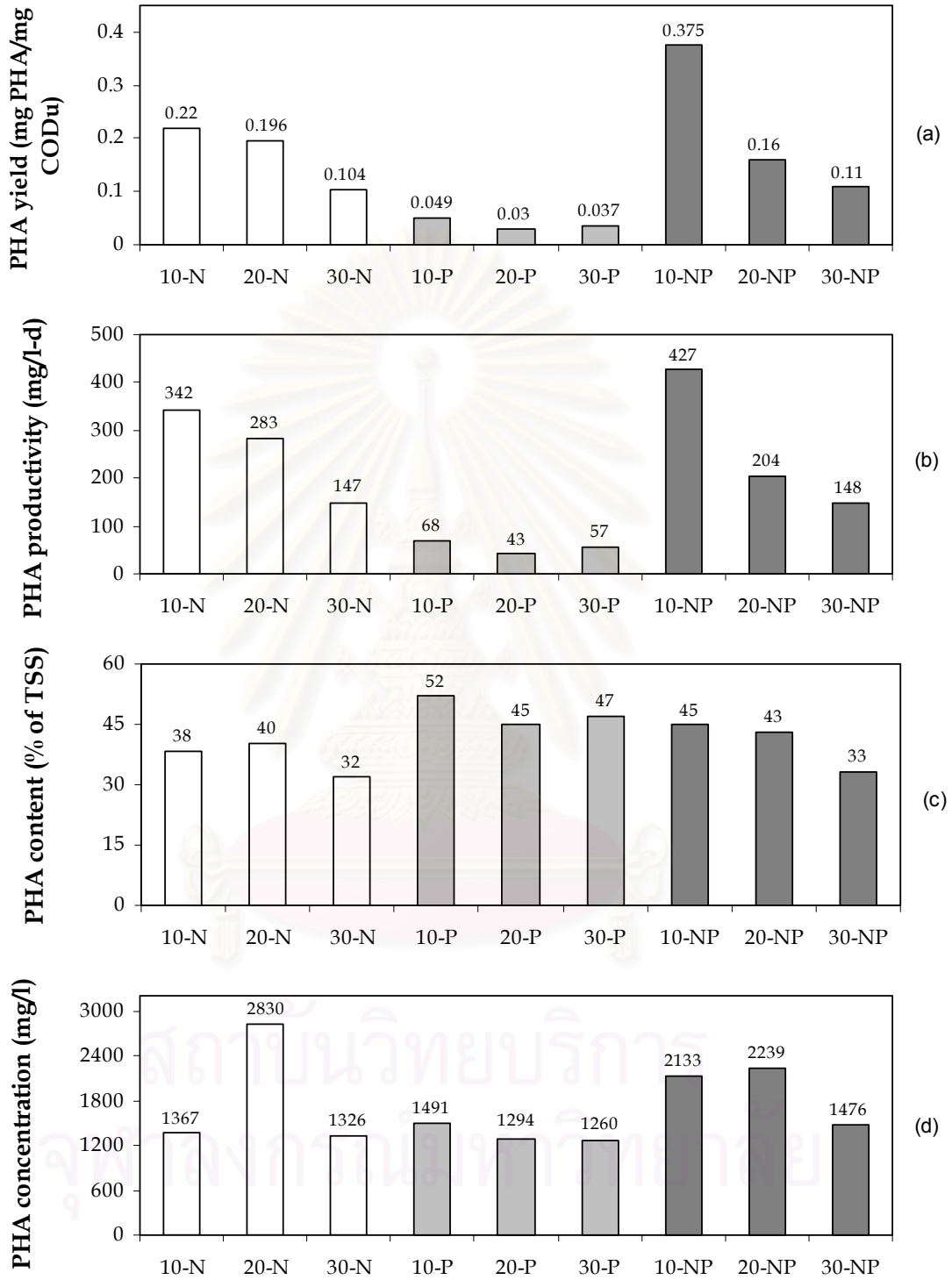
The PHA content is an indication of cell's ability to accumulate PHA in given condition. Choi and Lee (1999) mentioned that PHA content affected the PHA yield and the efficiency of recovery process. When the PHA content is high, low amount of non-PHA cell materials required to be separated, resulting in the reduction of recovery cost. Also a low PHA content indicates that some of carbon substrate would be wasted on non-PHA materials and/or other metabolites. Van Wegen et al. (1998) stated that variations in PHA content of $\pm$10% could contribute about US$1.6 difference per kilogram of PHA.

The PHA concentration illustrates the amount of PHA produced by unit volume, which actually depends on cell (or biomass) concentration. Kim et al. (1994a)

reported that they could increase the final PHA concentration from 92 to 121 g/l by the prolongation of cultivation time till cell concentration increased from 55 to 70 g/l before nitrogen was limited.

The production of PHA from nine experiments described in section 4.1-4.3 is summarized in Figure 4.37. In case of PHA yield, the best result of 0.38 mg PHA/mg COD_u (Figure 4.37a) was obtained in the experiment at 10°C with N&P limitation. In comparison with the theoretical yield of 0.45 mg PHA/mg COD calculated by Yamane (1993) or the observed yield of 0.42 mg PHA/mg sucrose (equivalent to 0.37 mg PHA/mg COD) reported by Wang and Lee (1997), PHA yield per COD utilized during this experiment (10°C with N&P limitation) was quite promising. In the case of P limitation, PHA yields ranged from 0.03 to 0.05 mg PHA/mg COD, which are very much lower due to longer time of PHA accumulation is required. Therefore, P limitation is not recommended for PHA accumulation with activated sludge biomass. However, not only nutrient limitation, but also temperature affects PHA yield, i.e., high temperature results in low PHA yield. Hence, though N&P limitation seems to stimulate PHA production better than other nutrient limitations, PHA production with N&P limitation at high temperature is still not recommended.

The PHA productivity shown in this study (Figure 4.37b) was significantly lower than those reported by Wang and Lee (1997) and Choi and Lee (1999) whose studies were based on using pure culture, because of a lower biomass concentration in this study. In commercial production or some studies with pure culture, biomass concentration used for cultivation was substantially high, e.g., the final biomass concentration of 164 g/l was obtained during the study by Kim et al. (1994a) and 143 g/l by Yamane et al. (1996), whereas the biomass concentration in an activated sludge system operated in these experiments was always lower than 5000 mg/l during growth phase and never exceeded 8000 mg/l. Also, concentration of carbon substrate fed in this first-part study was only about 660 mg/l COD while Kim et al. (1994a, 1994b) and Yamane et al. (1996) maintained the concentration of glucose in their culture broth at 10-20 g/l. However, among nine experiments here, the experiment at 10°C with N&P



Note: 10-N (at 10°C with N limitation), 20-N (at 20°C with N limitation), 30-N (at 30°C with N limitation), 10-P (at 10°C with P limitation), 20-P (at 20°C with P limitation), 30-P (at 30°C with P limitation), 10-NP (at 10°C with N&P limitation), 20-NP (at 20°C with N&P limitation), 30-NP (at 30°C with N&P limitation).

Figure 4.37: Comparison of PHA production between nine experiments with synthetic wastewater

limitation provided the best PHA productivity of 427 mg/l-d. Punrattanasin (2001) also obtained the similar PHA productivity of 497 mg/l-d. While the low PHA productivities obtained from the experiments with P limitation confirmed that P limitation was not suitable PHA accumulation process with activated sludge. Similar to PHA yield, temperature also affects the PHA concentration, resulting in lower PHA concentration at higher operating temperature.

Figure 4.37c illustrated that the PHA contents obtained from the experiment with P limitation were somewhat higher than others, especially, from the experiment at 10^{o}C, 52 % of TSS. This PHA content was quite similar to the value of 53 % of TSS obtained from activated sludge biomass with P limitation by Punrattanasin (2001). However, these PHA contents were very much lower in comparison with PHA content of 80% of cell dry weight obtained by pure culture, *Alcaligenes eutrophus*, with P limitation in Ryu et al. (1997). In addition, the experiments with N&P limitation had a little lower PHA contents (the highest content was 45 % of TSS in the experiment at 10^{o}C) than with P limitation. On the other hand, in this study effects due to different nutrient limitations and temperature were not so distinct, when compared to the first two scenarios.

However, as PHA content in the experiment with P limitation obtained when their biomass concentrations were declining, their PHA concentrations, i.e., 1491, 1294 and 1260 mg PHA/l at 10, 20 and 30^{o}C, respectively, were somewhat lower than those in experiments with N&P limitation, i.e., 2133, 2239 and 1476 mg PHA/l (Figure 4.37d). The best PHA concentration of 2830 mg PHA/l was obtained from the experiment at 20^{o}C with nitrogen limitation due to its high biomass concentration of 7548 mg/l. Regarding this PHA content parameter, the setup with N&P limitation and low temperature was still the best among the others.

Choi and Lee (1999) concluded that PHA content was the most important factor affecting the performance and economics of PHA production because of its effects on PHA yield and recovery efficiency. However, the results gained from this study were

a little different; for example, the experiments with P limitation had higher PHA contents, but very lower PHA yields and productivities than others. The cause of this occurrence was the biomass required much longer time to obtain high PHA content while prolonged cultivation with P limitation caused instability of biomass and consequently the loss of biomass with supernatant withdrawal. Hence, in this study, we have to conclude that P limitation was not suitable for PHA production using activated sludge biomass though they produce higher PHA contents. Consider the experiments at 30^{o}C; PHA productions with combined N&P limitation or only N limitation were much alike. This will be an advantage for future study because finding the ideal real wastewater (high carbon with low nitrogen and phosphorus) for using in PHA production would be more flexible.

4.5 PHA production at 20°C with candy (glucose & sucrose) wastewater

4.5.1 The experiment 20C-1x

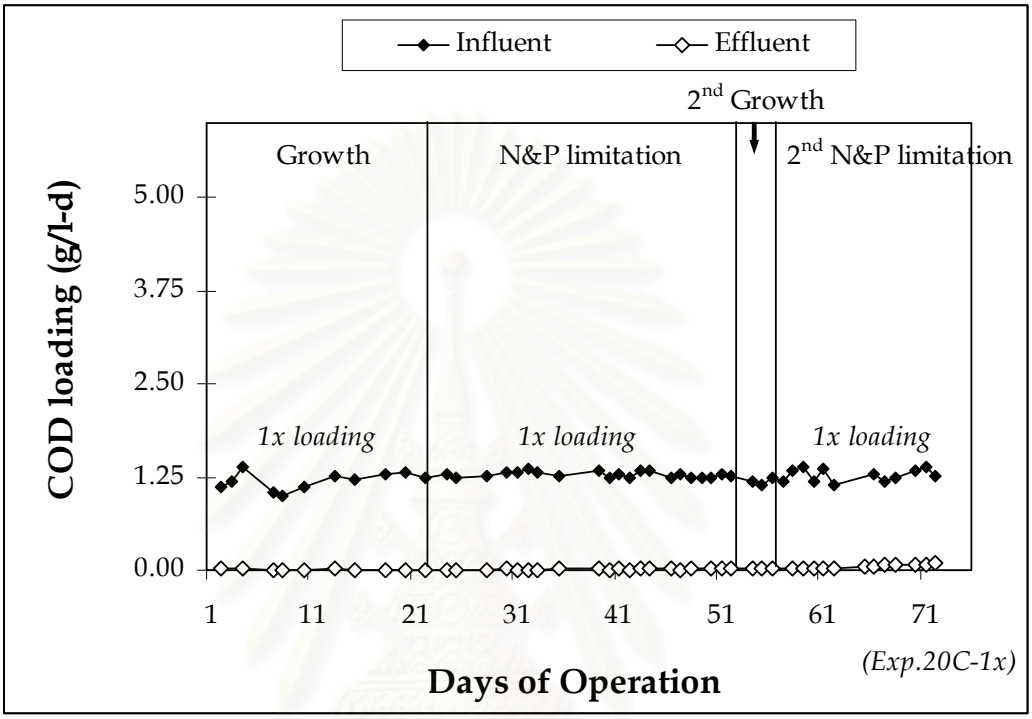


Figure 4.38: Profiles of influent and effluent COD loading for the experiment 20C-1x.

This experiment was operated at 20°C and the COD loading was kept constant at 1.25 g/l-d throughout the experiment (Figure 4.38). It is from now on referred to as the experiment 20C-1x. The growth phase was operated for 22 days before starting the nutrients limitation phase. Figure 4.39 shows that the biomass concentration was quite constant during the growth phase because the sludge retention time (SRT) was kept at 20 days. The biomass concentration was 8780 mg/l on the day before PHA accumulation phase was started, and gradually increased to about 15000 mg/l on day 41 of operation because of no sludge wasting, and then maintained at this level till the second growth phase was started. After the second nutrients limitation phase was introduced, the biomass concentration drastically decreased because of its poor settleability as shown by the V30 values in Figure 4.40. Especially, during the second limitation, the biomass lost its ability to settle entirely as seen by the V30 values, which reached 1000 ml/1000 ml.

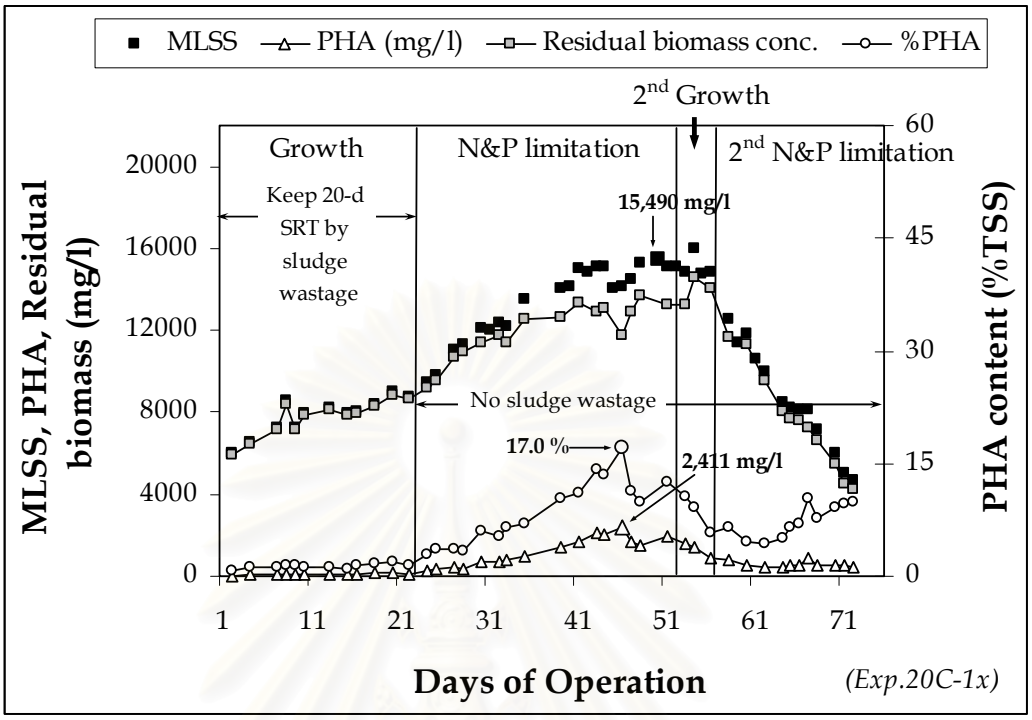


Figure 4.39: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment 20C-1x.

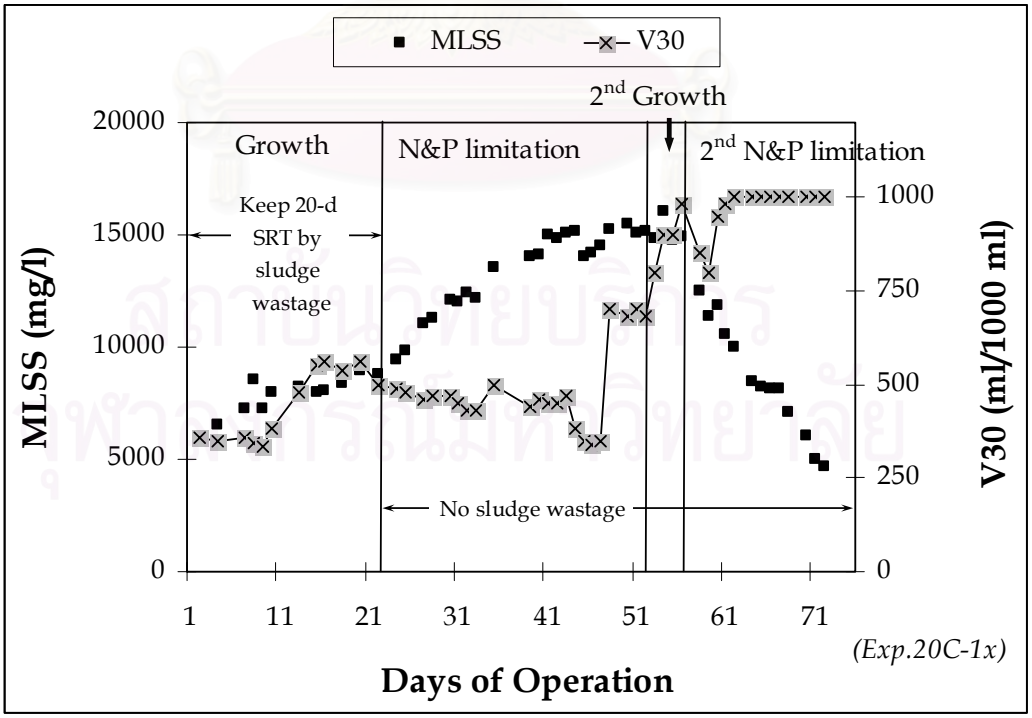


Figure 4.40: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment 20C-1x.

The PHA content slowly increased from 1.4 % of TSS on the last day of the growth phase to the maximum value of 17.0 % of TSS on day 46 of operation (24 days after nutrients were limited). The second growth and nutrients limitation phases were started on days 53 and 57 of operation, respectively. The maximum PHA content of this second limitation phase could reach only 10.4 % of TSS at 11 days after the limitation started. The PHA concentration increased with the same pattern as PHA content course. The maximum PHA concentration of 2411 mg/l was also obtained on the day 46 of operation (same day as maximum PHA content). The PHA concentration decreased gradually after the second limitation started because of the decrease in biomass concentration as mentioned above.

Lee (1996a) mentioned that during the nutrient limitation period of fed-batch culture, an increase of biomass concentration was caused by intracellular PHA accumulation, whereas the residual biomass concentration, defined as the biomass concentration minus the PHA concentration and denoted the cell growth, was almost constant. During this first nutrients-limitation condition of this run, the residual biomass concentration gradually increased for some time, then, remained almost constant while the fraction of PHA approached the maximum level. Considering the time course of effluent COD shown in Figure 4.38, it illustrates that high COD utilization existed throughout the run and most of utilized COD was transformed to the cell growth and maintenance channels. During the second nutrients limitation period, the residual biomass concentration sharply decreased because of the loss of biomass with supernatant withdrawal.

4.5.2 The experiment 20C-2x

This experiment was operated at 20°C and with the COD loading of 1.25 g/l-d for only during growth phase. But when the nutrients limitation was applied, the COD loading was increased to 2.5 g/l-d, so the name experiment 20C-2x (Figure 4.41). Biomass in this system was also cultivated during the growth phase for 22 days with SRT maintained at 20 days. Its concentration on the last day of this phase was 8820 mg/l and increased to 19420 mg/l on day 35 of operation (Figure 4.42). Then, the biomass concentration sharply decreased to about 3000 mg/l on the last day of operation as the sludge entirely lost its settleability as shown by V30 increase to 1000 ml/1000 ml (Figure 4.43).

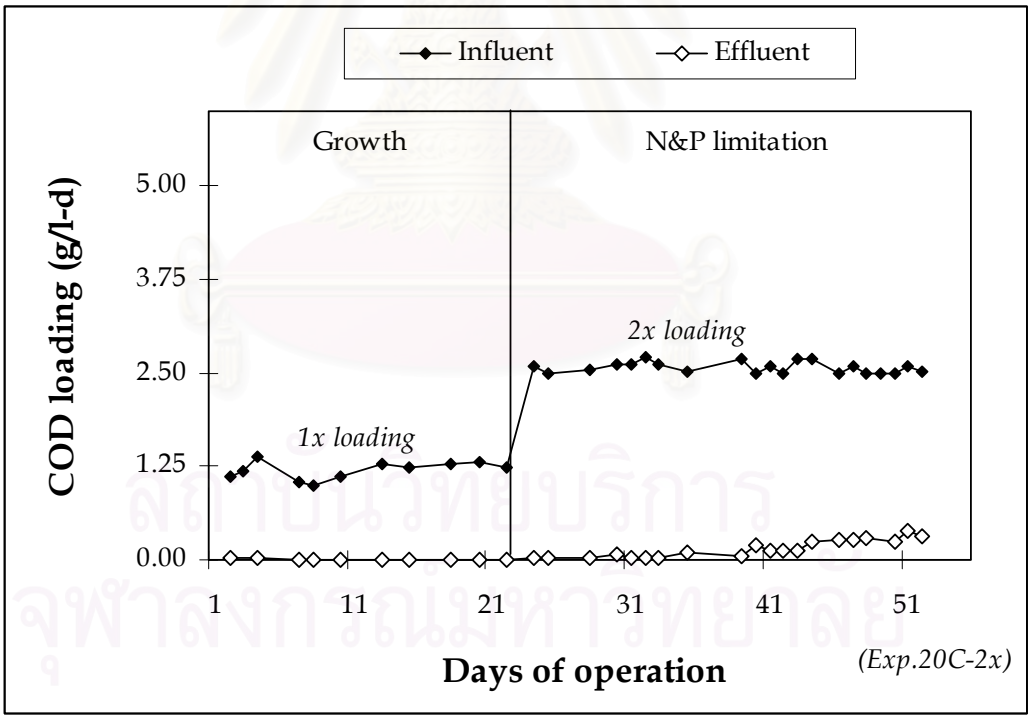


Figure 4.41: Profiles of influent and effluent COD loading for the experiment 20C-2x.

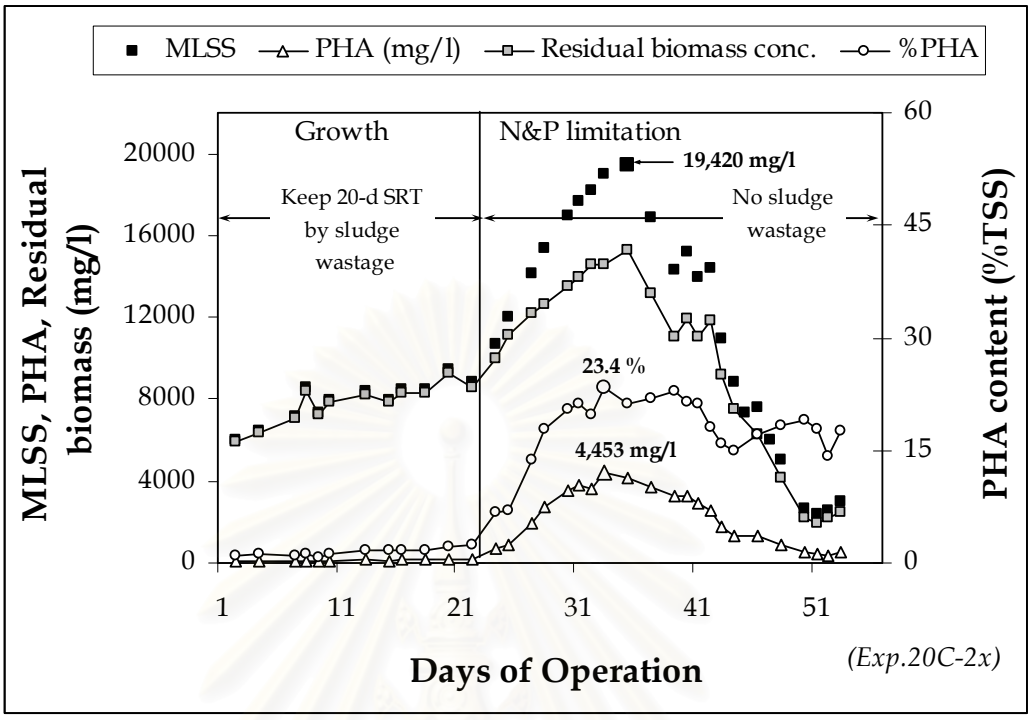


Figure 4.42: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment 20C-2x.

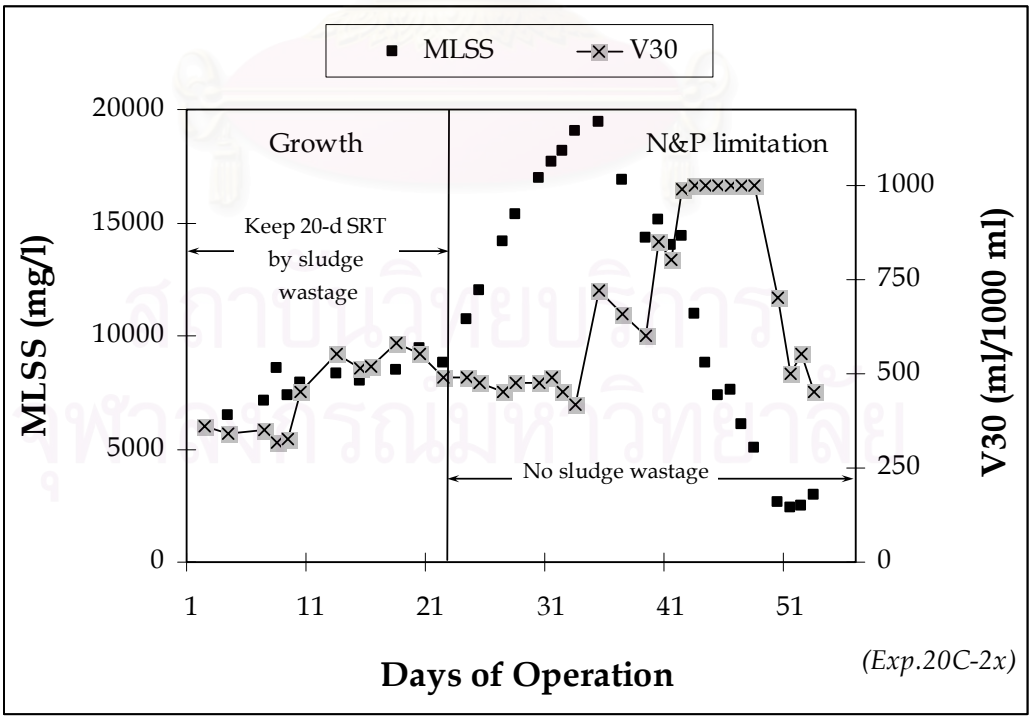


Figure 4.43: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment 20C-2x.

After the nutrients limitation started, the PHA content gradually increased to the maximum value of 23.4% of TSS on the day 33 of operation (11 days after nutrients were limited). This maximum PHA content (23.4 %) was somewhat higher than that from the experiment 20C-1x (17.0 %), this should be due to higher excess carbon during PHA accumulation phase. The maximum PHA concentration of 4453 mg/l occurred on the same day as the maximum content did. This PHA concentration was almost two times higher than that obtained from the experiment 20C-1x. Then they gradually decreased as biomass concentration decreased.

After nutrients limitation condition started, the residual biomass concentration gradually increased to the maximum value on the same day as the maximum biomass concentration of 19420 mg/l obtained. Unlike the experiment 20C-1x, the residual biomass concentration once increased to the maximum value could not remain constant; they started to decrease due to the reduction of biomass concentration. An increase of COD loading fed during nutrients limitation period enhanced the accumulation of PHA as shown by more PHA content and concentration obtained, in comparison with those in the previous experiment. However, the transformation of utilized carbon substrate still proceeded into both channels of cell growth and PHA accumulation.

4.6 PHA production at room temperature with candy (glucose & sucrose) wastewater

4.6.1 The experiment RT-1x

This run was operated at Thailand room temperatures, RT (28-31^{o}C) and with the constant COD loading of 1.25 g/l-d (1x) throughout the experiment (Figure 4.44). It is therefore referred to as the experiment RT-1x from now on. Biomass in this system was also cultivated for 22 days during the growth phase with SRT maintained at 20 days. Its concentration on the last day of this phase was 8480 mg/l and increased to 12140 mg/l on day 39 of operation (Figure 4.45). Then, they decreased a little to 10670 mg/l on the last day of operation. Although there was an increase in V30 values (Figure 4.46), the settled biomass layer was still mostly lower than the supernatant withdrawal level. Therefore, the loss of biomass was not critical.

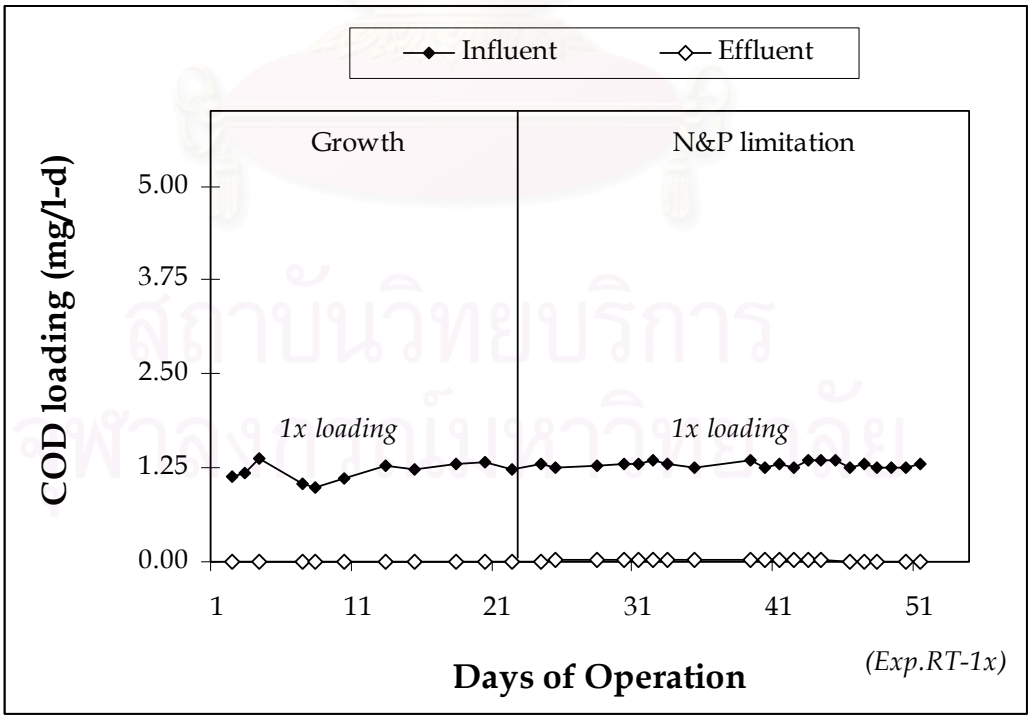


Figure 4.44: Profiles of influent and effluent COD loading for the experiment RT-1x.

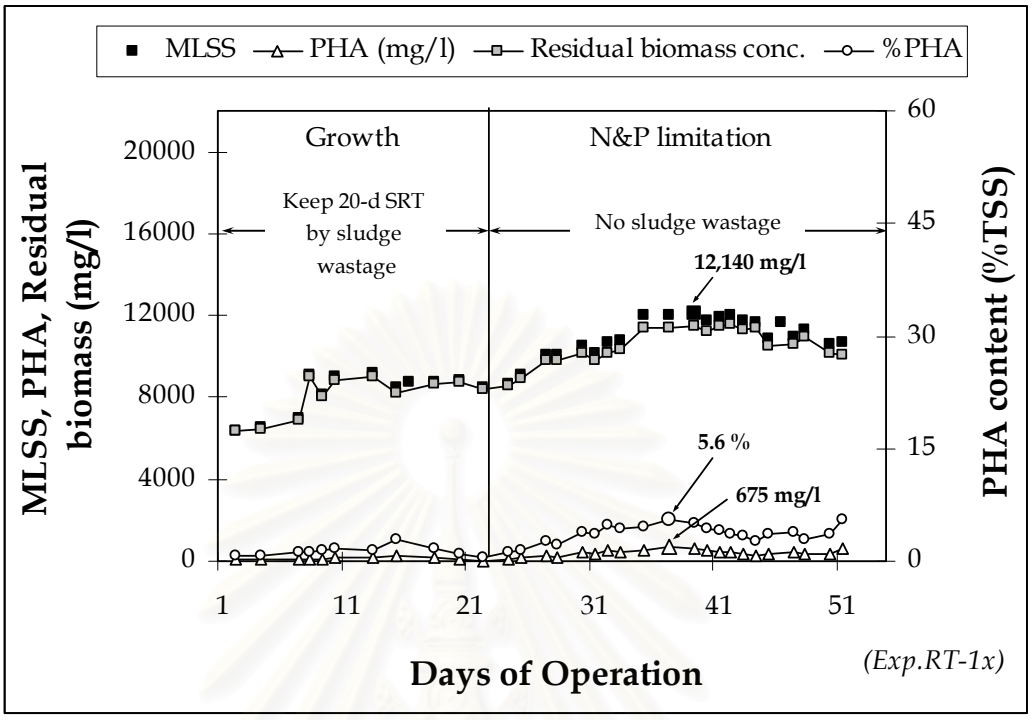


Figure 4.45: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment RT-1x.

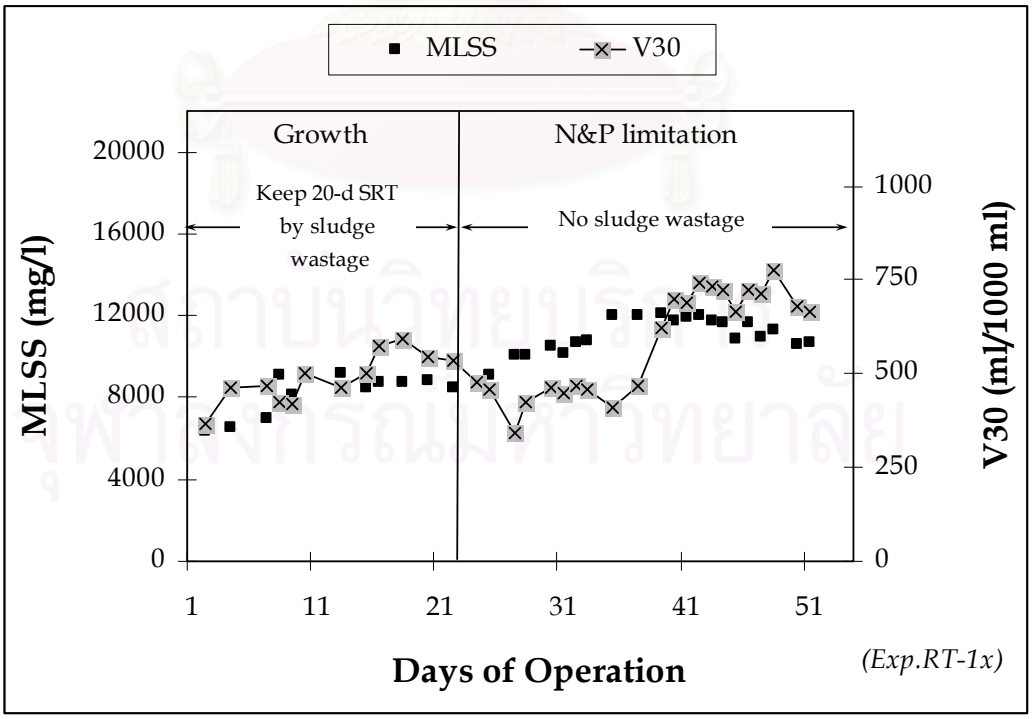


Figure 4.46: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment RT-1x.

There was quite small amount of PHA accumulation during this experiment. The maximum PHA content obtained from this system was only 5.6 % of TSS on day 37 of operation (15 days after nutrients were limited). The maximum PHA concentration was only 675 mg/l on the same day as the maximum PHA content. This amount of PHA accumulation was much lower than those obtained from the experiment 20C-1x, which was operated with the same COD loading, but at 20^{o}C.

From Figure 4.45, the residual biomass concentration gradually increased while there was very low PHA accumulation during nutrients limitation period. This also means that more of utilized carbon substrate was converted for cell growth than to PHA material. When compared between this experiment (RT-1x) and the experiment 20C-1x, the carbon substrate utilization in both runs was almost identical, whereas the PHA production at room temperature was much less. Braunegg et al. (1998) and Kessler and Witholt (2001) mentioned that the synthesis and degradation of PHA occurred simultaneously. On the observation with *Alcaligenes eutrophus*, Doi et al. (1992) has concluded that the intracellular PHA accumulation is concomitantly with its degradation, resulting in cyclic pathway of PHA metabolism. Seemingly, PHA in bacteria cells acted like a buffer of carbon source for cell growth and metabolism. As we have known, bacteria will have higher growth rates and need more energy for cell maintenance and activities at higher temperature, therefore, biomass in the experiment at higher temperature (room temperature ranging from 28 to 31^{o}C) may waste more carbon substrate for their cell growth and metabolism than biomass cultured at lower temperature, 20^{o}C. Also, at this room temperature, biomass might need to degrade their intracellular PHA for more carbon supply, resulting in low total PHA accumulation.

4.6.2 The experiment RT-2x

This experiment was also operated at room temperatures (28-31^{o}C) and fed with the COD loadings of 1.25 g/l-d (1x) during the growth phase, but with 2.5 g/l-d COD loading (2x) during the nutrients limitation phase as shown by a time course of influent COD loading in Figure 4.47. The growth phase was conducted for 23 days with SRT of 20 days. From Figure 4.48, the biomass concentration was quite constant and was 9550 mg/l before the nutrients limitation started. The biomass concentration increased at faster rate than that in the experiment RT-1x and reach 15460 mg/l on day 38 of operation (15 days after the nutrients were limited). The decrease in biomass concentration also occurred in this experiment as the V30 sharply increased till they finally reached 1000 ml/1000 ml (Figure 4.49).

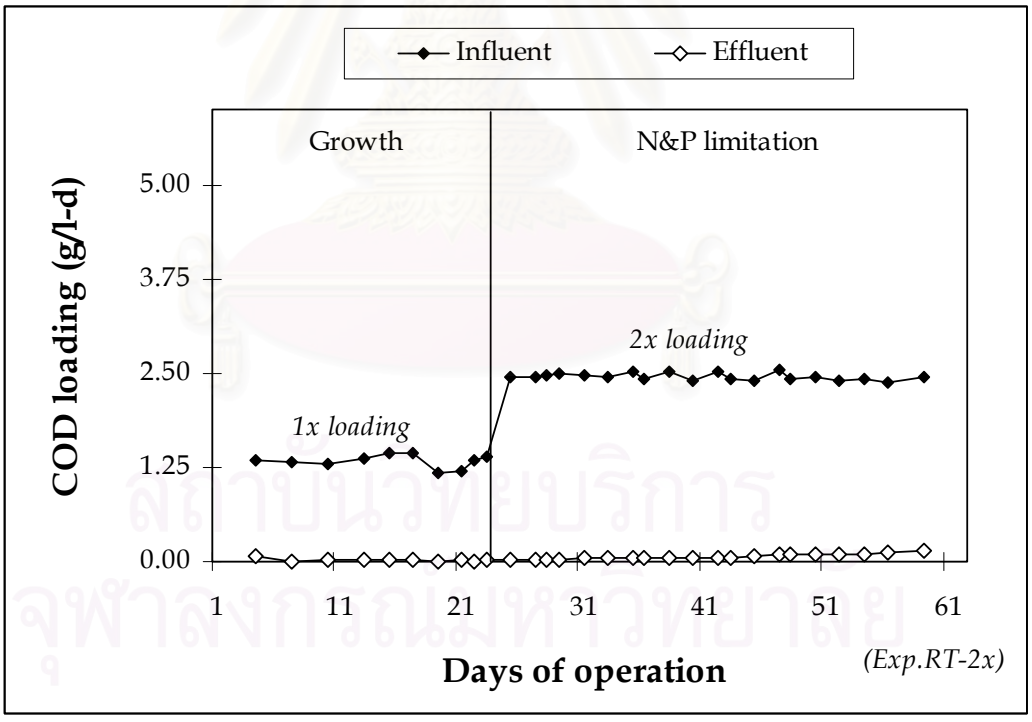


Figure 4.47: Profiles of influent and effluent COD loading for the experiment RT-2x.

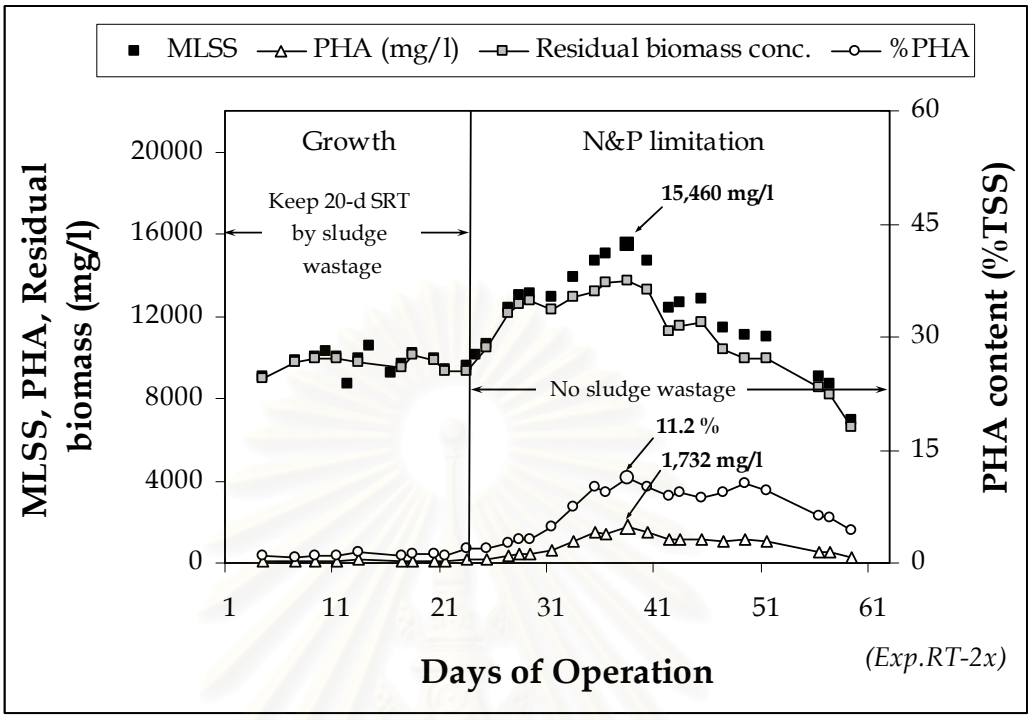


Figure 4.48: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment RT-2x.

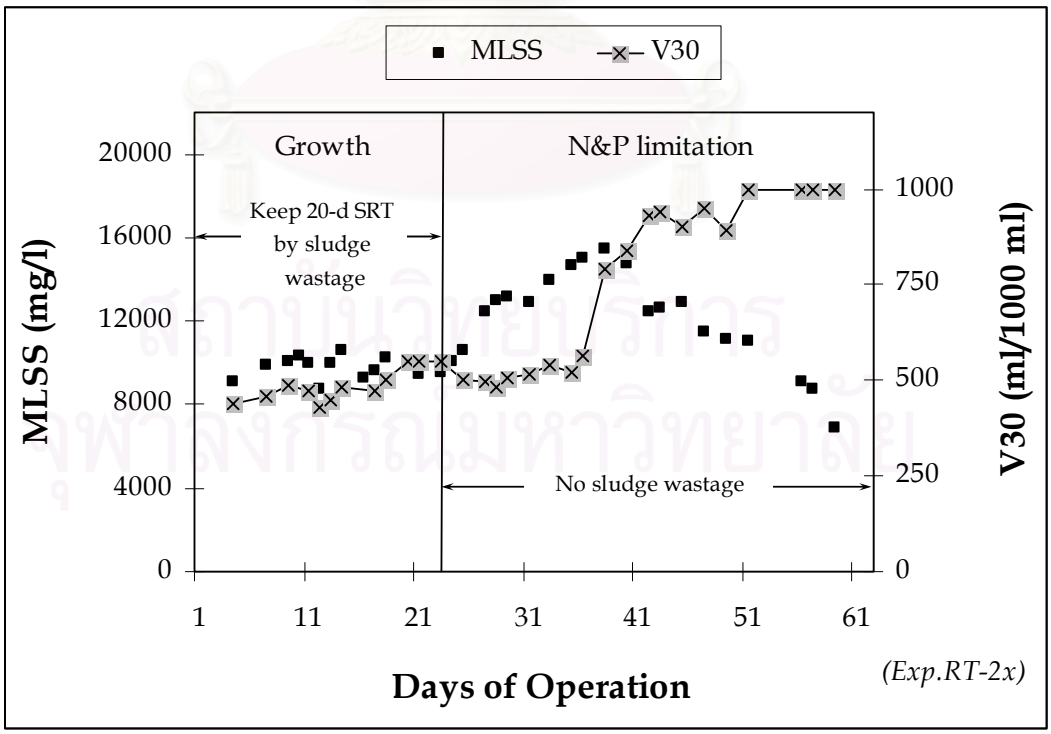


Figure 4.49: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment RT-2x.

The result shows that a 2 folds increase of COD loading during nutrients limitation could enhance PHA production by increasing of both cell concentration and fraction of PHA in biomass cell. The PHA content and concentration gradually increased to the maximum values of 11.2 % of TSS and 1732 mg/l, respectively, on the day 38 of operation. Both of PHA content and concentration in this run were over two times higher than those obtained from the previous run (RT-1x), this could mean that the fed COD loading of 1.25 g/l-d could not provide enough excess carbon for effective culturing at room temperature (28-31^{o}C), resulting in low PHA accumulation. However, in comparison with the experiment 20C-2x that operated with the same pattern of influent COD loading, the PHA accumulated at room temperature was still much lower, clearly indicating again the temperature's adverse effect.

The residual biomass course in Figure 4.48 shows that it gradually increased during the early period of nutrients limitation. This is due to carbon substrate being utilized for cell growth much more than for PHA synthesis. Then, the residual biomass-increasing rate reduced concurrently with an increase of PHA content; thereafter the residual biomass was almost constant as the PHA content reaching its peak. This means that during this later period, biomass converted more carbon substrate into PHA material than for cell growth.

It can be seen that, though more carbon substrate fed during nutrients limitation could stimulate higher PHA accumulation in comparison with the lower carbon input, more of carbon substrate was wasted for cell growth and metabolism than that at lower operating temperature, 20^{o}C. Also, as mentioned in previous section, there might be more intracellular PHA degradation occurred in bacteria cultured at room temperature than at lower temperature, 20^{o}C, resulting in lower PHA accumulation.

4.6.3 The experiment RT-3x

The operating condition of this run was the same as the previous ones; that is the fed COD loading was 1.25 g/l-d (1x) and the SRT was kept at 20 days. But, when nutrients were limited, three times (3x) COD loading, 3.75 g/l-d, was however fed (Figure 4.50) into the system in order to even more enhance excess carbon condition, and consequently as well as hopefully, more PHA production. As seen from Figure 4.51, the biomass concentration increased from 9550 to 18040 mg/l on day 36 of operation (13 days after the nutrients limitation started). Figure 4.52 illustrates that the V30 values quickly increased to 920 ml/1000 ml within only 10 days of nutrients limitation phase and to 1000 ml/1000 ml after the maximum biomass concentration was reached. This caused the loss of biomass with the supernatant withdrawal, thus, the biomass concentration drastically decreased.

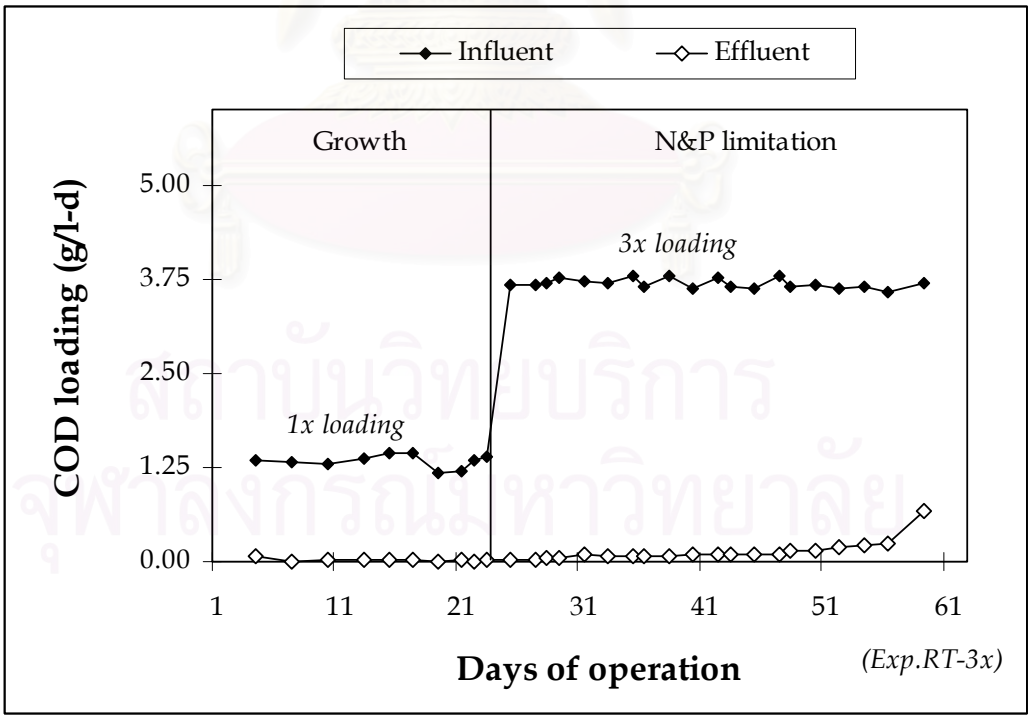


Figure 4.50: Profiles of influent and effluent COD loading for the experiment RT-3x.

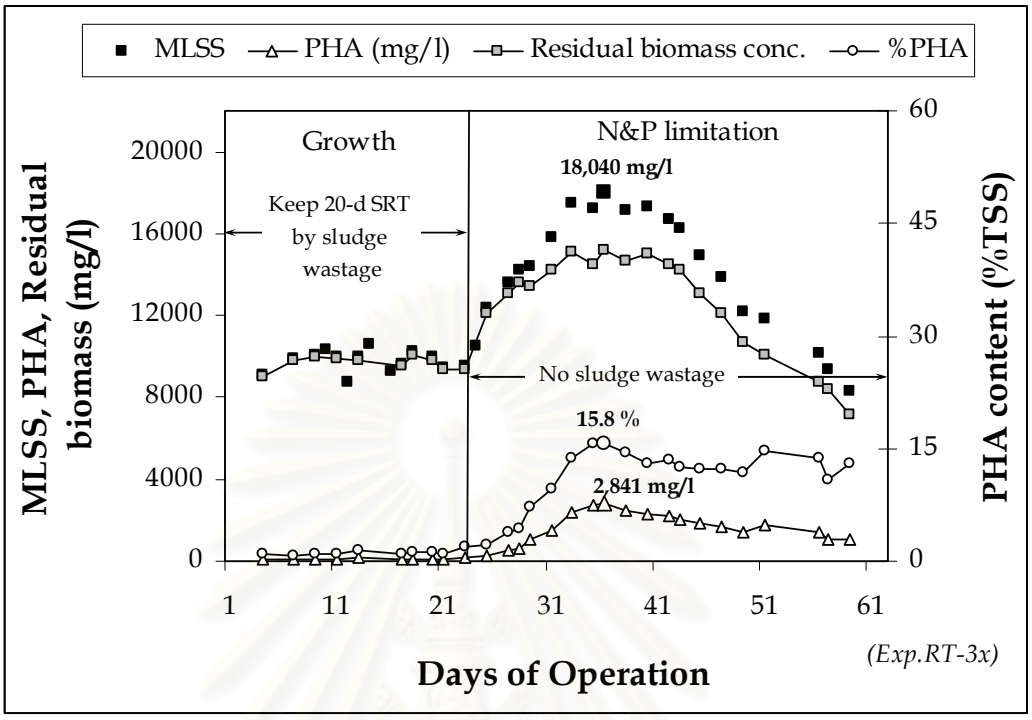


Figure 4.51: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment RT-3x.

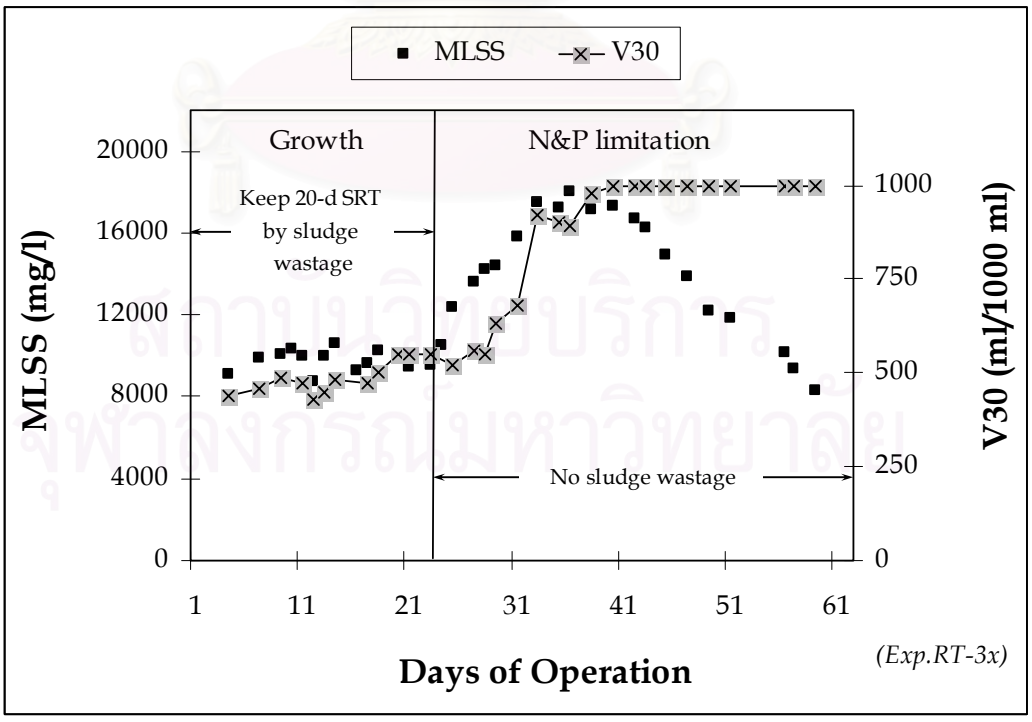


Figure 4.52: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment RT-3x.

The PHA accumulation in this experiment was better than those from the last two experiments, namely, RT-1x and RT-2x. The maximum PHA content of 15.8 % of TSS was obtained on day 36 of operation (or 13 days after nutrients limitation started). Also, the maximum PHA concentration of 2841 mg/l occurred on the same day, and then gradually decreased as the cell concentration drastically declined.

A time course of the residual biomass concentration was similar to the ones in previous experiments. That is, it quickly increased during the early period of nutrients limitation phase then the increase rate was reduced, while accumulated PHA increased and the residual biomass concentration stayed almost constant as the PHA content reaching the maximum level. Subsequently, the residual biomass concentration, however, sharply decreased, as biomass was largely lost from the system.

4.6.4 The experiment RT-4x

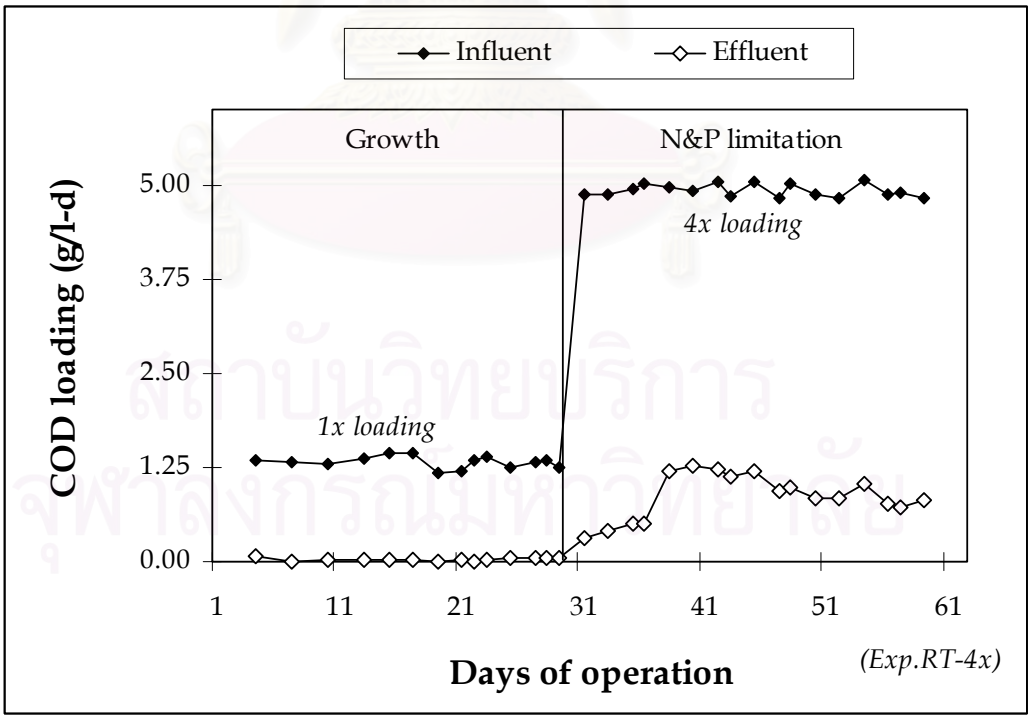


Figure 4.53: Profiles of influent and effluent COD loading for the experiment RT-4x.

Similarly to the experiments RT-2x and RT-3x, the growth phase of this experiment was conducted under the same condition, but it was in this specific case operated for 29 days before the limitation was introduced (23 days for RT-2x and RT-3x). Once nutrients were limited, the feed loading was raised four times (experiment RT-4x) to 5.0 g/l-d (Figure 4.53). The biomass concentration largely increased from 9550 to 21860 mg/l on day 40 of operation (11 days after the nutrients limitation started) as shown in Figure 4.54. With rapid and huge increase of biomass concentration coupled with loss of their settleability, the V30 values kept increasing till they reached 1000 ml/1000 ml (Figure 4.55). Therefore, the biomass drastically decreased ever since.

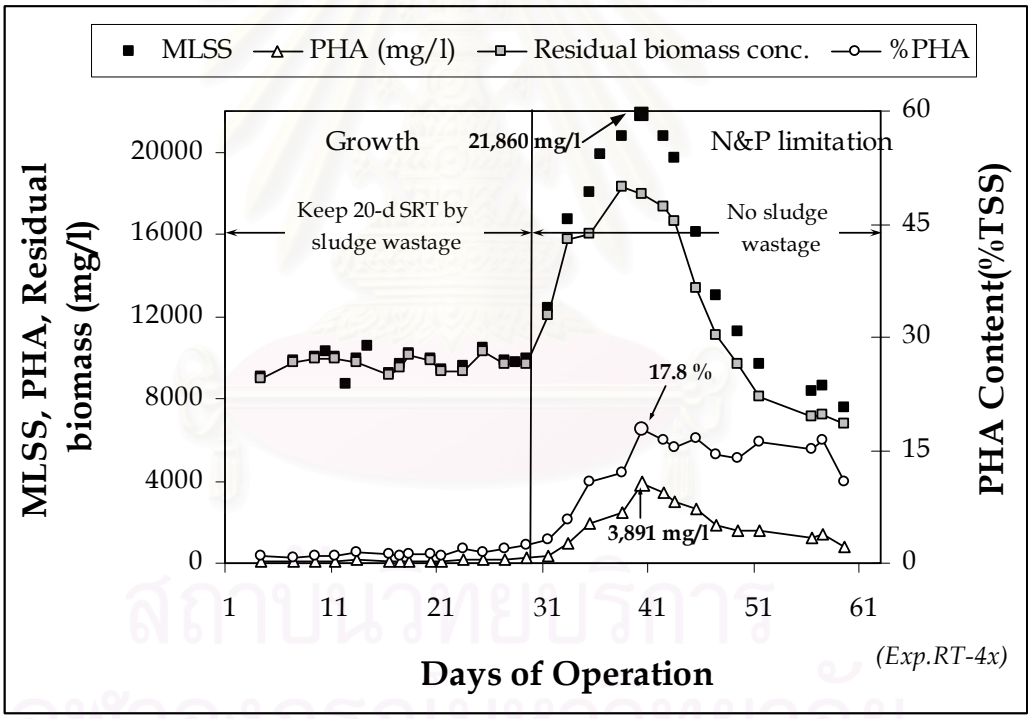


Figure 4.54: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment RT-4x.

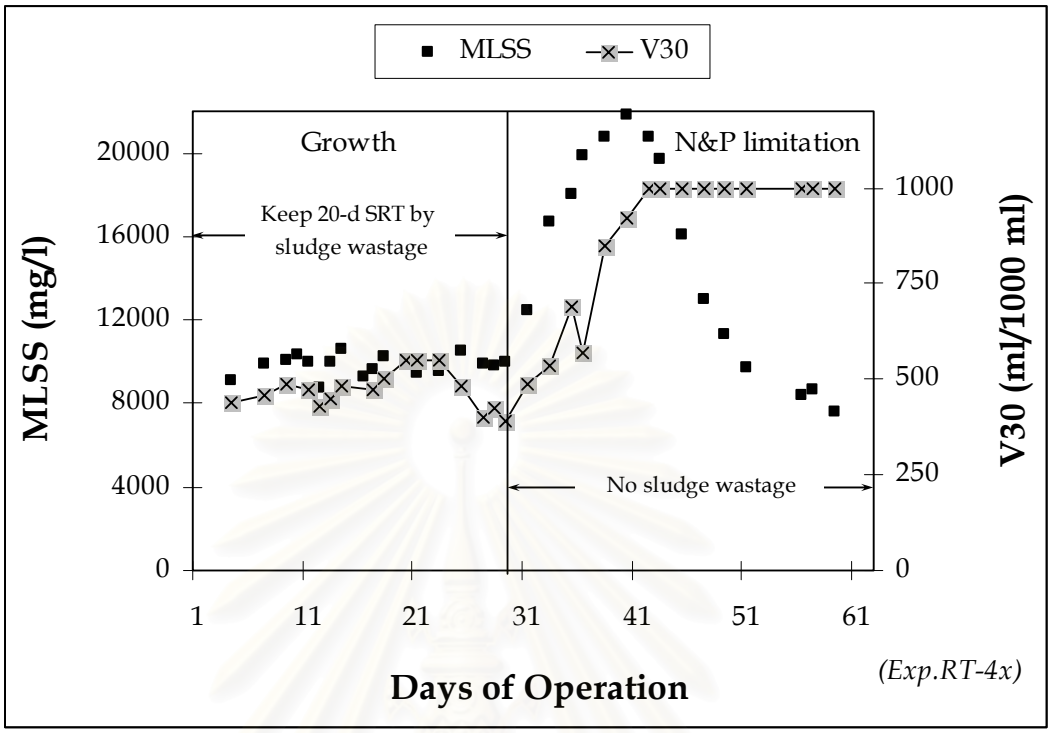


Figure 4.55: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment RT-4x.

The maximum PHA content was 17.8 % of TSS and required only 11 days of nutrients limitation phase. Also, the PHA concentration of 3891 mg/l was obtained on the same day of operation. In comparison with the PHA accumulations in other experiments conducted at the same room temperature, this experiment had higher PHA content and PHA concentration.

The residual biomass concentration sharply increased similarly to cell concentration, but the rate was reduced while the PHA content was rising and reaching its peak. After that, the residual biomass concentration drastically reduced because of the loss of biomass. The 4x COD loading could therefore enhance a little higher PHA content, but most carbon would go to the biomass production.

4.6.5 The experiment RT-6x

The growth phase of this experiment was operated for 23 days and with the 1x COD loading of 1.25 g/l-d. During the nutrients limitation phase, the feed loading was enhanced to 7.5 g/l-d, six times of the one used in growth phase (referred as to the experiment RT-6x). This system showed the sign of failure, such as the effluent was turbid, since the nutrient limitations started. Also, as can be seen from Figure 4.56, the system could not utilize the much excess carbon at this very high organic loading, resulting in increase of effluent COD concentration. In addition, the biomass apparently became whitish and its concentration increased only very little (Figure 4.57) while its V30 kept increasing (Figure 4.58). The combination of those occurrences shows the sign of overloading and failure of the system, therefore this particular system (RT-6x) was operated for only 35 days before it was terminated. It could be noted that though an increase in the carbon substrate could raise the PHA production, the fed COD loading of 7.5 g/l-d was too high for this size of system.

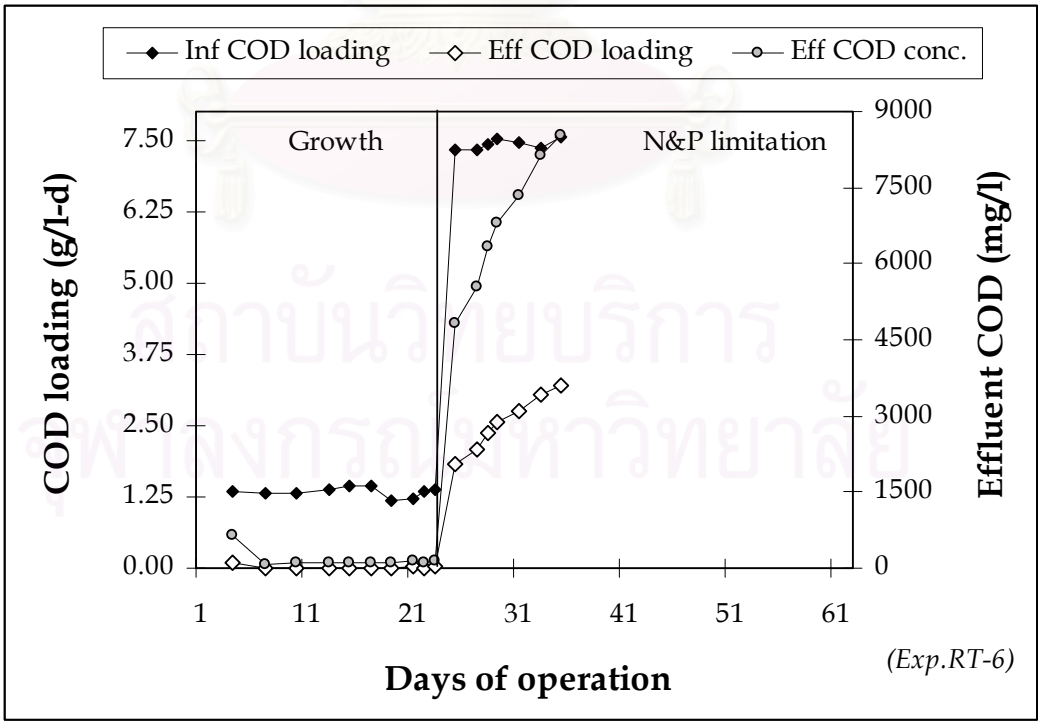


Figure 4.56: Profiles of COD loading and effluent COD concentration for the experiment RT-6x.

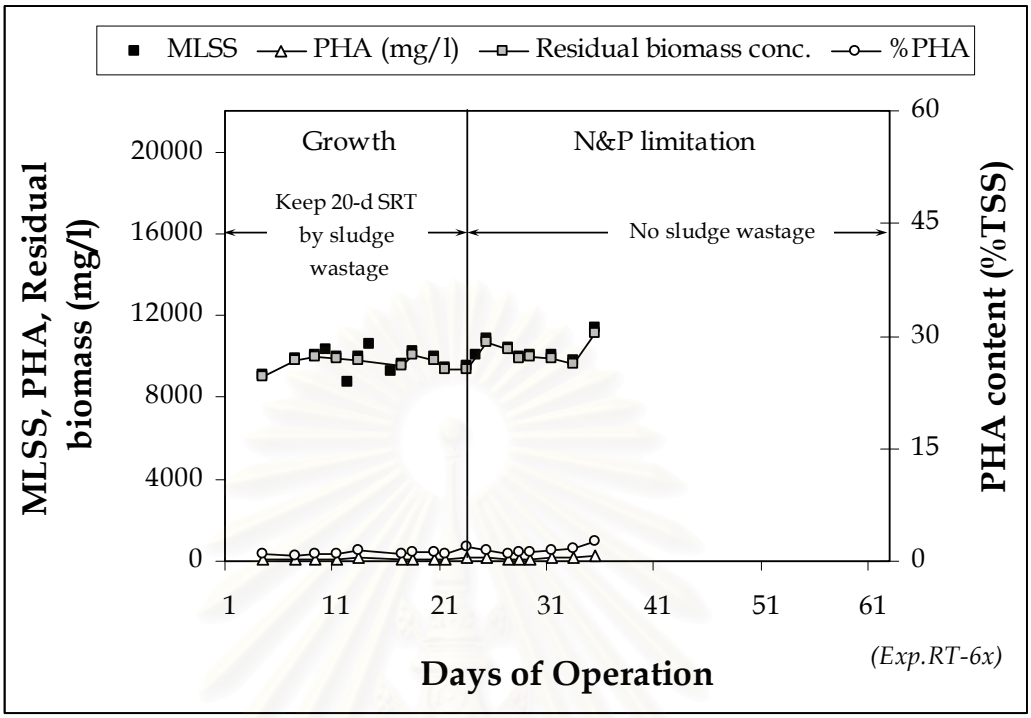


Figure 4.57: Profiles of PHA content (%TSS), PHA concentration (mg/l), MLSS (mg/l) and residual biomass (mg/l) for the experiment RT-6x.

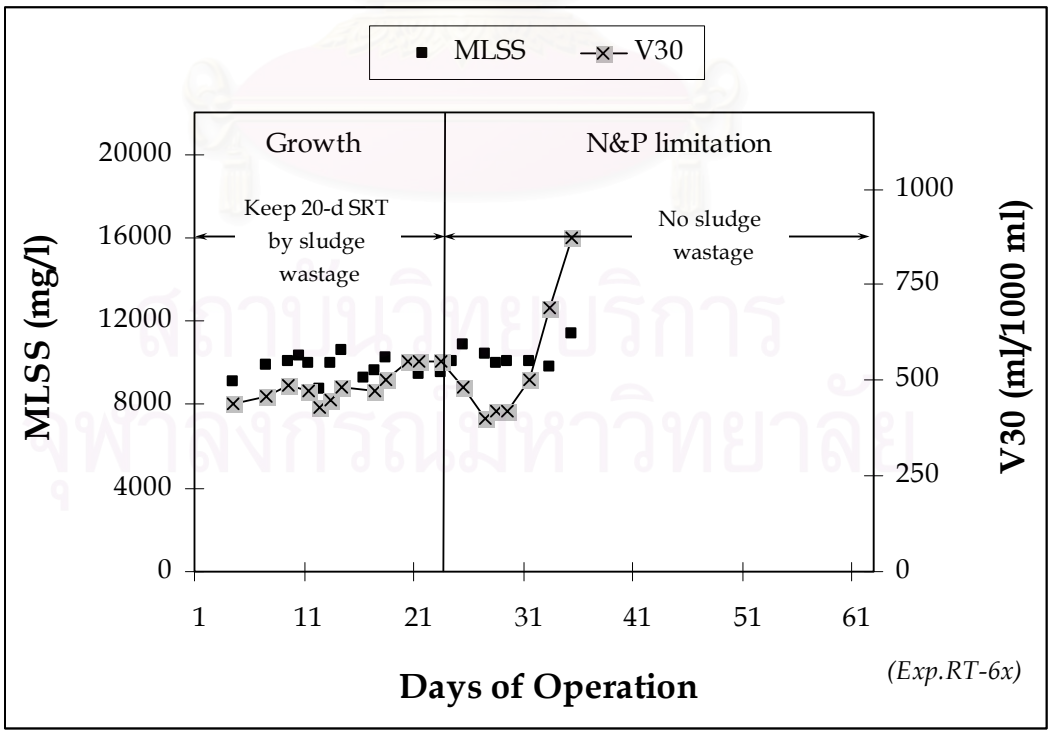


Figure 4.58: Profiles of MLSS (mg/l) and V30 (ml/1000 ml) for the experiment RT-6x.

4.7 The potential of using candy wastewater for PHA production

Figure 4.59 shows that the yields of PHA obtained from using the candy wastewater as a substrate were mostly low, ranging from only 0.036 to 0.159 mg PHA/mg COD_u. The best result of 0.159 mg PHA/mg COD_u was obtained from the experiment at 20°C with 2.5 g/l-d COD loading during nutrients limitation stage (experiment 20C-2x) while in other 20°C experiment (20C-1x), with 1.25 g/l-d COD loading, PHA yield of only 0.078 mg PHA/mg COD_u was achieved. The PHA yields resulted from the experiments at room temperature was even very much lower than those at 20°C, the highest one was only 0.083 mg PHA/mg COD_u obtained from the experiment fed with as high as 5 g/l-d COD loadings during nutrients limitation step. However, the results also show that the increase of COD loading could affect the yield of PHA. The COD loading of 1.25 g/l-d was too low to provide sufficient excess carbon during PHA accumulation period, especially at higher temperature like the Thailand room temperature of 28-31°C.

The PHA contents ranging from 5.6 to 23.4 % of TSS resulted from experiments with glucose-sucrose based candy wastewater were somewhat lower than those obtained from the experiments using acetate-propionate synthetic wastewater in the previous part. The highest PHA content of 23.4 % of TSS and the highest PHA yield of 0.159 mg PHA/mg COD_u) obtained from the experiment 20C-2x are shown in Figure 4.60. The significantly low PHA content in the experiment RT-1x, 5.6 % of TSS shows that carbon substrate was mostly channeled towards cell growth, not PHA material production. Shi et al. (1997), who studied the flux of carbon substrate distributions during PHB synthesis in *Alcaligenes eutrophus*, reported that the flux of carbon substrate to TCA cycle was nearly constant or only slightly decreased with the depletion of nitrogen source. It can be said then that the biomass in this study might as well needs some constant amount of carbon to supply for TCA cycle as their respiration is aerobically like *Alcaligenes eutrophus*. Hence, most of carbon substrate from the COD loading of 1.25 g/l-d might be transformed into TCA cycle, especially at higher experimental temperature, and left not enough carbon for conversion into

PHA. Therefore, the increase carbon substrate supplied to the system enhanced the PHA production as illustrated by the increase of both PHA yield and content in Figures 4.59 and 4.60.

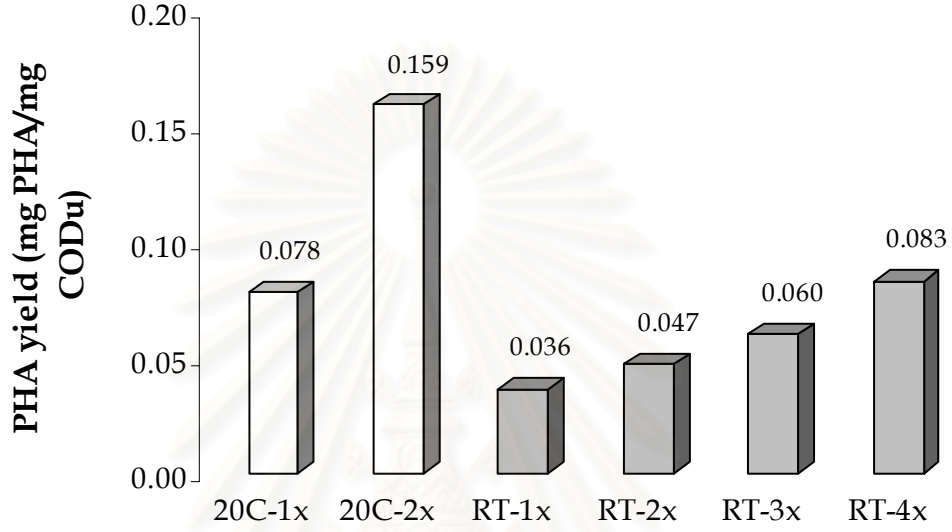


Figure 4.59: The yield of PHA on substrate utilized obtained from the experiments using candy wastewater.

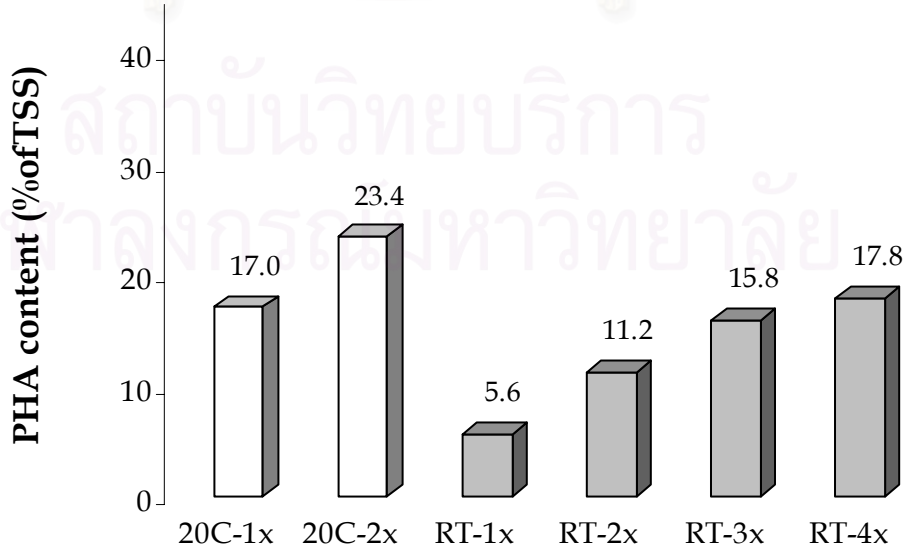


Figure 4.60: The PHA content obtained from the experiments using candy wastewater.

Figure 4.61 shows the quite high PHA concentration, ranging from 2411 to 4453 mg/l and from 675 to 3891 mg/l, obtained from the candy-wastewater experiments at 20°C and room temperature (28-31°C), respectively. In comparison with the experiments with acetate-propionate synthetic wastewater, these PHA concentrations (from the experiments using glucose-sucrose candy wastewater) were somewhat higher because of the larger amount of their biomass concentration. As we have known that the culturing temperature affects the biomass concentration, i.e. the less cell concentration will be obtained from the higher temperature because biomass needs more carbon and energy for their growth and intracellular metabolism, the less biomass concentration obtained from room temperature (28-31°C) experiments consequently results in less PHA concentration when compared with the 20°C experiments under the same condition.

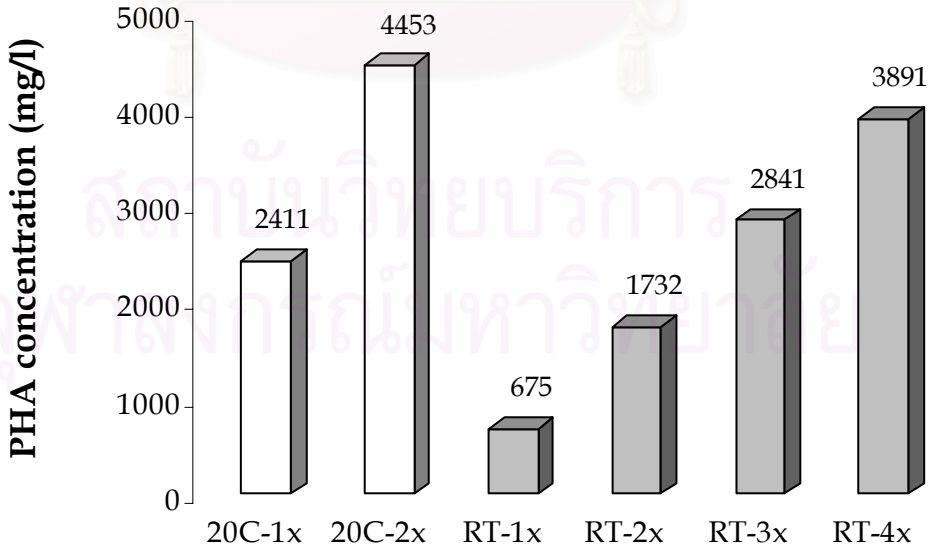


Figure 4.61: The PHA concentration obtained from the experiments using candy wastewater.

The highest PHA productivity of 405 mg/l-d was also obtained from the experiment 20C-2x (at 20°C with 2x COD loading during nutrients limitation). But the productivity obtained from another experiment, 20C-1x, at the same operating temperature was much lower; it was only 100 mg/l-d (Figure 4.62). This is because the PHA productivity was involved with the duration of PHA accumulation; the experiment 20C-1x required more time to obtain the maximum PHA production, i.e. 24 days, while the experiment 20C-2x needed only 11 days as shown in Figure 4.63, resulting in more than two times higher of PHA productivity. For the experiments operated at room temperature, the highest PHA productivity of 354 mg/l-d was obtained from the experiment RT-4x. Two factors involving this high productivity were the large amount of biomass concentration (21860 mg/l) and the less day required for accumulating PHA (as shown in Figure 4.63) although the yield of PHA was not so high (0.083 mg PHA/mg COD_u).

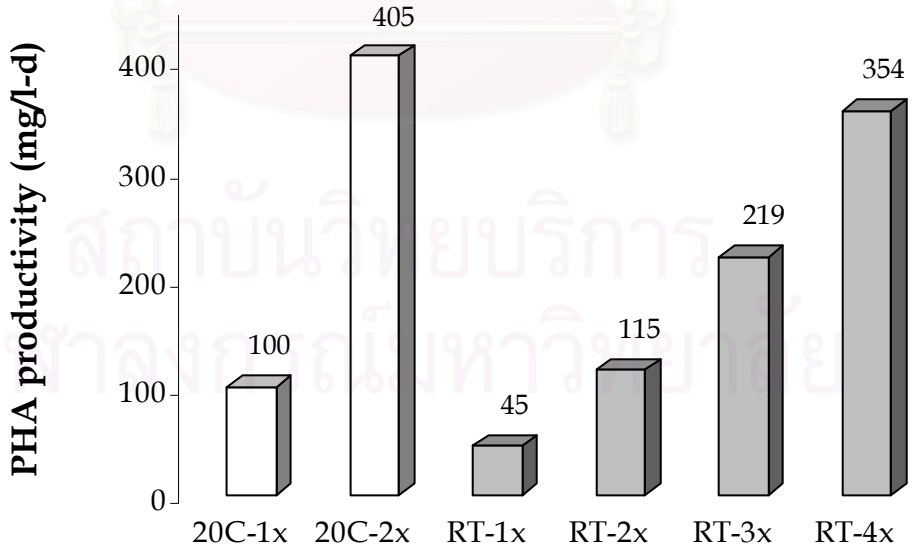
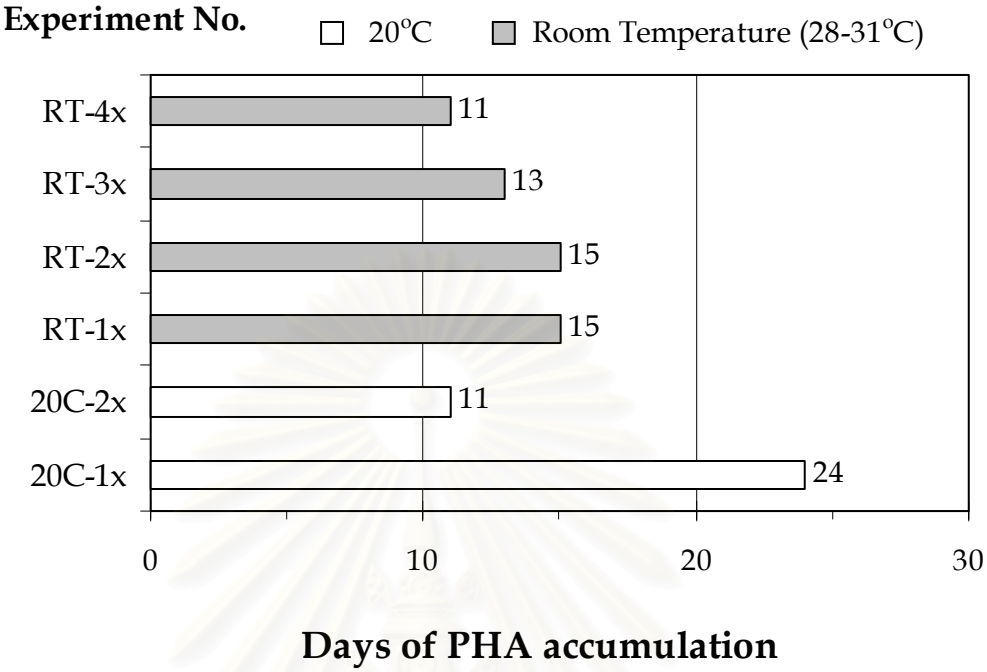


Figure 4.62: The PHA productivity obtained from the experiments using candy wastewater.



Note: The days of PHA accumulation considered when each experiment obtained the maximum PHA production.

Figure 4.63: Comparison of the number of PHA accumulation days among the experiments using candy wastewater.

4.8 Comparison of PHA production between using 10000 mg/l COD, glucose & sucrose based candy wastewater and 660 mg/l COD, acetate-propionate synthetic wastewater

Table 4.5: Comparison of PHA production at 20°C

Parameters \ Experiment.	Using synthetic wastewater (660 mg/l COD)		Using candy wastewater (10000 mg/l COD)
	20°C with N limitation	20°C with N&P limitation	20C-2x with N&P limitation
COD loading (g/l-d)	1.58*	1.58*	2.5**
PHA yield (mg PHA/mg COD_u)	0.196	0.16	0.159
PHA content (% of TSS)	40	43	23.4
PHA concentration (mg/l)	2830	2239	4453
PHA productivity (mg/l-d)	283	204	405
Corresponding MLSS (mg/l)***	7548	5255	19030
Days to accumulate Max. PHA production (days)	10	11	11

Note: *Calculated from COD concentration of 660 mg/l feeding with hydraulic retention time of 10 hrs.
**Considering COD loading fed during nutrients limitation period.
***Corresponding MLSS is MLSS on the day of maximum PHA production.

Considering the experiments at 20°C (data summarized in Table 4.5), the PHA content of 23.4 % of TSS, occurred in the run using candy wastewater, was lower than those of 40 and 43 % of TSS obtained from the two runs using synthetic wastewater. This low PHA content in the experiment 20C-2x shows that biomass in the system converted the carbon substrate into non-PHA materials more than to PHA material. However, Beun et al. (2002) mentioned that in the production of PHB using mixed activated sludge culture, PHB content will hardly reach the level of 80 % of TSS achieved by

using pure culture. They obtained a maximum PHA content of around 40 % of TSS by culturing mixed activated sludge on acetate, which is nearly the amount gained from this study. In addition, Chua et al. (2003) studied PHA accumulation by using activated sludge, which was operated at 'cooler' room temperature between 18-25^{o}C with municipal wastewater supplemented with 30 mg C/l of acetate as influent. The highest PHA content achieved from their study was only 30% of cell dry weight, which is a little higher than that of 23.4 % of TSS obtained by using candy wastewater in this study. However, high PHA content, more than 80 % of cell dry weight, is still required for an economical PHA production process due to its effect on recovery process, which is one of major costs of PHA production process. Thereby, the capability in PHA production of activated sludge needs to be further improved.

The PHA yield from the experiment 20C-2x (with candy wastewater) and that using synthetic wastewater with N&P limitation at 20^{o}C were almost identical; that is 0.159 and 0.16 mg PHA/mg COD_u, respectively, though the types of carbon substrate and COD loading feeding were different. As expected, the yields of PHA obtained from this study were much lower than the theoretical yield of 0.42 mg PHA/mg COD of glucose and 0.45 mg PHA/mg COD of acetic acid calculated by Yamane (1993). The similar PHA yields on substrate utilized mentioned above means that the biomass in this study produced the same amount of PHA by consuming the same amount of COD of carbon substrate. Thereby, the cost of PHA production could be reduced as the industrial wastewater was practically cheaper than synthetic wastewater.

As PHA concentration is associated with biomass concentration, the larger amount of MLSS is the higher PHA concentration can be achieved. Although PHA content (23.4%) from the experiment with the candy wastewater was lower than those with synthetic wastewater (40 and 43%), the PHA concentration was contrarily higher due to the almost two times higher MLSS (data shown in Table 4.5). The PHA productivity resulted from the experiment 20C-2x (using candy wastewater) was also higher due to the fact that the system produced higher PHA concentration whereas the times consumed for PHA accumulation were surprisingly the same. From the results

obtained from this study, it can be suggested that the PHA accumulation at 20^{o}C temperature require around 10 days to reach the maximum PHA production. This could be said that the type of substrate and COD loading would not affect the duration of PHA accumulation, but PHA content and biomass concentration, resulting in different PHA production.

For the experiments at 30^{o}C and room temperature (Table 4.6), the PHA content obtained from the experiment using glucose-sucrose based candy wastewater was only 17.8 % of TSS, which was lower than those of 30 and 33 % of TSS obtained from the experiments using acetate-propionate synthetic wastewater with only N limitation and N&P limitation, respectively. The yield of PHA from the experiments at this range of operating temperatures was quite low; the PHA yield from candy wastewater as a substrate (0.083 mg PHA/mg COD_u) was a little lower than from synthetic wastewater (0.10 and 0.11 mg PHA/mg COD_u). Collins (1987) and Shi et al. (1997) explained that substrates, which produce acetyl CoA via enzyme pyruvate dehydrogenase (PDH), could not be converted to product with the efficiency higher than 67% because there must be carbon loss via CO_2 released during the reaction from pyruvate to acetyl CoA. Therefore, substrate of which its product, acetyl CoA, is converted from pyruvate should yield less amounts of PHA. This could be the explanation for the lower PHA yield obtained by using glucose-sucrose based candy wastewater. That is, the industrial wastewater used in this study mainly contained carbohydrates of which their acetyl CoA is produced by conversion from pyruvate and some carbon must be lost through released CO_2. Whereas the synthetic wastewater comprised of acetate which directly transformed into acetyl CoA via no intermediate and propionate which formed acetyl CoA by the elimination of carbonyl carbon from propionyl CoA without any loss of CO_2 during the reactions.

Although this glucose-sucrose based candy wastewater provides lower yield of PHA, it is an attractive substrate for PHA production because of its cheaper cost compared to those of virgin acetate and propionate. Also, the operating condition can be modified to improve PHA production, e.g., an increase of substrate concentration to 10000 mg/l

COD and loading to 5 g/l-d COD could result in increasing of biomass concentration to as high 21840 mg/l, which is almost five times higher than those of using 660 mg/l COD synthetic wastewater in this study (Table 4.6). Thence, high PHA concentration of 3891 mg/l and productivity of 354 mg/l-d were obtained in comparison with those concentrations of 1326 and 1476 mg/l and productivities of 147 and 148 mg/l-d obtained by using synthetic wastewater.

Table 4.6: Comparison of PHA production between at 30°C and Thailand room temperature (28-31°C).

Experiment. / Parameters	Using synthetic wastewater (660 mg/l COD)		Using industrial wastewater (10000 mg/l COD)
	30°C with N limitation	30°C with N&P limitation	RT-4 with N&P limitation
Operating temperature (°C)	30	30	28-31
COD loading (g/l-d)	1.58*	1.58*	5.0**
PHA yield (mg PHA/mg COD_u)	0.104	0.11	0.083
PHA content (% of TSS)	30	33	17.8
PHA concentration (mg/l)	1326	1476	3891
PHA productivity (mg/l-d)	147	148	354
Corresponding MLSS (mg/l)***	4421	4542	21860
Days to accumulate Max. PHA production (days)	9	10	11

Note: *Calculated from COD concentration of 660 mg/l feeding with hydraulic retention time of 10 hrs.
**Considering COD loading fed during nutrients limitation period.
***Corresponding MLSS is MLSS on the day of maximum PHA production.

From the above results, low PHA content and yield obtained by using glucose-sucrose based candy wastewater as substrate indicated that biomass converted more carbon

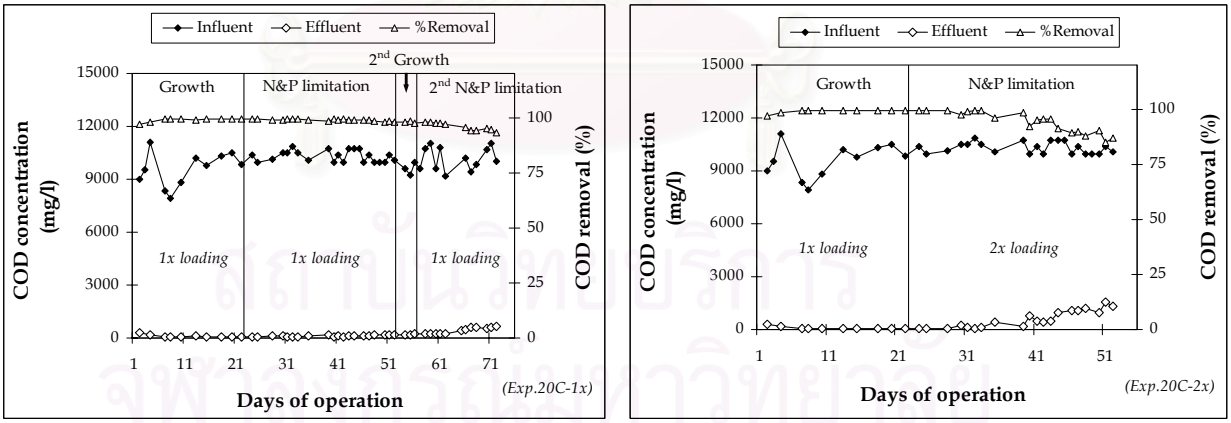
substrate into non-PHA materials. In addition to PHA, glycogen and glycogen-like materials can also be accumulated in cells as carbohydrate reserves. Glycogen is accumulated under the same condition as PHA; that is, utilizable nitrogen is limited and the excess carbon is available (Dawes, 1986). In general carbohydrate metabolism, a precursor for the biosynthesis of glycogen is glucose 6-phosphate, which is also the first intermediate of the Embden-Meyerhof Pathway (EMP) converting glucose into pyruvate. Also, the enzyme ADP glucose pyrophosphorylase, which is a key regulatory enzyme of glycogen biosynthesis in most bacteria, is activated by certain EMP intermediates such as fructose 6-phosphate, fructose 1,6-diphosphate, glycerate 3-phosphate or pyruvate (Dawes, 1986). Therefore, glycogen synthesis should be more likely to occur when substrate is carbohydrate than organic acids. Hence, this candy wastewater, which is carbohydrate-based, might be converted into glycogen more than PHA, resulting in low PHA content and yield, even while increasing cell concentration.

Although PHA production resulted by using industrial carbohydrate wastewater was not substantially high enough to draw the attention for commercial PHA production, the double benefit of treating wastewater and converting waste to valuable product is still interesting. The optimization of operating conditions in activated sludge using industrial carbohydrate wastewater needs however to be further studied and improved.

Other sources of industrial wastewater can also be of interest if they are of acetate or propionate origin, e.g., elastic industry, vinegar industry, etc. These wastewaters can be a good carbon provider for the PHA production as well.

4.9 COD removal in wastewater treatment aspect

Although PHA production is the main objective in this study, the point of wastewater treatment should not be overlooked, otherwise, the benefit of using wastewater as renewable waste for PHA production would be regretfully reduced. Figure 4.64a shows that during the N&P limitation phase, COD removal efficiency was maintain not less than 90% throughout the experiment 20C-1x. Also, for the experiment 20C-2x, of which COD loading increased 2 folds during N&P limitation phase, the COD removal efficiency was higher than 90% till the day 45 (numeric data shown in Appendices), then COD removal slightly declined. The decrease of COD removal occurred after the maximum PHA content of 23.4 % of TSS in this experiment was obtained for awhile. This is similar to the results described in section 4.1 to 4.3, in which COD removal (or utilization) will start to decrease when biomass accumulates peak PHA content. Therefore, if the timing to harvest PHA biomass was selected appropriately, the high PHA production could be obtained concurrently with high COD removal efficiency.

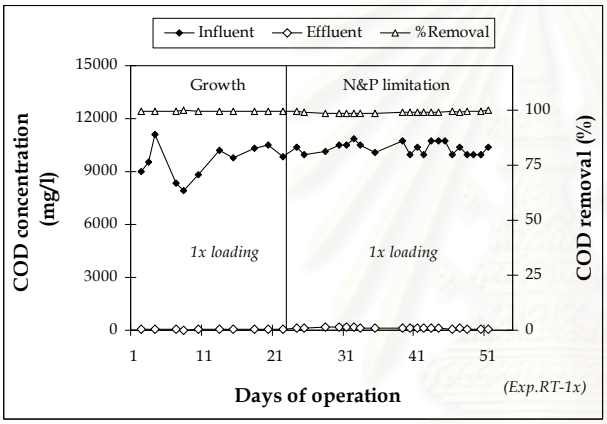


(a) The experiment 20C-1x (b) The experiment 20C-2x

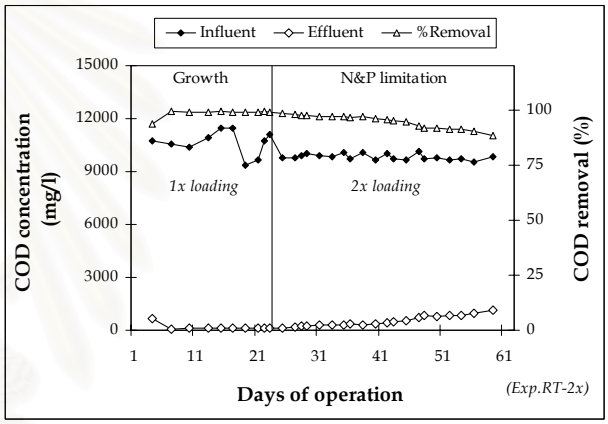
Figure 4.64: Profile of COD concentration and removal efficiency in the experiments using candy wastewater at 20°C.

For the experiments at room temperature, Figure 4.65 shows the same occurrence as the experiments at 20°C. That is, the COD removals in three experiments of RT-1x,

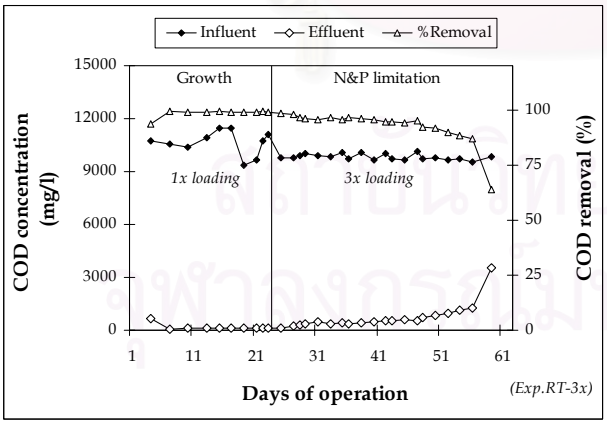
RT-2x and RT-3x were mostly maintained above 90% even during the N&P limitation phase. The COD removal efficiency slightly decrease after peak of PHA content was obtained. In the experiment RT-4x, the COD removal decreased since the N&P limitation started, and on the day of peak PHA occurred, the COD removal efficiency was only 48% (data shown in Appendices). Since the PHA content obtained from this experiment was only 17.8 % of TSS, which is not high enough for commercial PHA production, the PHA production at room temperature concomitantly with wastewater treatment aspect needs to be further studied.



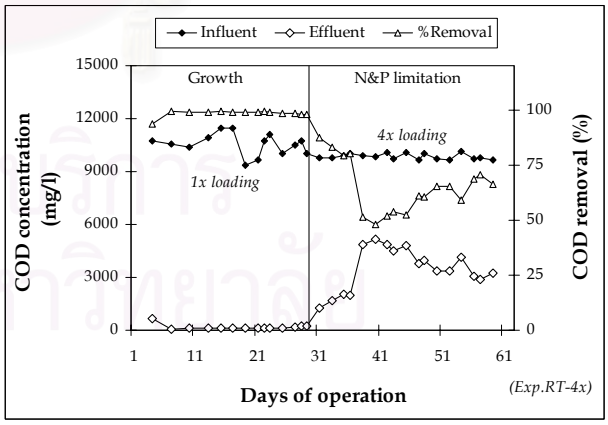
(a) The experiment RT-1x



(b) The experiment RT-2x



(c) The experiment RT-3x



(d) The experiment RT-4x

Figure 4.65: Profile of COD concentration and removal efficiency in the experiments using candy wastewater at Thailand room temperature (28-31^{o}C).

CHAPTER 5

CONCLUSIONS

The objective of the study with synthetic wastewater was to investigate the effects of temperature on PHA production by mixed cultures of activated sludge biomass. Based upon the results of the study, the conclusions were listed as the followings;

1. The activated sludge biomass is capable of accumulating substantial amount of PHA and candy wastewater is possible to be used as a substrate for PHA production. As wastewater is an inexpensive source of substrate, the PHA production for biodegradable plastics by using activated sludge biomass and wastewater as a substrate could as well be the economic solution to produce biopolymer for biodegradable plastics. In such cases, the price of biodegradable plastics will be lessened and can be more competitive to using virgin acetate as substrate.

2. Temperature can have a significant effect on PHA production, i.e., at the low temperature of 10^{o}C, high PHA content and PHA productivity can be accumulated while that of the 20^{o}C system accumulated similarly high PHA content, but with lower PHA productivity due to the requirement of longer PHA production time. The PHA production of 30^{o}C biomass was not as good as those at two other lower temperatures.

3. The operating procedure, i.e., two consecutive times of growth & nutrient limitation phases, and temperature also affects the type of copolymer, not only because of the type of substrate.

4. The experiment with N&P limitation at 10^{o}C produced the highest PHA productivity and PHA yield in comparison to the experiments with either N or P limitations at the same temperature. For the temperatures of 20 and 30^{o}C, N&P

limitation and only N limitation made not much difference in PHA productivity and yield. The P limitation condition provided the lowest PHA productivity and yield in all experimental temperatures and, as a result, was not recommended.

5. Using glucose-sucrose based candy wastewater as the carbon substrate obviously produced lower PHA contents than acetate-propionate synthetic-wastewater counterpart. One reason is that the carbohydrate substrates lose some carbon as CO_2 released during the conversion of pyruvate to acetyl-CoA, a precursor for PHA production, while organic acids is more readily incorporated into PHA production.

6. Although the PHA contents from the 10000 mg/l COD candy wastewater case were relatively lower than those from using 660 mg/l COD acetate-propionate synthetic wastewater, the PHA productivities were somewhat higher because of very much higher cell concentrations. This shows the positive possibility to produce PHA from industrial wastewater, but ways to maximize PHA contents, however, need to be improved.

Suggestions for future works

1. To increase the PHA contents, any carbohydrate based industrial wastewater should be biologically acidified, i.e., fermented, before used as a substrate for the production of PHA by activated sludge biomass.

2. In case where carbohydrate wastewaters are used as input substrate, bulking problem may arise during the nutrients limitation stage. Since maintaining the cell concentration in the reactor is very important in the PHA production scheme, physical separation of cells and, in turn, PHA via a membrane system can be very promising in such circumstances.

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APPENDICES

Experiment 1 : 10C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
24 Jul 02	1												
25 Jul 02	2	608		5,744		1,076	940	0.87		11.9	128		948
26 Jul 02	3												
27 Jul 02	4	611	9	5,775	99	2,313	2,121	0.92	216				
28 Jul 02	5		15	5,718	98	2,665	2,429	0.91	135				
29 Jul 02	6	611	6	5,805	99	3,069	2,780	0.91	140	11.9	365		2,704
30 Jul 02	7		15	5,720	98	3,523	3,155	0.90	152				
31 Jul 02	8	648	9	6,138	99	3,554	3,213	0.90		7.0	249		3,305
1 Aug 02	9												
2 Aug 02	10	608	22	5,626	96	3,892	3,532	0.91	103	7.6	296		3,596
3 Aug 02	11												
4 Aug 02	12	625	16	5,850	98								
5 Aug 02	13												
6 Aug 02	14	657	16	6,150	98	4,558	4,176	0.92	81	30.0	1,367	342	3,190
7 Aug 02	15												
8 Aug 02	16	619	371	2,385	40	3,520	3,335	0.95	85	38.4	1,352	225	2,168
9 Aug 02	17												
10 Aug 02	18												
11 Aug 02	19	615	529	825	14	3,600	3,415	0.95	90	37.7	1,357	151	2,243
12 Aug 02	20												
13 Aug 02	21	620	370	2,400	40	3,707	3,465	0.93	156	37.2	1,379	125	2,328
14 Aug 02	22												
15 Aug 02	23	645	94	5,292	85	4,453	4,188	0.94	63	39.4	1,755		2,699
16 Aug 02	24												
17 Aug 02	25												
18 Aug 02	26												
19 Aug 02	27	663	62	5,776	91	5,766	5,356	0.93	62	32.4	1,868	623	3,898

Experiment 1 : 10C, N limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
20 Aug 02	28	677	55	5,973	92								
21 Aug 02	29	646	249	3,812	61	5,898	5,545	0.94	70	34.5	2,035	407	3,863
22 Aug 02	30												
23 Aug 02	31	610	361	2,389	41	5,627	5,295	0.94	64	36.3	2,043	292	3,585
24 Aug 02	32												
25 Aug 02	33												
26 Aug 02	34	628	385	2,332	39	5,457	5,114	0.94	78	24.7	1,348	135	4,109
27 Aug 02	35												
28 Aug 02	36	604	373	2,219	38	5,268	4,928	0.94	74				

Experiment 2 : 20C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
7 Jun 02	1												
8 Jun 02	2												
9 Jun 02	3												
10 Jun 02	4	615	22	5701	97	2,857	2,278	0.80	98	0.7	20		2,837
11 Jun 02	5												
12 Jun 02	6												
13 Jun 02	7	621		5720		3,638							
14 Jun 02	8	650	25	6000	96	4,180	3,135	0.75	108	0.7	29		4,150
15 Jun 02	9												
16 Jun 02	10	639	25	5894	96	4,963	3,747	0.75	81	1.0	50		4,914
17 Jun 02	11												
18 Jun 02	12												
19 Jun 02	13	631	19	5880	97	4,919	3,723	0.76	85	1.6	79		4,840
20 Jun 02	14												
21 Jun 02	15	633	19	5894	97	4,966	3,761	0.76	70	1.0	50		4,916
22 Jun 02	16												
23 Jun 02	17												
24 Jun 02	18	621	6	5903	99	5,455	4,075	0.75	64	2.1	115		5,340
25 Jun 02	19												
26 Jun 02	20	646	33	5888	95	5,335	3,970	0.74	56	1.2	64		5,271
27 Jun 02	21												
28 Jun 02	22												
29 Jun 02	23	620	21	5746	97	5,327	3,951	0.74	49	0.9	48		5,279
30 Jun 02	24												
1 Jul 02	25												
2 Jul 02	26	598	21	5540	96	5,081	3,933	0.77	47	2.5	127		4,954
3 Jul 02	27												

Experiment 2 : 20C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
4 Jul 02	28	641	27	5892	96	3,991	3,230	0.81	48	4.4	176		3,816
5 Jul 02	29												
6 Jul 02	30												
7 Jul 02	31	618	36	5585	94	4,059	3,636	0.90	64	4.6	187		3,872
8 Jul 02	32												
9 Jul 02	33												
10 Jul 02	34	612	27	5615	96	4,775	4,292	0.90	63	21.1	1,008	336	3,767
11 Jul 02	35												
12 Jul 02	36												
13 Jul 02	37	634	21	5888	97	5,586	5,114	0.92	50	30.7	1,715	286	3,871
14 Jul 02	38												
15 Jul 02	39	630	61	5469	90	6,750	6,119	0.91	44	32.4	2,187	273	4,563
16 Jul 02	40												
17 Jul 02	41	656	148	4879	78	7,548	6,913	0.92	42	37.5	2,830	283	4,717
18 Jul 02	42		89	5445									
19 Jul 02	43	645	242	3871	63	7,054	6,531	0.93	46	40.1	2,829	236	4,225
20 Jul 02	44		113	5110									
21 Jul 02	45	634	326	2949	48	7,174	6,774	0.94	60	37.4	2,683	192	4,491
22 Jul 02	46												
23 Jul 02	47	614	431	1756	30	6,083	5,717	0.94	60	35.3	2,147	134	3,936
24 Jul 02	48												
25 Jul 02	49	602	403	1906	33	5,717	5,375	0.94	67	34.5	1,972	110	3,744
26 Jul 02	50												
27 Jul 02	51	605	397	1993	34	5,287	5,026	0.95	61	32.0	1,692	85	3,595
28 Jul 02	52												
29 Jul 02	53	611	16	5715	97	5,291	4,955	0.94	119	19.5	1,032		4,259
30 Jul 02	54		30			4,447	4,200	0.94	164				

Experiment 2 : 20C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
31 Jul 02	55	600	24	5527	96	3,807	3,526	0.93	202	10.2	388		3,419
1 Aug 02	56												
2 Aug 02	57												
3 Aug 02	58	633	40	5686	94	3,487	3,188	0.91	169	25.2	879	293	2,609
4 Aug 02	59	619	41	5550	93								
5 Aug 02	60												
6 Aug 02	61	650	33	5932	95	4,100	3,706	0.90	124	31.5	1,292	215	2,809
7 Aug 02	62												
8 Aug 02	63	613	219	3778	64	4,435	4,165	0.94	115	29.9	1,326	166	3,109
9 Aug 02	64												
10 Aug 02	65												
11 Aug 02	66	655	423	2221	35	4,223	3,885	0.92	130	27.5	1,161	106	3,062
12 Aug 02	67												
13 Aug 02	68	600	460	1344	23	4,163	3,874	0.93	127	23.1	962	74	3,201
14 Aug 02	69												
15 Aug 02	70	645	494	1452	23	3,607	3,269	0.91	152	20.3	732	49	2,875

Experiment 3 : 30C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
7 Jun 02	1												
8 Jun 02	2												
9 Jun 02	3												
10 Jun 02	4	628	18	5849	97	4,037	3,086	0.76	99	0.5	20		4,017
11 Jun 02	5												
12 Jun 02	6												
13 Jun 02	7	666	29	6113	96	4,524			82	0.8	36		4,488
14 Jun 02	8	650		5964									
15 Jun 02	9												
16 Jun 02	10	623	28	5715	96	4,061	3,183	0.78	167	1.8	73		3,988
17 Jun 02	11												
18 Jun 02	12												
19 Jun 02	13	644	41	5790	94	3,940	2,987	0.76	89	1.8	71		3,869
20 Jun 02	14												
21 Jun 02	15	645	50	5715	92	3,867	2,910	0.75	59	1.1	43		3,824
22 Jun 02	16												
23 Jun 02	17												
24 Jun 02	18	621	24	5731	96	4,140	3,155	0.76	60	2.5	104		4,037
25 Jun 02	19												
26 Jun 02	20	604	30	5518	95	3,748	2,900	0.77	69	2.5	94		3,654
27 Jun 02	21												
28 Jun 02	22												
29 Jun 02	23	626	42	5601	93	3,874	3,161	0.82	72	2.3	89		3,785
30 Jun 02	24												
1 Jul 02	25												
2 Jul 02	26	623	27	5716	96	4,118	3,509	0.85	85	3.9	161		3,958
3 Jul 02	27												

Experiment 3 : 30C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
4 Jul 02	28	635	34	5775	95	3,826	3,279	0.86	107	2.8	107		3,719
5 Jul 02	29												
6 Jul 02	30												
7 Jul 02	31	612	45	5440	93	4,163	3,700	0.89	159	16.8	699	233	3,464
8 Jul 02	32												
9 Jul 02	33												
10 Jul 02	34	600	45	5324	92	4,807	4,364	0.91	148	27.2	1,308	218	3,500
11 Jul 02	35												
12 Jul 02	36												
13 Jul 02	37	634	59	5518	91	4,421	4,090	0.93	149	30.0	1,326	147	3,094
14 Jul 02	38												
15 Jul 02	39	612	218	3782	64	3,664	3,464	0.95	186	28.4	1,041	95	2,623
16 Jul 02	40												
17 Jul 02	41	643	148	4753	77	2,746	2,592	0.94	357	31.4	862	66	1,884
18 Jul 02	42												
19 Jul 02	43	632	332	2880	47	2,669	2,579	0.97	216	31.7	846	56	1,823
20 Jul 02	44												
21 Jul 02	45	666	266	3840	60	2,953	2,894	0.98	228	30.4	898	53	2,055
22 Jul 02	46												
23 Jul 02	47	626	329	2858	48	3,095	2,926	0.95	229	30.7	950	50	2,145
24 Jul 02	48												
25 Jul 02	49	608	367	2310	40	3,165	3,000	0.95	196	20.1	636		2,529
26 Jul 02	50												
27 Jul 02	51	605	70	5130	88	3,300	3,129	0.95	182	6.2	205		3,095
28 Jul 02	52												
29 Jul 02	53	605	25	5563	96	3,906	3,529	0.90	146	16.2	633	316	3,273
30 Jul 02	54												

Experiment 3 : 30C, N limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
31 Jul 02	55	606	24	5585	96	4,148	3,687	0.89	132	21.9	908	227	3,239
1 Aug 02	56												
2 Aug 02	57	639	40	5745	94	5,087	4,400	0.87	94	26.5	1,348	225	3,739
3 Aug 02	58												
4 Aug 02	59	638	59	5550	91								
5 Aug 02	60												
6 Aug 02	61	676	36	6150	95	6,090	5,045	0.83	76	25.6	1,559	156	4,531
7 Aug 02	62												
8 Aug 02	63	652	58	5698	91	6,055	5,036	0.83	83	23.4	1,417	118	4,638
9 Aug 02	64												
10 Aug 02	65												
11 Aug 02	66	628	50	5554	92	5,660	4,573	0.81	87	21.6	1,223	82	4,437
12 Aug 02	67												
13 Aug 02	68	600	43	5344	93	6,069	4,969	0.82	79	19.0	1,153	68	4,916
14 Aug 02	69												
15 Aug 02	70	666	57	5841	91	5,871	4,750	0.81	76	17.1	1,004	53	4,867

Experiment 4 : 10C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
21 Jan 02	1												
22 Jan 02	2	610	13	5,730	98	3,353	2,522	0.75	93				
23 Jan 02	3												
24 Jan 02	4	622	19	5,788	97	3,410	2,600	0.76	59	6.0	205		3,205
25 Jan 02	5		26	5,726	96	3,540	2,740	0.78	63				
26 Jan 02	6					3,940	3,080	0.78	59	11.7	461		3,479
27 Jan 02	7												
28 Jan 02	8	623	13	5,854	98	3,730	2,945	0.79	61	8.7	325		3,405
29 Jan 02	9												
30 Jan 02	10	627	32	5,714	95	4,160	3,170	0.76	57	6.1	254		3,906
31 Jan 02	11												
1 Feb 02	12	611	20	5,682	97	3,850	3,115	0.81	59	8.1	312		3,538
2 Feb 02	13		26	5,620	96	3,820	3,015	0.79	64	6.9	264		3,556
3 Feb 02	14												
4 Feb 02	15		26	5,858	96	3,713	2,973	0.80	66	7.5	279		3,435
5 Feb 02	16	636	50	5,624	92								
6 Feb 02	17	598	19	5,563	97	3,700	3,165	0.85	69	11.8	437		3,263
7 Feb 02	18												
8 Feb 02	19												
9 Feb 02	20	626	68	5,358	89	3,473	3,092	0.89	70	13.7	476		2,997
10 Feb 02	21												
11 Feb 02	22	626	37	5,656	94	3,514	3,109	0.88	68	10.7	376		3,138
12 Feb 02	23												
13 Feb 02	24												
14 Feb 02	25	578	25	5,305	96	4,500	4,273	0.95	73	14.0	630	210	3,870
15 Feb 02	26	648	38	5,851	94	5,193	4,907	0.94	77	10.2	530	132	4,664
16 Feb 02	27	593	63	5,088	89	5,220	4,960	0.95	84	10.0	522	104	4,698

Experiment 4 : 10C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
17 Feb 02	28												
18 Feb 02	29	647	31	5,910	95	5,453	5,207	0.95	105	15.0	818	117	4,635
19 Feb 02	30	619	57	5,399	91	5,187	4,973	0.96	110	11.4	591	74	4,595
20 Feb 02	31	619	57	5,399	91	4,369	4,213	0.96	121	14.5	633	70	3,735
21 Feb 02	32												
22 Feb 02	33	609	19	5,663	97	3,947	3,880	0.98	139	14.2	560	51	3,386
23 Feb 02	34	599	52	5,249	91					17.3	683	57	
24 Feb 02	35	642	26	5,914	96	3,720	3,587	0.96	156	16.6	618	48	3,102
25 Feb 02	36												
26 Feb 02	37	603	13	5,670	98	3,078	3,033	0.99		25.1	773	52	2,305
27 Feb 02	38	614				3,053	2,973	0.97	180	30.5	931	58	2,122
28 Feb 02	39	622	25	5,730	96	3,527	3,393	0.96	164	31.8	1,121	66	2,405
1 Mar 02	40	603	51	5,303	92	3,900	3,790	0.97	144	32.6	1,271	71	2,629
2 Mar 02	41												
3 Mar 02	42												
4 Mar 02	43	668	164	4,838	75	2,629	2,505	0.95	205	47.0	1,235	59	1,393
5 Mar 02	44	643	302	3,266	53	2,867	2,762	0.96	199	52.0	1,491	68	1,376
6 Mar 02	45		296	3,326	54	2,130	2,090	0.98	258	38.6	822	36	1,308
7 Mar 02	46	658	262	3,801	60								
8 Mar 02	47												
9 Mar 02	48	659	317	3,281	52	2,290	2,220	0.97	279	46.8	1,072	41	1,218
10 Mar 02	49												
11 Mar 02	50	651	368	2,717	43	2,140	1,990	0.93	266	42.2	903	32	1,237
12 Mar 02	51												
13 Mar 02	52												
14 Mar 02	53	636											
15 Mar 02	54	617	346	2,600	44	2,029	1,952	0.96	311	52.0	1,055	33	974

Experiment 4 : 10C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
16 Mar 02	55												
17 Mar 02	56												
18 Mar 02	57	650	313	3,240	52	1,855	1,773	0.96	313	34.7	644	18	1,211
19 Mar 02	58												
20 Mar 02	59	659	463	1,889	30	1,476	1,419	0.96	406	36.2	534	14	942
21 Mar 02	60												
22 Mar 02	61	649	514	1,298	21	1,313	1,253	0.95	434	24.4	320	8	993
23 Mar 02	62												
24 Mar 02	63												
25 Mar 02	64												
26 Mar 02	65	634	517	1,122	18	1,196	1,123	0.94	502	22.7	272	6	925
27 Mar 02	66												
28 Mar 02	67	630	479	1,451	24	930	910	0.98	591	30.8	286	6	644
29 Mar 02	68												
30 Mar 02	69												
31 Mar 02	70	615	517	945	16	1,124	990	0.88	587	33.3	374	8	750
1 Apr 02	71												
2 Apr 02	72	629	533	914	15	1,125	1,058	0.94	596	32.9	370	7	755
3 Apr 02	73												
4 Apr 02	74	625	494	1,260	21	1,300	1,109	0.85	415	33.0	429	8	871
5 Apr 02	75	608	533	714	12	904	800	0.88	464	35.7	323		581
6 Apr 02	76	608	335	2,620	45	1,117	975	0.87	403	29.2	326		791
7 Apr 02	77	639	366	2,620	43	1,292	1,125	0.87	348	32.1	415		877
8 Apr 02	78	615	344	2,602	44	1,408	1,300	0.92	348	27.5	387		1,021
9 Apr 02	79	624	364	2,498	42	1,333	1,185	0.89					
10 Apr 02	80	624	490	1,287	21	1,288	1,144	0.89	350	27.1	349		939
11 Apr 02	81												

Experiment 4 : 10C, P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
12 Apr 02	82	631	38	5,700	94	1,766	1,669	0.95	362	27.8	491	164	1,275
13 Apr 02	83												
14 Apr 02	84												
15 Apr 02	85	630	38	5,684	94	2,477	2,269	0.92	371	47.8	1,184	197	1,293
16 Apr 02	86												
17 Apr 02	87	645	277	3,530	57	1,808	1,769	0.98	404				
18 Apr 02	88												
19 Apr 02	89												
20 Apr 02	90	619	363	2,460	41	2,208	2,108	0.95	378	43.4	958	87	1,250
21 Apr 02	91												
22 Apr 02	92	638	175	4,440	73	2,069	1,950	0.94	401	42.3	875	67	1,194
23 Apr 02	93												
24 Apr 02	94												
25 Apr 02	95	644	260	3,684	60	1,624	1,560	0.96	462	35.5	577	36	1,047

Experiment 5 : 20C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
4 Apr 02	1	619	50	5,460	92								
5 Apr 02	2	614	93	5,001	85	3,485	2,795	0.80	77	6.8	237		3,248
6 Apr 02	3												
7 Apr 02	4	620	81	5,180	87	2,568	2,254	0.88	80	7.2	185		2,383
8 Apr 02	5												
9 Apr 02	6	624	59	5,432	91	2,232	1,964	0.88	116	12.4	277		1,955
10 Apr 02	7	626	32	5,698	95	2,516	2,191	0.87	119	9.6	241		2,274
11 Apr 02	8												
12 Apr 02	9	613	31	5,580	95	2,555	2,255	0.88	157	7.7	197		2,358
13 Apr 02	10												
14 Apr 02	11												
15 Apr 02	12	611	6	5,805	99	3,514	3,121	0.89	191	8.9	313		3,202
16 Apr 02	13												
17 Apr 02	14	652	32	5,946	95	2,793	2,585	0.93	211	8.4	235		2,558
18 Apr 02	15												
19 Apr 02	16												
20 Apr 02	17	663	25	6,120	96	2,247	2,053	0.91	320				
21 Apr 02	18												
22 Apr 02	19	631	13	5,940	98	2,818	2,316	0.82	225	6.8	192		2,626
23 Apr 02	20												
24 Apr 02	21					3,677							
25 Apr 02	22	618	33	5,620	95	3,579			84	4.6	165		3,414
26 Apr 02	23												
27 Apr 02	24												
28 Apr 02	25												
29 Apr 02	26												
30 Apr 02	27												

Experiment 5 : 20C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
1 May 02	28												
2 May 02	29	620	37	5,596	94	4,458	3,747	0.84	76	9.5	423		4,034
3 May 02	30												
4 May 02	31	620	12	5,834	98	4,288	3,708	0.86	80	10.9	467		3,821
5 May 02	32												
6 May 02	33												
7 May 02	34												
8 May 02	35	641	7	6,093	99	5,407	4,904	0.91	80	3.2	173	43	5,234
9 May 02	36												
10 May 02	37	649	50	5,745	92	6,000	5,606	0.93	80	9.1	546	91	5,454
11 May 02	38												
12 May 02	39	622	6	5,912	99	5,480	5,103	0.93	91	9.0	493	62	4,987
13 May 02	40												
14 May 02	41												
15 May 02	42	655	44	5,866	93	5,490	5,179	0.94	109	14.4	791	72	4,699
16 May 02	43												
17 May 02	44												
18 May 02	45												
19 May 02	46	609	25	5,610	96	5,677				10.0	568	38	5,109
20 May 02	47												
21 May 02	48	609	22	5,640	96	5,657				16.1	911	54	4,746
22 May 02	49												
23 May 02	50												
24 May 02	51												
25 May 02	52	603	32	5,481	95	5,222				21.0	1,097	52	4,125
26 May 02	53												
27 May 02	54												
28 May 02	55												

Experiment 5 : 20C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
29 May 02	56												
30 May 02	57	610	29	5,577	95	4,091	4,000	0.98	115	24.0	982	38	3,109
31 May 02	58												
1 Jun 02	59												
2 Jun 02	60												
3 Jun 02	61	618	231	3,715	63	4,108	3,946	0.96	166	31.5	1,294	43	2,814
4 Jun 02	62												
5 Jun 02	63	622	130	4,724	79	3,218	3,091	0.96	227	33.7	1,085	34	2,134
6 Jun 02	64												
7 Jun 02	65	613	56	5,340	91	3,048	2,952	0.97	200	35.8	1,091	32	1,957
8 Jun 02	66												
9 Jun 02	67												
10 Jun 02	68	615	74	5,199	88	2,947	2,895	0.98	231	36.1	1,064	29	1,883
11 Jun 02	69												
12 Jun 02	70												
13 Jun 02	71	646	141	4,854	78	3,181			261	34.0	1,082	27	2,099
14 Jun 02	72	613											
15 Jun 02	73												
16 Jun 02	74	626	65	5,388	90	1,933	1,900	0.98	403	42.3	818	19	1,116
17 Jun 02	75												
18 Jun 02	76												
19 Jun 02	77	606	34	5,490	94	2,182	2,118	0.97	367	44.0	960	21	1,222
20 Jun 02	78												
21 Jun 02	79	620	43	5,537	93	2,167	2,122	0.98	420	45.0	975	20	1,192
22 Jun 02	80												
23 Jun 02	81												
24 Jun 02	82	597	278	3,066	54	1,640	1,580	0.96	213				
25 Jun 02	83												

Experiment 5 : 20C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
26 Jun 02	84	622	415	1,991	33	1,352	1,248	0.92	111	44.8	606	11	747
27 Jun 02	85												
28 Jun 02	86												
29 Jun 02	87	595	328	2,570	45	1,029	867	0.84	194	23.6	243	4	786
30 Jun 02	88												
1 Jul 02	89												
2 Jul 02	90	611	113	4,778	82	1,848	1,714	0.93	307	10.4	192	3	1,655
3 Jul 02	91												
4 Jul 02	92	592	116	4,573	80	1,233	1,058	0.86	590	8.4	104		1,130
5 Jul 02	93												
6 Jul 02	94	602	121	4,618	80	958	853	0.89					
7 Jul 02	95	588	115	4,538	80	682	618	0.91	1220	7.4	50		632
8 Jul 02	96												
9 Jul 02	97												
10 Jul 02	98	594	76	4,975	87	1,209	1,122	0.93	761	18.0	218	73	991
11 Jul 02	99												
12 Jul 02	100												
13 Jul 02	101	569	77	4,722	86	1,392	1,367	0.98	632	28.8	401	67	991
14 Jul 02	102												
15 Jul 02	103	630	288	3,287	54	1,440	1,387	0.96	511	29.3	422	53	1,018
16 Jul 02	104												
17 Jul 02	105	636	62	5,508	90	1,794	1,743	0.97	432	27.3	490	49	1,304
18 Jul 02	106												
19 Jul 02	107	652	81	5,481	88	2,172	2,152	0.99	405	28.2	613	51	1,560
20 Jul 02	108												
21 Jul 02	109	627	90	5,161	86	1,940	1,907	0.98	408	32.0	621	44	1,319
22 Jul 02	110												
23 Jul 02	111	602	239	3,483	60	1,510	1,434	0.95	461	29.6	447	28	1,063

Experiment 5 : 20C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
24 Jul 02	112												
25 Jul 02	113	620											
26 Jul 02	114												
27 Jul 02	115	605											
28 Jul 02	116		36	5,458	94	1,631	1,569	0.96	417				
29 Jul 02	117	624	31	5,684	95	1,765	1,718	0.97	322	31.7	559	25	1,205
30 Jul 02	118												
31 Jul 02	119	606	36	5,469	94	2,477	2,426	0.98	263	33.6	832	35	1,645
1 Aug 02	120												
2 Aug 02	121												
3 Aug 02	122	620	50	5,477	92	2,279	2,242	0.98	276	34.5	786	29	1,493
4 Aug 02	123	606	34	5,490	94								
5 Aug 02	124												
6 Aug 02	125	631	81	5,276	87	2,165	2,071	0.96	253	34.0	736	25	1,429
7 Aug 02	126												
8 Aug 02	127	658	74	5,605	89	2,606	2,500	0.96	184	30.8	803	25	1,803
9 Aug 02	128												
10 Aug 02	129												
11 Aug 02	130	621	46	5,522	93	2,733	2,633	0.96	227	31.8	869	25	1,864
12 Aug 02	131												
13 Aug 02	132	613	60	5,312	90	2,709	2,582	0.95	225	33.4	905	24	1,804
14 Aug 02	133												
15 Aug 02	134	639	34	5,808	95	2,570	2,440	0.95	218	34.1	876	22	1,694

Experiment 6 : 30C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
21 Jan 02	1												
22 Jan 02	2	660	25	6,095	96	4,400	3,670	0.83					
23 Jan 02	3												
24 Jan 02	4	658	26	6,070	96	4,430	3,730	0.84					
25 Jan 02	5		32	6,008	95	4,287	3,753	0.88	147	9.4	403		3,884
26 Jan 02	6					4,293	3,793	0.88	130	7.2	309		3,984
27 Jan 02	7												
28 Jan 02	8	636	26	5,854	96	3,767	3,427	0.91	189	9.1	343		3,424
29 Jan 02	9												
30 Jan 02	10	653	26	6,021	96	3,540	3,227	0.91	184	8.2	290		3,250
31 Jan 02	11												
1 Feb 02	12	670	20	6,244	97	3,107	2,900	0.93	229	5.6	174		2,933
2 Feb 02	13		26	6,181	96	3,127	2,853	0.91	205	6.9	216		2,911
3 Feb 02	14												
4 Feb 02	15		46	5,671	93	2,760	2,520	0.91	268	9.3	257		2,503
5 Feb 02	16	636	88	5,261	86								
6 Feb 02	17	655	94	5,382	86								
7 Feb 02	18									13.4	370		2,400
8 Feb 02	19												
9 Feb 02	20	670	37	6,073	94	2,770	2,520	0.91	269	3.3	91		2,679
10 Feb 02	21												
11 Feb 02	22	626	43	5,596	93	2,000	1,930	0.96	400	2.0	40		1,960
12 Feb 02	23												
13 Feb 02	24												
14 Feb 02	25	635	25	5,851	96	2,290	1,920	0.84					
15 Feb 02	26	641	32	5,851	95	2,210	2,070	0.94					
16 Feb 02	27	649	44	5,805	93	2,570	2,230	0.87	195	3.3	85		2,485

Experiment 6 : 30C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
17 Feb 02	28												
18 Feb 02	29	647	69	5,547	89	2,680	2,230	0.83	153	4.3	115		2,565
19 Feb 02	30												
20 Feb 02	31	640	69	5,480	89	3,380	2,767	0.82	178	7.6	257		3,123
21 Feb 02	32												
22 Feb 02	33	627	13	5,898	98	3,913	3,233	0.83	160				
23 Feb 02	34	629	7	5,980	99	4,307	3,560	0.83	163				
24 Feb 02	35	637	26	5,869	96	3,973	3,227	0.81	189	9.3	370		3,604
25 Feb 02	36												
26 Feb 02	37	614	6	5,837	99	3,773	3,067	0.81					
27 Feb 02	38	608				3,533	2,887	0.82	88	9.5	336		3,198
28 Feb 02	39												
1 Mar 02	40	622	25	5,730	96	3,920	3,340	0.85	77	8.8	345		3,575
2 Mar 02	41												
3 Mar 02	42					3,227	2,787	0.86					
4 Mar 02	43	643	25	5,926	96	3,293	2,793	0.85	88	4.5	148		3,145
5 Mar 02	44	630	50	5,563	92	3,613	3,077	0.85	77	2.7	98		3,515
6 Mar 02	45		38	5,684	94	3,573	3,060	0.86	78	2.3	82		3,491
7 Mar 02	46	646	19	6,021	97	3,515	3,100	0.88	63	3.6	127		3,389
8 Mar 02	47												
9 Mar 02	48	634	32	5,778	95	3,639	3,290	0.90	121	3.0	109	36	3,530
10 Mar 02	49	651	38	5,887	94	3,542	3,303	0.93	133	1.8	64	16	3,478
11 Mar 02	50	649	32	5,930	95	3,632	3,303	0.91	165	2.1	76	15	3,556
12 Mar 02	51	658	44	5,897	93	3,400	3,107	0.91	185	1.5	51	8	3,349
13 Mar 02	52												
14 Mar 02	53	636											
15 Mar 02	54	655	6	6,229	99	3,300	3,160	0.96	211	9.6	317	35	2,983
16 Mar 02	55	649	6	6,168	99	3,387	3,155	0.93	177	8.8	298	30	3,089

Experiment 6 : 30C, P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
17 Mar 02	56												
18 Mar 02	57	663	50	5,880	92	3,827	3,567	0.93	180	9.5	364	30	3,463
19 Mar 02	58												
20 Mar 02	59	656	69	5,640	90	3,810	3,380	0.89	160	8.9	339	24	3,471
21 Mar 02	60												
22 Mar 02	61	643	26	5,917	96	4,327	3,740	0.86	159	9.8	424	27	3,903
23 Mar 02	62												
24 Mar 02	63												
25 Mar 02	64												
26 Mar 02	65	634	37	5,730	94	4,740	3,573	0.75	154	21.1	1,000	50	3,740
27 Mar 02	66												
28 Mar 02	67	636	38	5,745	94	4,533	3,347	0.74	163	27.8	1,260	57	3,273
29 Mar 02	68												
30 Mar 02	69												
31 Mar 02	70	622	43	5,553	93	3,272	2,488	0.76	226	33.7	1,103	44	2,169
1 Apr 02	71												
2 Apr 02	72	629	13	5,912	98	2,853	2,193	0.77	210	36.5	1,041	39	1,812
3 Apr 02	73												
4 Apr 02	74	625	25	5,760	96	2,958	2,292	0.77	274	36.1	1,068	37	1,890
5 Apr 02	75												
6 Apr 02	76	626	31	5,715	95	2,543	2,079	0.82	346	34.6	880	28	1,663
7 Apr 02	77												
8 Apr 02	78	615	13	5,776	98	1,874	1,607	0.86	443	37.7	707	21	1,168
9 Apr 02	79												
10 Apr 02	80	626	26	5,760	96	1,664	1,455	0.87	541	47.4	789	23	875
11 Apr 02	81												
12 Apr 02	82												
13 Apr 02	83												

Experiment 6 : 30C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
14 Apr 02	84												
15 Apr 02	85	592	176	3,991	70	885	785	0.89	384	43.0	380	10	504
16 Apr 02	86												
17 Apr 02	87	639	187	4,335	71	767	667	0.87	717	38.8	297	7	469
18 Apr 02	88												
19 Apr 02	89												
20 Apr 02	90	606	94	4,920	85	1,150	1,040	0.90	852	38.4	442	10	708
21 Apr 02	91												
22 Apr 02	92	606	50	5,340	92	633	622	0.98	821	37.1	235	5	398
23 Apr 02	93												
24 Apr 02	94												
25 Apr 02	95	631	208	4,059	67	422	389	0.92	805	27.2	115	2	307
26 Apr 02	96												
27 Apr 02	97												
28 Apr 02	98	626	226	3,840	64								
29 Apr 02	99												
30 Apr 02	100	630	302	3,149	52								
1 May 02	101												
2 May 02	102	602	285	3,036	53	540	520	0.96	1241	16.4	89	2	451
3 May 02	103												
4 May 02	104	620	31	5,656	95	760	680	0.89	895	14.5	110		650
5 May 02	105												
6 May 02	106	632	35	5,731	94								
7 May 02	107												
8 May 02	108	640	13	6,015	98	1,357	1,207	0.89	516	3.1	42		1,315
9 May 02	109												
10 May 02	110	636	38	5,745	94	1,426	1,270	0.89	617	10.6	151	76	1,275
11 May 02	111												

Experiment 6 : 30C, P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
12 May 02	112	622	44	5,547	93	1,317	1,233	0.94	592	21.5	283	71	1,034
13 May 02	113												
14 May 02	114												
15 May 02	115	630	44	5,624	93	1,418	1,236	0.87	564	20.7	294	42	1,125
16 May 02	116												
17 May 02	117												
18 May 02	118												
19 May 02	119	603	41	5,400	93	2,534				23.5	595	54	1,938
20 May 02	120												
21 May 02	121	609	159	4,320	74	2,200				22.6	497	38	1,702
22 May 02	122												
23 May 02	123												
24 May 02	124												
25 May 02	125	619	294	3,120	53	2,198				29.9	657	39	1,541
26 May 02	126												
27 May 02	127												
28 May 02	128												
29 May 02	129												
30 May 02	130	622	35	5,638	94	2,026	1,817	0.90	429	30.9	626	28	1,400
31 May 02	131												
1 Jun 02	132												
2 Jun 02	133												
3 Jun 02	134	630	48	5,584	92	2,343	1,852	0.79	389	40.7	954	37	1,390
4 Jun 02	135												
5 Jun 02	136	648	38	5,851	94	2,248	1,810	0.81	418	28.5	641	23	1,607

Experiment 7 : 10C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
21 Jan 02	1												
22 Jan 02	2	610	19	5,669	97	3,200	2,324	0.72	96				
23 Jan 02	3												
24 Jan 02	4	622	32	5,664	95	3,307	2,475	0.75	56	6.9	228		3,079
25 Jan 02	5		13	5,849	98	3,640	2,790	0.76	60				
26 Jan 02	6					3,830	2,945	0.77	60	7.7	295		3,535
27 Jan 02	7												
28 Jan 02	8	623	20	5,791	97	3,300	2,720	0.82	67	9.0	297		3,003
29 Jan 02	9												
30 Jan 02	10	627	32	5,714	95	3,760	2,965	0.79	64	6.8	256		3,504
31 Jan 02	11												
1 Feb 02	12	611	20	5,682	97	4,060	3,275	0.81	61	5.2	211		3,849
2 Feb 02	13		39	5,495	94	4,027	3,252	0.81	76	5.9	238		3,789
3 Feb 02	14												
4 Feb 02	15		33	5,796	95	3,627	3,002	0.83	84	6.4	232		3,395
5 Feb 02	16	636	50	5,624	92								
6 Feb 02	17	598	31	5,443	95	3,660	3,240	0.88	83				
7 Feb 02	18												
8 Feb 02	19												
9 Feb 02	20	626	43	5,596	93	3,380	3,063	0.90	78	13.7	463		2,917
10 Feb 02	21												
11 Feb 02	22	626	74	5,299	88	3,647	3,225	0.89	66	13.9	507		3,140
12 Feb 02	23												
13 Feb 02	24												
14 Feb 02	25	591	32	5,370	95	4,453	4,207	0.94	65	23.6	1,051	350	3,402
15 Feb 02	26	641	349	2,804	46	4,793	4,580	0.96	61	36.9	1,769	442	3,025
16 Feb 02	27	580	274	2,937	53	4,740	4,620	0.97	68	45.0	2,133	427	2,607

Experiment 7 : 10C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
17 Feb 02	28												
18 Feb 02	29	641	265	3,617	59	4,960	4,800	0.97	60	37.5	1,860	266	3,100
19 Feb 02	30	600	397	1,946	34	4,867	4,713	0.97	66	44.3	2,156	269	2,711
20 Feb 02	31	600	428	1,643	29	5,033	4,920	0.98	58	44.8	2,255	251	2,778
21 Feb 02	32												
22 Feb 02	33	609	410	1,915	33	4,940	4,880	0.99	61	42.0	2,075	189	2,865
23 Feb 02	34	612	377	2,261	38	5,300	5,193	0.98	57	43.9	2,327	194	2,973
24 Feb 02	35	648	410	2,290	37	5,053	4,933	0.98	56	39.8	2,011	155	3,042
25 Feb 02	36												
26 Feb 02	37	603	429	1,676	29	4,740	4,633	0.98		42.6	2,019	135	2,721
27 Feb 02	38	616				4,540	4,413	0.97	64	41.0	1,861	116	2,679
28 Feb 02	39	622	457	1,585	27	4,580	4,393	0.96	63	41.3	1,892	111	2,688
1 Mar 02	40	611	451	1,536	26	4,567	4,453	0.98	66	42.3	1,932	107	2,635
2 Mar 02	41												
3 Mar 02	42												
4 Mar 02	43	668	290	3,628	57	4,865	4,716	0.97	82	44.1	2,145	102	2,719
5 Mar 02	44	668	365	2,903	45	5,240	5,073	0.97	78	42.5	2,227	101	3,013
6 Mar 02	45		409	2,479	39	4,865	4,742	0.97	88	41.5	2,019	88	2,846
7 Mar 02	46	664	467	1,893	30								
8 Mar 02	47												
9 Mar 02	48	646	463	1,756	28	4,871	4,658	0.96	132	37.9	1,846	71	3,025
10 Mar 02	49												
11 Mar 02	50	651	476	1,681	27	4,820	4,673	0.97	151	38.8	1,870	67	2,950
12 Mar 02	51												
13 Mar 02	52												
14 Mar 02	53	636											
15 Mar 02	54	668	447	2,117	33	4,053	3,887	0.96	178	44.9	1,820	57	2,233

Experiment 7 : 10C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
16 Mar 02	55												
17 Mar 02	56												
18 Mar 02	57	644	488	1,500	24	3,445	3,284	0.95	209	23.5	810	23	2,636
19 Mar 02	58												
20 Mar 02	59	656	526	1,248	20	3,156	3,013	0.95	200	29.7	937	25	2,219
21 Mar 02	60												
22 Mar 02	61	662	525	1,322	21	3,140	3,013	0.96	220	19.8	622	16	2,518
23 Mar 02	62												
24 Mar 02	63												
25 Mar 02	64												
26 Mar 02	65	637	509	1,222	20	3,245	3,116	0.96	191	21.7	704	16	2,541
27 Mar 02	66	655	375	2691	43	3,108	2,927	0.94	172	21.9	681		2,427
28 Mar 02	67	643	227	3,991	65	3,127	2,940	0.94	160	15.6	488		2,639
29 Mar 02	68		163	4,603	75								
30 Mar 02	69		31	5,790	95	4,250	3,931	0.93		16.6	706		3,545
31 Mar 02	70	634	37	5,730	94	4,252	3,923	0.92	151	15.6	663		3,588
1 Apr 02	71												
2 Apr 02	72	635	38	5,730	94	3,673	3,400	0.93	139	17.8	654	327	3,019
3 Apr 02	73												
4 Apr 02	74	606	113	4,740	81	3,265	3,052	0.93	178	16.4	535	134	2,729
5 Apr 02	75												
6 Apr 02	76	639	310	3,155	51	3,267	3,093	0.95	184	14.9	487	81	2,780
7 Apr 02	77												
8 Apr 02	78	615	383	2,221	38	3,386	3,297	0.97	165	16.7	565	71	2,821
9 Apr 02	79												
10 Apr 02	80	637	387	2,398	39	2,673	2,553	0.96	224	17.4	465	47	2,208
11 Apr 02	81												

Experiment 7 : 10C, N&P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
12 Apr 02	82	610	381	2,198	38	3,194	3,025	0.95	197	15.0	479	40	2,715
13 Apr 02	83												
14 Apr 02	84												
15 Apr 02	85	611	309	2,903	49	3,166	2,986	0.94	185	15.2	481	32	2,684
16 Apr 02	86												
17 Apr 02	87	658	426	2,230	35	3,306	3,229	0.98	166	9.2	304	18	3,002
18 Apr 02	88												
19 Apr 02	89												
20 Apr 02	90	606	425	1,740	30	3,277	3,155	0.96	131	11.9	390	20	2,887
21 Apr 02	91												
22 Apr 02	92	600	394	1,980	34	3,717	3,578	0.96	145	12.3	457	21	3,260
23 Apr 02	93												
24 Apr 02	94												
25 Apr 02	95	624	410	2,060	34	3,850	3,657	0.95	112	10.7	412	16	3,438

Experiment 8 : 20C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
4 Apr 02	1	619	50	5,460	92								
5 Apr 02	2	614	93	5,001	85	3,485	2,795	0.80	77	6.8	237		3,248
6 Apr 02	3												
7 Apr 02	4	620	81	5,180	87	2,568	2,254	0.88	80	7.2	185		2,383
8 Apr 02	5												
9 Apr 02	6	624	59	5,432	91	2,232	1,964	0.88	116	12.4	277		1,955
10 Apr 02	7	626	32	5,698	95	2,516	2,191	0.87	119	9.6	241		2,274
11 Apr 02	8												
12 Apr 02	9	613	31	5,580	95	2,555	2,255	0.88	157	7.7	197		2,358
13 Apr 02	10												
14 Apr 02	11												
15 Apr 02	12	611	6	5,805	99	3,514	3,121	0.89	191	8.9	313		3,202
16 Apr 02	13												
17 Apr 02	14	652	32	5,946	95	2,793	2,585	0.93	211	8.4	235		2,558
18 Apr 02	15												
19 Apr 02	16												
20 Apr 02	17	663	25	6,120	96	2,247	2,053	0.91	320				
21 Apr 02	18												
22 Apr 02	19	631	13	5,940	98	2,818	2,316	0.82	225	6.8	192		2,626
23 Apr 02	20												
24 Apr 02	21					3,677							
25 Apr 02	22	618	33	5,620	95	3,579			84	4.6	165		3,414
26 Apr 02	23												
27 Apr 02	24												
28 Apr 02	25												
29 Apr 02	26												
30 Apr 02	27												

Experiment 8 : 20C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
1 May 02	28												
2 May 02	29	620	37	5,596	94	4,458	3,747	0.84	76	9.5	423		4,034
3 May 02	30												
4 May 02	31	626	6	5,953	99	4,151	3,591	0.87	82	8.9	369		3,782
5 May 02	32												
6 May 02	33												
7 May 02	34												
8 May 02	35	635	13	5,966	98	5,185	4,749	0.91	68	13.2	684	171	4,500
9 May 02	36												
10 May 02	37	636	126	4,898	80	5,976	5,653	0.95	59	24.5	1,464	244	4,512
11 May 02	38												
12 May 02	39	622	159	4,450	74	6,054	5,741	0.95	61	33.3	2,016	252	4,038
13 May 02	40												
14 May 02	41												
15 May 02	42	640	321	3,060	50	5,255	4,985	0.95	74	42.6	2,239	204	3,016
16 May 02	43												
17 May 02	44												
18 May 02	45												
19 May 02	46	609	266	3,300	56	6,302				22.2	1,399	93	4,903
20 May 02	47												
21 May 02	48	597	300	2,850	50	5,706				20.4	1,164	68	4,542
22 May 02	49												
23 May 02	50												
24 May 02	51												
25 May 02	52	603	369	2,250	39	4,830				15.6	753	36	4,077
26 May 02	53												
27 May 02	54												
28 May 02	55												

Experiment 8 : 20C, N&P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
29 May 02	56												
30 May 02	57	641	410	2,225	36	4,222	4,044	0.96	118	14.9	629	24	3,593
31 May 02	58												
1 Jun 02	59												
2 Jun 02	60												
3 Jun 02	61	639	418	2,123	35	4,600	4,283	0.93	107	10.8	497	17	4,103
4 Jun 02	62												
5 Jun 02	63	654	470	1,768	28	4,286	4,007	0.94	105	9.1	390	12	3,896
6 Jun 02	64												
7 Jun 02	65	613	125	4,680	80	4,607	4,243	0.92	124	3.9	180		4,427
8 Jun 02	66												
9 Jun 02	67												
10 Jun 02	68	631	46	5,612	93	2,587	2,393	0.93	205	3.3	85		2,501
11 Jun 02	69	609	34	5,524	94	2,544			216	3.6	92		2,452
12 Jun 02	70												
13 Jun 02	71	659	35	5,990	95	3,114			183	16.5	514	257	2,600
14 Jun 02	72	613											
15 Jun 02	73												
16 Jun 02	74	614	47	5,447	92	3,974	3,784	0.95	126	24.7	981	196	2,992
17 Jun 02	75												
18 Jun 02	76												
19 Jun 02	77	619	253	3,510	59	4,147	3,924	0.95	109	26.7	1,107	138	3,040
20 Jun 02	78												
21 Jun 02	79	620	298	3,096	52	4,277	4,062	0.95	94	17.8	761	76	3,516
22 Jun 02	80												
23 Jun 02	81												
24 Jun 02	82	603	257	3,324	57	5,333	5,067	0.95	85	14.7	784	60	4,549
25 Jun 02	83												

Experiment 8 : 20C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
26 Jun 02	84	616	284	3,186	54	4,408	4,129	0.94	98	11.8	520	35	3,888
27 Jun 02	85												
28 Jun 02	86												
29 Jun 02	87	632	159	4,533	75	4,813	4,481	0.93	87	8.0	385	21	4,428
30 Jun 02	88												
1 Jul 02	89												
2 Jul 02	90	641	205	4,192	68	4,650	4,329	0.93	105	10.7	498	24	4,152
3 Jul 02	91												
4 Jul 02	92	636	238	3,823	63	3,542	3,258	0.92	136	12.8	453	20	3,088

Experiment 9 : 30C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
21 Jan 02	1												
22 Jan 02	2	660	19	6,156	97	4,303	3,587	0.83					
23 Jan 02	3												
24 Jan 02	4	658	26	6,070	96	3,770	3,260	0.86					
25 Jan 02	5		32	6,008	95	4,120	3,573	0.87	153	9.3	383		3,737
26 Jan 02	6					4,073	3,607	0.89	130	11.1	452		3,621
27 Jan 02	7												
28 Jan 02	8	636	20	5,917	97	3,787	3,400	0.90	177	9.3	352		3,435
29 Jan 02	9												
30 Jan 02	10	653	38	5,898	94	3,827	3,427	0.90	162	4.7	180		3,647
31 Jan 02	11												
1 Feb 02	12	670	26	6,181	96	3,667	3,347	0.91	164	8.2	301		3,366
2 Feb 02	13		20	6,244	97	3,907	3,493	0.89	150	7.8	305		3,602
3 Feb 02	14												
4 Feb 02	15		39	5,733	94	3,433	3,107	0.90	181	7.4	254		3,179
5 Feb 02	16	636	38	5,745	94								
6 Feb 02	17	655	50	5,805	92	3,820	3,487	0.91	157				
7 Feb 02	18									4.0	153		3,667
8 Feb 02	19												
9 Feb 02	20	670				2,740	2,540	0.93	265	6.0	164		2,576
10 Feb 02	21												
11 Feb 02	22	626	43	5,596	93	2,320	2,190	0.94	356	2.1	49		2,271
12 Feb 02	23												
13 Feb 02	24												
14 Feb 02	25	635	32	5,790	95	1,933	1,773	0.92					
15 Feb 02	26	641	19	5,973	97	1,660	1,527	0.92					
16 Feb 02	27	649	38	5,866	94	1,910	1,680	0.88	393	4.0	76		1,834

Experiment 9 : 30C, N&P limitation

Date	Days	COD (mg/l)				MLSS	MLVSS	SS/VSS	SVI	PHA			Res. biom
		Inf	Eff	mg/day	%rem.	(mg/l)	(mg/l)	(mg/l)	(ml/g SS)	(%TSS)	(mg/l)	(mg/l-d)	(mg/l)
17 Feb 02	28												
18 Feb 02	29	647	88	5,366	86	2,420	2,113	0.87	351	5.8	140		2,280
19 Feb 02	30												
20 Feb 02	31	640	57	5,601	91	3,467	2,867	0.83	213	7.0	243		3,224
21 Feb 02	32												
22 Feb 02	33	627	13	5,898	98	3,527	3,007	0.85	227				
23 Feb 02	34	629	7	5,980	99	3,180	2,773	0.87	252				
24 Feb 02	35	637	26	5,869	96	2,794	2,406	0.86	197	6.2	173		2,621
25 Feb 02	36												
26 Feb 02	37	614	6	5,837	99	2,860	2,547	0.89					
27 Feb 02	38	608				2,480	2,240	0.90	238	7.4	184		2,296
28 Feb 02	39												
1 Mar 02	40	622	25	5,730	96	3,713	3,273	0.88	110	7.6	282		3,431
2 Mar 02	41												
3 Mar 02	42					3,333	2,887	0.87					
4 Mar 02	43	643	31	5,866	95	3,540	2,933	0.83	79	4.4	156		3,384
5 Mar 02	44	630	69	5,382	89	3,453	2,860	0.83	72	3.1	107		3,346
6 Mar 02	45		38	5,684	94	3,484	2,845	0.82	72	4.0	139		3,345
7 Mar 02	46	634	19	5,898	97	3,473	2,927	0.84	60	4.0	139		
8 Mar 02	47												
9 Mar 02	48	640	32	5,839	95	3,729	3,329	0.89	64	6.4	239	80	3,490
10 Mar 02	49	629	25	5,790	96	4,000	3,656	0.91	63	9.4	376	94	3,624
11 Mar 02	50	654	32	5,973	95	4,439	4,071	0.92	56	14.4	639	128	3,800
12 Mar 02	51	664	50	5,895	92	4,560	4,240	0.93	53	14.9	679	113	3,881
13 Mar 02	52												
14 Mar 02	53	649											
15 Mar 02	54	611	258	3,386	58	4,981	4,794	0.96	60	27.6	1,375	153	3,606

Experiment 9 : 30C, N&P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
16 Mar 02	55	617	233	3,689	62	4,542	4,368	0.96	66	32.5	1,476	148	3,066
17 Mar 02	56												
18 Mar 02	57	613	300	3,000	51	4,520	4,347	0.96	65	22.5	1,017	85	3,503
19 Mar 02	58												
20 Mar 02	59	619	294	3,120	53	4,594	4,406	0.96	76	19.6	900	64	3,693
21 Mar 02	60												
22 Mar 02	61	636	269	3,525	58	5,187	4,920	0.95	77	14.2	737	46	4,450
23 Mar 02	62												
24 Mar 02	63												
25 Mar 02	64												
26 Mar 02	65	622	271	3,367	56	5,302	5,007	0.94	102	9.8	520	26	4,782
27 Mar 02	66												
28 Mar 02	67	649	50	5,745	92	5,327	5,053	0.95	111	15.6	831	38	4,496
29 Mar 02	68												
30 Mar 02	69												
31 Mar 02	70	605	43	5,392	93	4,177	3,954	0.95	129	15.4	643	26	3,534
1 Apr 02	71												
2 Apr 02	72	629	38	5,669	94	4,160	3,933	0.95	156	18.4	765	28	3,395
3 Apr 02	73												
4 Apr 02	74	631	38	5,700	94	4,032	3,768	0.93	157	12.9	520	18	3,512
5 Apr 02	75	608	25	5,596	96	3,215	2,992	0.93	227	10.0	322		2,894
6 Apr 02	76	626	43	5,596	93	2,496	2,378	0.95	288	6.7	167		2,329
7 Apr 02	77	626	31	5,715	95	2,383	2,225	0.93	269	5.5	131		2,252
8 Apr 02	78	615	26	5,649	96	2,179	2,034	0.93	312	4.7	102		2,077
9 Apr 02	79	618	33	5,620	95	2,400	2,200	0.92					
10 Apr 02	80	632	32	5,760	95	2,200	2,045	0.93	136	6.5	143		2,057
11 Apr 02	81												

Experiment 9 : 30C, N&P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
12 Apr 02	82												
13 Apr 02	83												
14 Apr 02	84												
15 Apr 02	85	611	6	5,805	99	5,070	4,826	0.95	59	31.1	1,577	263	3,493
16 Apr 02	86												
17 Apr 02	87	619	58	5,388	91	5,073	4,952	0.98	67	24.4	1,238	155	3,835
18 Apr 02	88												
19 Apr 02	89												
20 Apr 02	90	638	37	5,760	94	5,553	5,329	0.96	86	21.0	1,166	106	4,387
21 Apr 02	91												
22 Apr 02	92	612	50	5,400	92	4,505	4,253	0.94	93	17.3	779	60	3,726
23 Apr 02	93												
24 Apr 02	94												
25 Apr 02	95	624	26	5,744	96	4,252	3,970	0.93	101	16.9	719	45	3,533
26 Apr 02	96												
27 Apr 02	97												
28 Apr 02	98	626	33	5,693	95								
29 Apr 02	99												
30 Apr 02	100	617	31	5,626	95								
1 May 02	101												
2 May 02	102	633	43	5,656	93	4,318	3,909	0.91	100	10.1	436	19	3,882
3 May 02	103												
4 May 02	104	626	56	5,477	91	4,600	4,263	0.93	85	14.2	653	26	3,947
5 May 02	105												
6 May 02	106												
7 May 02	107												
8 May 02	108	635	86	5,268	86	3,344	2,911	0.87	84	14.4	482	17	2,863

Experiment 9 : 30C, N&P limitation

Date	Days	COD (mg/l)				MLSS (mg/l)	MLVSS (mg/l)	SS/VSS (mg/l)	SVI (ml/g SS)	PHA			Res. biom (mg/l)
		Inf	Eff	mg/day	%rem.					(%TSS)	(mg/l)	(mg/l-d)	
9 May 02	109												
10 May 02	110	630	183	4,294	71	3,368	3,000	0.89	89	19.1	643	21	2,725
11 May 02	111												
12 May 02	112	610	171	4,206	72	3,647	3,318	0.91	96	18.1	660	20	2,987
13 May 02	113												
14 May 02	114												
15 May 02	115	611	69	5,201	89	3,789	3,337	0.88	90	14.9	565	16	3,225

The experiment 20C-1x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
25 Mar 03	1															
26 Mar 03	2	9000	1.13	282	0.04	1.09	97	5,970	5,080	0.85	360	152	0.7	42		5,928
27 Mar 03	3	9527	1.19													
28 Mar 03	4	11084	1.39	209	0.03	1.36	98	6,510	5,780	0.89	350	131	1.3	85		6,425
29 Mar 03	5															
30 Mar 03	6															
31 Mar 03	7	8356	1.04	69	0.01	1.04	99	7,250	6,490	0.90	360	119	1.2	87		7,163
1 Apr 03	8	7941	0.99	53	0.01	0.99	99	8,550	7,520	0.88	345	99	1.5	128		8,422
2 Apr 03	9							7,240	6,420	0.89	335	115	1.4	101		7,139
3 Apr 03	10	8847	1.11	75	0.01	1.10	99	7,970	7,050	0.88	380	88	1.1	88		7,882
4 Apr 03	11															
5 Apr 03	12															
6 Apr 03	13	10185	1.27	99	0.01	1.26	99	8,220	7,280	0.89	480	71	1.2	99		8,121
7 Apr 03	14															
8 Apr 03	15	9782	1.22	71	0.01	1.21	99	7,960	7,080	0.89	550	69	0.9	72		7,888
9 Apr 03	16							8,080	7,180	0.89	560	69	1.5	121		7,959
10 Apr 03	17															
11 Apr 03	18	10328	1.29	57	0.01	1.28	99	8,410	7,460	0.89	535	64	1.6	135		8,275
12 Apr 03	19															
13 Apr 03	20	10512	1.31	58	0.01	1.31	99	8,980	7,990	0.89	560	62	1.9	171		8,809
14 Apr 03	21															
15 Apr 03	22	9818	1.23	65	0.01	1.22	99	8,780	7,880	0.90	500	57	1.4	123		8,657
16 Apr 03	23															
17 Apr 03	24	10380	1.30	61	0.01	1.29	99	9,430	8,500	0.90	490	52	2.9	273	137	9,157
18 Apr 03	25	9934	1.24	63	0.01	1.23	99	9,850	8,920	0.91	480	49	3.5	345	115	9,505
19 Apr 03	26															
20 Apr 03	27							11,060	10,060	0.91	460	42	3.7	409	82	10,651

The experiment 20C-1x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
21 Apr 03	28	10161	1.27	95	0.01	1.26	99	11,290	10,300	0.91	470	42	3.3	373	62	10,917
22 Apr 03	29															
23 Apr 03	30	10476	1.31	118	0.01	1.29	99	12,080	11,070	0.92	470	39	6.0	725	91	11,355
24 Apr 03	31	10476	1.31	76	0.01	1.30	99	11,980	10,980	0.92	450	38				
25 Apr 03	32	10857	1.36	84	0.01	1.35	99	12,380	11,350	0.92	430	35	5.4	669	67	11,711
26 Apr 03	33	10476	1.31	80	0.01	1.30	99	12,200	11,200	0.92	430	35	6.6	805	73	11,395
27 Apr 03	34															
28 Apr 03	35	10099	1.26	99	0.01	1.25	99	13,540	12,550	0.93	500	37	7.1	961	74	12,579
29 Apr 03	36															
30 Apr 03	37															
1 May 03	38															
2 May 03	39	10752	1.34	184	0.02	1.32	98	14,050	13,180	0.94	440	31	10.3	1,447	85	12,603
3 May 03	40	9984	1.25	61	0.01	1.24	99	14,150	13,220	0.93	460	33				
4 May 03	41	10368	1.30	100	0.01	1.28	99	15,010	14,000	0.93	450	30	11.2	1,681	88	13,329
5 May 03	42	9984	1.25	77	0.01	1.24	99	14,860	13,980	0.94	450	30				
6 May 03	43	10752	1.34	108	0.01	1.33	99	15,070	14,150	0.94	470	31	14.2	2,140	102	12,930
7 May 03	44	10752	1.34	100	0.01	1.33	99	15,150	14,300	0.94	380	25	13.4	2,030	92	13,120
8 May 03	45	10752						14,020	13,350	0.95	350	25				
9 May 03	46	9984	1.25	115	0.01	1.23	99	14,180	13,410	0.95	340	24	17.0	2,411	100	11,769
10 May 03	47	10368	1.30	92	0.01	1.28	99	14,510	13,660	0.94	350	24	11.4	1,654	66	12,856
11 May 03	48	9984	1.25	161	0.02	1.23	98	15,260	14,350	0.94	700	46	10.0	1,526	59	13,734
12 May 03	49	9984	1.25													
13 May 03	50	9984	1.25	207	0.03	1.22	98	15,490	14,780	0.95	680	44				
14 May 03	51	10368	1.30	163	0.02	1.28	98	15,120	14,360	0.95	700	46	12.6	1,905	66	13,215
15 May 03	52	10080	1.26	192	0.02	1.24	98	15,130	14,360	0.95	680	45				
16 May 03	53							14,800	13,700	0.93	800	54	10.7	1,584		13,216
17 May 03	54	9600	1.20	184	0.02	1.18	98	16,020	14,900	0.93	900	56	9.1	1,458		14,562

The experiment 20C-1x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
18 May 03	55	9216	1.15	154	0.02	1.13	98	14,760	13,800	0.93	900	61				
19 May 03	56	9984	1.25	238	0.03	1.22	98	14,880	13,720	0.92	980	66	5.7	848		14,032
20 May 03	57	9600	1.20													
21 May 03	58	10752	1.34	246	0.03	1.31	98	12,520	11,460	0.92	850	68	6.6	826	413	11,694
22 May 03	59	11059	1.38	253	0.03	1.35	98	11,400	10,560	0.93	800	70				
23 May 03	60	9600	1.20	264	0.03	1.17	97	11,840	11,000	0.93	950	80	4.5	533	133	11,307
24 May 03	61	10800	1.35	264	0.03	1.32	98	10,580	9,800	0.93	980	93				
25 May 03	62	9200	1.15	264	0.03	1.12	97	9,960	9,180	0.92	1000	100	4.3	428	71	9,532
26 May 03	63															
27 May 03	64							8,500	7,967	0.94	1000	118	5.0	425	53	8,075
28 May 03	65			409	0.05	1.16		8,200	7,833	0.96	1000	122	6.5	533	59	7,667
29 May 03	66	10230	1.28	468	0.06	1.16	95	8,133	7,700	0.95	1000	123	7.0	569	57	7,564
30 May 03	67	9443	1.18	570	0.07	1.14	94	8,133	7,767	0.95	1000	123	10.4	846	77	7,287
31 May 03	68	9836	1.23	585	0.07	1.14	94	7,133	6,867	0.96	1000	140	7.7	549	46	6,584
1 Jun 03	69															
2 Jun 03	70	10667	1.33	514	0.06	1.27	95	6,033	5,900	0.98	1000	166	9.2	555	40	5,478
3 Jun 03	71	11048	1.38	590	0.07	1.31	95	5,033	4,933	0.98	1000	199	9.7	488	33	4,545
4 Jun 03	72	10030	1.25	681	0.09	1.17	93	4,700	4,533	0.96	1000	213	10.0	470	29	4,230

The experiment 20C-2x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
25 Mar 03	1															
26 Mar 03	2	9000	1.13	273	0.03	1.09	97	5,990	5,110	0.85	360	152	0.9	54		5,936
27 Mar 03	3	9527	1.19													
28 Mar 03	4	11084	1.39	167	0.02	1.36	98	6,470	5,730	0.89	340	130	1.1	71		6,399
29 Mar 03	5															
30 Mar 03	6															
31 Mar 03	7	8356	1.04	64	0.01	1.04	99	7,160	6,400	0.89	350	119	1.0	72		7,088
1 Apr 03	8	7941	0.99	53	0.01	0.99	99	8,530	7,580	0.89	315	96	1.3	111		8,419
2 Apr 03	9							7,340	6,420	0.87	325	112	0.8	59		7,281
3 Apr 03	10	8847	1.11	77	0.01	1.10	99	7,960	7,070	0.89	450	82	1.2	96		7,864
4 Apr 03	11															
5 Apr 03	12															
6 Apr 03	13	10185	1.27	73	0.01	1.26	99	8,350	7,450	0.89	550	66	1.8	150		8,200
7 Apr 03	14															
8 Apr 03	15	9782	1.22	67	0.01	1.21	99	7,970	7,060	0.89	515	65	1.6	128		7,842
9 Apr 03	16							8,440	7,530	0.89	520	62	1.7	143		8,297
10 Apr 03	17															
11 Apr 03	18	10328	1.29	53	0.01	1.28	99	8,460	7,500	0.89	580	69	1.6	135		8,325
12 Apr 03	19															
13 Apr 03	20	10512	1.31	60	0.01	1.31	99	9,480	8,500	0.90	550	58	2.1	199		9,281
14 Apr 03	21															
15 Apr 03	22	9818	1.23	63	0.01	1.22	99	8,820	7,870	0.89	490	56	2.4	212		8,608
16 Apr 03	23															
17 Apr 03	24	10380	2.60	53	0.01	2.58	99	10,700	9,730	0.91	490	46	6.7	717	358	9,983
18 Apr 03	25	9934	2.48	57	0.01	2.47	99	12,000	11,000	0.92	475	40	7.1	852	284	11,148
19 Apr 03	26															
20 Apr 03	27							14,180	13,120	0.93	450	32	13.8	1,957	391	12,223

The experiment 20C-2x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
21 Apr 03	28	10161	2.54	66	0.02	2.52	99	15,350	14,300	0.93	475	31	17.8	2,732	455	12,618
22 Apr 03	29															
23 Apr 03	30	10476	2.62	253	0.06	2.56	98	16,970	15,840	0.93	475	28	20.6	3,496	437	13,474
24 Apr 03	31	10476	2.62	107	0.03	2.59	99	17,710	16,580	0.94	490	28	21.3	3,772	419	13,938
25 Apr 03	32	10857	2.71	80	0.02	2.69	99	18,180	17,150	0.94	450	24	19.8	3,600	360	14,580
26 Apr 03	33	10476	2.62	91	0.02	2.60	99	19,030	18,180	0.96	420	23	23.4	4,453	405	14,577
27 Apr 03	34															
28 Apr 03	35	10099	2.52	396	0.10	2.43	96	19,420	18,560	0.96	720	37	21.2	4,117	317	15,303
29 Apr 03	36															
30 Apr 03	37							16,877	16,080	0.95	660	39	22.0	3,713	248	13,164
1 May 03	38															
2 May 03	39	10752	2.69	154	0.04	2.65	99	14,333	13,600	0.95	600	42	22.8	3,268	192	11,065
3 May 03	40	9984	2.50	768	0.19	2.30	92	15,160	14,380	0.95	850	56	21.5	3,259	181	11,901
4 May 03	41	10368	2.59	499	0.12	2.47	95	13,980	13,300	0.95	800	57	21.1	2,950	155	11,030
5 May 03	42	9984	2.50	442	0.11	2.39	96	14,420	13,820	0.96	990	69	18.0	2,596	130	11,824
6 May 03	43	10752	2.69	499	0.12	2.56	95	10,967	10,400	0.95	1000	91	15.9	1,744	83	9,223
7 May 03	44	10752	2.69	960	0.24	2.45	91	8,800	8,533	0.97	1000	114	15.0	1,320	60	7,480
8 May 03	45	10752						7,367	7,167	0.97	1000	136				
9 May 03	46	9984	2.50	1075	0.27	2.23	89	7,620	7,240	0.95	1000	131	17.2	1,311	55	6,309
10 May 03	47	10368	2.59	1075	0.27	2.32	90	6,040	5,900	0.98	1000	166				
11 May 03	48	9984	2.50	1190	0.30	2.20	88	5,040	4,880	0.97	1000	198	18.3	922	35	4,118
12 May 03	49	9984	2.50													
13 May 03	50	9984	2.50	960	0.24	2.26	90	2,680	2,580	0.96	700	373	19.0	509	18	2,171
14 May 03	51	10368	2.59	1536	0.38	2.21	85	2,380	2,320	0.97	500	84	17.8	424	15	1,956
15 May 03	52	10080	2.52	1306	0.33	2.19	87	2,520	2,480	0.98	550	79	14.1	355	12	2,165
16 May 03	53							3,000	2,960	0.99	450	50	17.5	525	17	2,475

The experiment RT-1x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
25 Mar 03	1															
26 Mar 03	2	9000	1.13	68	0.01	1.12	99	6,400	5,630	0.88	367	151	0.7	45		6,355
27 Mar 03	3	9527	1.19													
28 Mar 03	4	11084	1.39	51	0.01	1.38	100	6,520	5,800	0.89	461	140	0.8	52		6,468
29 Mar 03	5															
30 Mar 03	6															
31 Mar 03	7	8356	1.04	43	0.01	1.04	99	7,000	6,340	0.91	467	124	1.2	84		6,916
1 Apr 03	8	7941	0.99	18	0.00	0.99	100	9,100	8,160	0.90	422	90	1.1	100		9,000
2 Apr 03	9							8,150	7,330	0.90	417	100	1.4	114		8,036
3 Apr 03	10	8847	1.11	49	0.01	1.10	99	8,970	8,180	0.91	500	78	1.8	161		8,809
4 Apr 03	11															
5 Apr 03	12															
6 Apr 03	13	10185	1.27	46	0.01	1.27	100	9,150	8,280	0.90	461	67	1.5	137		9,013
7 Apr 03	14															
8 Apr 03	15	9782	1.22	50	0.01	1.22	99	8,470	7,650	0.90	500	71	2.8	237		8,233
9 Apr 03	16							8,710	7,870	0.90	572	66				
10 Apr 03	17															
11 Apr 03	18	10328	1.29	71	0.01	1.28	99	8,790	7,990	0.91	594	68	1.7	149		8,641
12 Apr 03	19															
13 Apr 03	20	10512	1.31	79	0.01	1.30	99	8,870	8,040	0.91	544	61	1.0	89		8,781
14 Apr 03	21															
15 Apr 03	22	9818	1.23	81	0.01	1.22	99	8,480	7,640	0.90	533	63	0.5	42		8,438
16 Apr 03	23															
17 Apr 03	24	10380	1.30	90	0.01	1.29	99	8,680	7,725	0.89	475	55	1.1	95	48	8,585
18 Apr 03	25	9934	1.24	106	0.01	1.23	99	9,090	8,272	0.91	460	51	1.5	136	45	8,954
19 Apr 03	26															
20 Apr 03	27							10,040	9,036	0.90	340	34	2.7	271	54	9,769

The experiment RT-1x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
21 Apr 03	28	10161	1.27	172	0.02	1.25	98	10,060	9,054	0.90	425	42	2.1	211	35	9,849
22 Apr 03	29															
23 Apr 03	30	10476	1.31	177	0.02	1.29	98	10,550	9,706	0.92	461	44	3.9	411	51	10,139
24 Apr 03	31	10476	1.31	190	0.02	1.29	98	10,150	9,338	0.92	450	44	3.6	365	41	9,785
25 Apr 03	32	10857	1.36	160	0.02	1.34	99	10,650	9,692	0.91	466	44	4.7	501	50	10,149
26 Apr 03	33	10476	1.31	149	0.02	1.29	99	10,820	9,954	0.92	456	42	4.3	465	42	10,355
27 Apr 03	34															
28 Apr 03	35	10099	1.26	141	0.02	1.24	99	11,980	11,141	0.93	410	34	4.6	551	42	11,429
29 Apr 03	36															
30 Apr 03	37							12,060	11,095	0.92	467	39	5.6	675	45	11,385
1 May 03	38															
2 May 03	39	10752	1.34	146	0.02	1.33	99	12,140	11,290	0.93	622	51	5.1	619	36	11,521
3 May 03	40	9984	1.25	115	0.01	1.23	99	11,730	10,909	0.93	700	60	4.4	516	29	11,214
4 May 03	41	10368	1.30	123	0.02	1.28	99	11,940	10,985	0.92	689	58	4.0	478	25	11,462
5 May 03	42	9984	1.25	108	0.01	1.23	99	12,050	11,086	0.92	744	62	3.6	434	22	11,616
6 May 03	43	10752	1.34	115	0.01	1.33	99	11,710	10,890	0.93	733	63	3.3	386	18	11,324
7 May 03	44	10752	1.34	123	0.02	1.33	99	11,680	10,862	0.93	722	62	2.6	304	14	11,376
8 May 03	45	10752	1.34					10,900	10,137	0.93	667	61	3.5	382	17	10,519
9 May 03	46	9984	1.25	84	0.01	1.24	99	11,620	10,690	0.92	722	62				
10 May 03	47	10368	1.30	92	0.01	1.28	99	10,980	10,102	0.92	711	65	3.8	417	17	10,563
11 May 03	48	9984	1.25	69	0.01	1.24	99	11,270	10,481	0.93	778	69	3.0	338	13	10,932
12 May 03	49	9984	1.25													
13 May 03	50	9984	1.25	54	0.01	1.24	99	10,570	9,830	0.93	678	64	3.6	381	14	10,189
14 May 03	51	10368	1.30	31	0.00	1.29	100	10,670	9,816	0.92	667	63	5.6	598	21	10,072

The experiment RT-2x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
4 Jun 03	1															
5 Jun 03	2															
6 Jun 03	3															
7 Jun 03	4	10746	1.34	663	0.08	1.26	94	9,100	7,917	0.87	440	48	0.9	82		9,018
8 Jun 03	5															
9 Jun 03	6															
10 Jun 03	7	10567	1.32	79	0.01	1.31	99	9,860	8,677	0.88	460		0.8	79		9,781
11 Jun 03	8															
12 Jun 03	9							10,070	8,761	0.87	485		0.9	91		9,979
13 Jun 03	10	10388	1.30	115	0.01	1.28	99	10,300	9,167	0.89						
14 Jun 03	11							10,010	8,909	0.89	470	47	1.0	100		9,910
15 Jun 03	12							8,730	7,770	0.89	430	49				
16 Jun 03	13	10925	1.37	107	0.01	1.35	99	9,940	8,747	0.88	450	45	1.4	139		9,801
17 Jun 03	14							10,560	9,504	0.90	480	45				
18 Jun 03	15	11463	1.43	100	0.01	1.42	99									
19 Jun 03	16							9,260	8,241	0.89						
20 Jun 03	17	11463	1.43	107	0.01	1.42	99	9,640	8,580	0.89	470	49	1.0	96		9,544
21 Jun 03	18							10,230	9,207	0.90	500	49	1.2	123		10,107
22 Jun 03	19	9375	1.17	90	0.01	1.16	99									
23 Jun 03	20							9,950	9,020	0.91	550	55	1.3	129		9,821
24 Jun 03	21	9672	1.21	129	0.02	1.19	99	9,450	8,494	0.90	550	58	1.0	95		9,356
25 Jun 03	22	10746	1.34	93	0.01	1.33	99									
26 Jun 03	23	11104	1.39	136	0.02	1.37	99	9,550	8,728	0.91	550	58	1.9	181		9,369
27 Jun 03	24							10,080	9,108	0.90						
28 Jun 03	25	9761	2.44	143	0.02	2.42	99	10,620	9,664	0.91	500	47	1.8	191	96	10,429
29 Jun 03	26															
30 Jun 03	27	9761	2.44	193	0.02	2.42	98	12,440	11,445	0.92	495	40	2.7	336	84	12,104

The experiment RT-2x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
1 Jul 03	28	9882	2.47	233	0.03	2.44	98	13,020	11,978	0.92	480	37	3.1	404	81	12,616
2 Jul 03	29	10030	2.51	254	0.03	2.48	97	13,140	12,220	0.93	505	38	3.2	420	70	12,720
3 Jul 03	30															
4 Jul 03	31	9928	2.48	301	0.04	2.44	97	12,920	12,180	0.94	515	40	4.9	633	79	12,287
5 Jul 03	32															
6 Jul 03	33	9826	2.46	313	0.04	2.42	97	13,940	12,936	0.93	540	39	7.4	1,032	103	12,908
7 Jul 03	34															
8 Jul 03	35	10087	2.52	309	0.04	2.48	97	14,667	13,640	0.93	520	35	10.0	1,467	122	13,200
9 Jul 03	36	9706	2.43	362	0.05	2.38	96	15,040	14,138	0.94	565	38	9.3	1,399	108	13,641
10 Jul 03	37															
11 Jul 03	38	10100	2.53	310	0.04	2.49	97	15,460	14,687	0.95	790	51	11.2	1,732	115	13,728
12 Jul 03	39															
13 Jul 03	40	9639	2.41	364	0.05	2.36	96	14,740	14,003	0.95	840	57	10.1	1,489	88	13,251
14 Jul 03	41															
15 Jul 03	42	10033	2.51	433	0.05	2.45	96	12,420	11,675	0.94	930	75	9.0	1,118	59	11,302
16 Jul 03	43	9738	2.43	472	0.06	2.38	95	12,667	12,033	0.95	940	74	9.3	1,178	59	11,489
17 Jul 03	44															
18 Jul 03	45	9639	2.41	511	0.06	2.35	95	12,867	12,223	0.95	900	70	8.7	1,119	51	11,747
19 Jul 03	46															
20 Jul 03	47	10131	2.53	748	0.09	2.44	93	11,467	10,900	0.95	950	83	9.4	1,078	45	10,389
21 Jul 03	48	9738	2.43	816	0.10	2.33	92									
22 Jul 03	49							11,100	10,458	0.94	890	80	10.6	1,177	45	9,923
23 Jul 03	50	9783	2.45	808	0.10	2.34	92									
24 Jul 03	51							11,033	10,482	0.95	1000	91	9.7	1,070	38	9,963
25 Jul 03	52	9640	2.41	864	0.11	2.30	91									
26 Jul 03	53															
27 Jul 03	54	9738	2.43	848	0.11	2.33	91									

The experiment RT-2x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
28 Jul 03	55															
29 Jul 03	56	9541	2.39	944	0.12	2.27	90	9,100	8,736	0.96	1000	110	6.2	560	17	8,540
30 Jul 03	57							8,733	8,471	0.97	1000	115	6.1	528	16	8,205
31 Jul 03	58															
1 Aug 03	59	9836	2.46	1141	0.14	2.32	88	6,933	6,656	0.96	1000	144	4.2	291	8	6,642

The experiment RT-3x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
4 Jun 03	1															
5 Jun 03	2															
6 Jun 03	3															
7 Jun 03	4	10746	1.34	663	0.08	1.26	94	9,100	7,917	0.87	440	48	0.9	82		9,018
8 Jun 03	5															
9 Jun 03	6															
10 Jun 03	7	10567	1.32	79	0.01	1.31	99	9,860	8,677	0.88	460		0.8	79		9,781
11 Jun 03	8															
12 Jun 03	9							10,070	8,761	0.87	485		0.9	91		9,979
13 Jun 03	10	10388	1.30	115	0.01	1.28	99	10,300	9,167	0.89						
14 Jun 03	11							10,010	8,909	0.89	470	47	1.0	100		9,910
15 Jun 03	12							8,730	7,770	0.89	430	49				
16 Jun 03	13	10925	1.37	107	0.01	1.35	99	9,940	8,747	0.88	450	45	1.4	139		9,801
17 Jun 03	14							10,560	9,504	0.90	480	45				
18 Jun 03	15	11463	1.43	100	0.01	1.42	99									
19 Jun 03	16							9,260	8,241	0.89						
20 Jun 03	17	11463	1.43	107	0.01	1.42	99	9,640	8,580	0.89	470	49	1.0	96		9,544
21 Jun 03	18							10,230	9,207	0.90	500	49	1.2	123		10,107
22 Jun 03	19	9375	1.17	90	0.01	1.16	99									
23 Jun 03	20							9,950	9,020	0.91	550	55	1.3	129		9,821
24 Jun 03	21	9672	1.21	129	0.02	1.19	99	9,450	8,494	0.90	550	58	1.0	95		9,356
25 Jun 03	22	10746	1.34	93	0.01	1.33	99									
26 Jun 03	23	11104	1.39	136	0.02	1.37	99	9,550	8,728	0.91	550	58	1.9	181		9,369
27 Jun 03	24							10,480	9,469	0.90						
28 Jun 03	25	9761	3.66	145	0.02	3.64	99	12,360	11,240	0.91	520	42	2.3	278	139	12,082
29 Jun 03	26															
30 Jun 03	27	9761	3.66	213	0.03	3.63	98	13,570	12,530	0.92	560	41	3.8	516	129	13,054

The experiment RT-3x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
1 Jul 03	28	9882	3.71	329	0.04	3.66	97	14,230	13,030	0.92	550	39	4.3	612	122	13,618
2 Jul 03	29	10030	3.76	381	0.05	3.71	96	14,440	13,450	0.93	630	44	7.2	1,040	173	13,400
3 Jul 03	30															
4 Jul 03	31	9928	3.72	451	0.08	3.64	95	15,780	14,940	0.95	680	43	9.6	1,515	189	14,265
5 Jul 03	32															
6 Jul 03	33	9826	3.68	339	0.06	3.62	97	17,520	16,400	0.94	920	53	13.9	2,427	243	15,093
7 Jul 03	34															
8 Jul 03	35	10087	3.78	432	0.08	3.70	96	17,220	16,320	0.95	900	52	15.7	2,695	225	14,525
9 Jul 03	36	9706	3.64	353	0.07	3.57	96	18,040	17,180	0.95	890	49	15.8	2,841	219	15,199
10 Jul 03	37															
11 Jul 03	38	10100	3.79	420	0.08	3.71	96	17,160	16,320	0.95	980	57	14.5	2,488	166	14,672
12 Jul 03	39															
13 Jul 03	40	9639	3.61	452	0.08	3.53	95	17,320	16,400	0.95	1000	58	13.1	2,269	133	15,051
14 Jul 03	41															
15 Jul 03	42	10033	3.76	561	0.11	3.66	94	16,700	15,860	0.95	1000	60	13.5	2,246	118	14,454
16 Jul 03	43	9738	3.65	511	0.10	3.56	95	16,300	15,520	0.95	1000	61	12.7	2,062	103	14,238
17 Jul 03	44															
18 Jul 03	45	9639	3.61	570	0.11	3.51	94	14,933	14,133	0.95	1000	67	12.4	1,844	84	13,089
19 Jul 03	46															
20 Jul 03	47	10131	3.80	521	0.10	3.70	95	13,833	13,300	0.96	1000	72	12.2	1,688	70	12,146
21 Jul 03	48	9738	3.65	748	0.14	3.51	92									
22 Jul 03	49							12,167	11,612	0.95	1000	82	11.8	1,436	55	10,731
23 Jul 03	50	9783	3.67	826	0.15	3.51	92									
24 Jul 03	51							11,800	11,233	0.95	1000	85	14.8	1,746	62	10,054
25 Jul 03	52	9640	3.61	965	0.18	3.43	90									
26 Jul 03	53															
27 Jul 03	54	9738	3.65	1135	0.21	3.44	88									

The experiment RT-3x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
28 Jul 03	55															
29 Jul 03	56	9541	3.58	1259	0.24	3.34	87	10,167	9,800	0.96	1000	98	13.7	1,393	42	8,774
30 Jul 03	57							9,400	9,008	0.96	1000	96	10.9	1,025	30	8,375
31 Jul 03	58															
1 Aug 03	59	9836	3.69	3541	0.66	3.02	64	8,267	7,969	0.96	1000	108	13.1	1,083	30	7,184

The experiment RT-4x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
4 Jun 03	1															
5 Jun 03	2															
6 Jun 03	3															
7 Jun 03	4	10746	1.34	663	0.08	1.26	94	9,100	7,917	0.87	440		0.9	82		9,018
8 Jun 03	5															
9 Jun 03	6															
10 Jun 03	7	10567	1.32	79	0.01	1.31	99	9,860	8,677	0.88	460		0.8	79		9,781
11 Jun 03	8															
12 Jun 03	9							10,070	8,761	0.87	485		0.9	91		9,979
13 Jun 03	10	10388	1.30	115	0.01	1.28	99	10,300	9,167	0.89						
14 Jun 03	11							10,010	8,909	0.89	470	47	1.0	100		9,910
15 Jun 03	12							8,730	7,770	0.89	430	49				
16 Jun 03	13	10925	1.37	107	0.01	1.35	99	9,940	8,747	0.88	450	45	1.4	139		9,801
17 Jun 03	14							10,560	9,504	0.90	480	45				
18 Jun 03	15	11463	1.43	100	0.01	1.42	99									
19 Jun 03	16							9,260	8,241	0.89			1.3	120		9,140
20 Jun 03	17	11463	1.43	107	0.01	1.42	99	9,640	8,580	0.89	470	49	1.0	96		9,544
21 Jun 03	18							10,230	9,207	0.90	500	49	1.2	123		10,107
22 Jun 03	19	9375	1.17	90	0.01	1.16	99									
23 Jun 03	20							9,950	9,020	0.91	550	55	1.3	129		9,821
24 Jun 03	21	9672	1.21	129	0.02	1.19	99	9,450	8,494	0.90	550	58	1.0	95		9,356
25 Jun 03	22	10746	1.34	93	0.01	1.33	99									
26 Jun 03	23	11104	1.39	136	0.02	1.37	99	9,550	8,728	0.91	550	58	1.9	181		9,369
27 Jun 03	24															
28 Jun 03	25	10022	1.25	145	0.04	1.22	99	10,480	9,469	0.90	480	46	1.5	157		10,323
29 Jun 03	26															
30 Jun 03	27	10522	1.32	193	0.05	1.27	98	9,900	8,981	0.91	400	40	2.0	198		9,702

The experiment RT-4x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
1 Jul 03	28	10765	1.35	229	0.06	1.29	98	9,800	8,795	0.90	422	43				
2 Jul 03	29	10030	1.25	211	0.05	1.20	98	9,950	9,077	0.91	389	39	2.3	229		9,721
3 Jul 03	30															
4 Jul 03	31	9761	4.88	1254	0.31	4.57	87	12,440	11,175	0.90	489	39	3.1	386	193	12,054
5 Jul 03	32															
6 Jul 03	33	9761	4.88	1655	0.41	4.47	83	16,740	15,452	0.92	533	32	5.7	954	239	15,786
7 Jul 03	34															
8 Jul 03	35	9882	4.94	2041	0.51	4.43	79	18,020	16,832	0.93	689	38	10.9	1,964	327	16,056
9 Jul 03	36	10030	5.01	1982	0.50	4.52	80	19,864	18,724	0.94	570	29				
10 Jul 03	37															
11 Jul 03	38	9928	4.96	4836	1.21	3.76	51	20,780	19,740	0.95	850	41	11.9	2,473	275	18,307
12 Jul 03	39															
13 Jul 03	40	9826	4.91	5130	1.28	3.63	48	21,860	21,060	0.96	920	42	17.8	3,891	354	17,969
14 Jul 03	41															
15 Jul 03	42	10087	5.04	4853	1.21	3.83	52	20,780	19,940	0.96	1000	48	16.4	3,408	262	17,372
16 Jul 03	43	9706	4.85	4471	1.12	3.74	54	19,700	18,800	0.95	1000	51	15.4	3,034	217	16,666
17 Jul 03	44															
18 Jul 03	45	10100	5.05	4800	1.20	3.85	52	16,067	15,433	0.96	1000	62	16.5	2,651	166	13,416
19 Jul 03	46															
20 Jul 03	47	9639	4.82	3754	0.94	3.88	61	13,000	12,400	0.95	1000	77	14.5	1,885	105	11,115
21 Jul 03	48	10033	5.02	3951	0.99	4.03	61									
22 Jul 03	49							11,267	10,800	0.96	1000	89	13.9	1,560	78	9,706
23 Jul 03	50	9738	4.87	3361	0.84	4.03	65									
24 Jul 03	51							9,700	9,400	0.97	1000	103	16.1	1,562	71	8,138
25 Jul 03	52	9639	4.82	3361	0.84	3.98	65									
26 Jul 03	53															
27 Jul 03	54	10131	5.07	4131	1.03	4.03	59									

The experiment RT-4x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
28 Jul 03	55															
29 Jul 03	56	9738	4.87	3049	0.76	4.11	69	8,400	8,067	0.96	1000	119	15.1	1,268	47	7,132
30 Jul 03	57	9783	4.89	2877	0.72	4.17	71	8,633	8,233	0.95	1000	116	16.4	1,416	51	7,217
31 Jul 03	58															
1 Aug 03	59	9640	4.82	3263	0.82	4.00	66	7,600	7,400	0.97	1000	132	10.8	821	27	6,779

The experiment RT-6x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
4 Jun 03	1															
5 Jun 03	2															
6 Jun 03	3															
7 Jun 03	4	10746	1.34	663	0.08	1.26	94	9,100	7,917	0.87	440		0.9	82		9,018
8 Jun 03	5															
9 Jun 03	6															
10 Jun 03	7	10567	1.32	79	0.01	1.31	99	9,860	8,677	0.88	460		0.8	79		9,781
11 Jun 03	8															
12 Jun 03	9							10,070	8,761	0.87	485		0.9	91		9,979
13 Jun 03	10	10388	1.30	115	0.01	1.28	99	10,300	9,167	0.89						
14 Jun 03	11							10,010	8,909	0.89	470	47	1.0	100		9,910
15 Jun 03	12							8,730	7,770	0.89	430	49				
16 Jun 03	13	10925	1.37	107	0.01	1.35	99	9,940	8,747	0.88	450	45	1.4	139		9,801
17 Jun 03	14							10,560	9,504	0.90	480	45				
18 Jun 03	15	11463	1.43	100	0.01	1.42	99									
19 Jun 03	16							9,260	8,241	0.89						
20 Jun 03	17	11463	1.43	107	0.01	1.42	99	9,640	8,580	0.89	470	49	1.0	96		9,544
21 Jun 03	18							10,230	9,207	0.90	500	49	1.2	123		10,107
22 Jun 03	19	9375	1.17	90	0.01	1.16	99									
23 Jun 03	20							9,950	9,020	0.91	550	55	1.3	129		9,821
24 Jun 03	21	9672	1.21	129	0.02	1.19	99	9,450	8,494	0.90	550	58	1.0	95		9,356
25 Jun 03	22	10746	1.34	93	0.01	1.33	99									
26 Jun 03	23	11104	1.39	136	0.02	1.37	99	9,550	8,728	0.91	550	58	1.9	181		9,369
27 Jun 03	24							10,080	9,108	0.90						
28 Jun 03	25	9761	7.32	4836	1.81	5.51	50	10,860	11,800	0.92	480	44	1.5	163	81	10,697
29 Jun 03	26															
30 Jun 03	27	9761	7.32	5552	2.08	5.24	43	10,440	11,650	0.94	400	38	1.0	104	26	10,336

The experiment RT-6x

Date	Days	Inf. COD		Eff. COD		CODu	Removal	MLSS	MLVSS	SS/VSS	V30	SVI	PHA			Res.biom
		mg/l	g/l-d	mg/l	g/l-d	g/l-d	%	(mg/l)	(mg/l)	(mg/l)	(ml/1000 ml)	(ml/g SS)	(%TSS)	(mg/L)	(mg/L/d)	(mg/l)
1 Jul 03	28	9882	7.41	6353	2.38	5.03	36	10,020	11,260	0.94	420	42	1.1	110	22	9,910
2 Jul 03	29	10030	7.52	6806	2.55	4.97	32	10,100	14,300	0.95	420	42	1.3	131	22	9,969
3 Jul 03	30															
4 Jul 03	31	9928	7.45	7343	2.75	4.69	26	10,040	11,400	0.95	500	50	1.4	141	18	9,899
5 Jul 03	32															
6 Jul 03	33	9826	7.37	8130	3.05	4.32	17	9,840	11,000	0.93	690	70	1.8	177	18	9,663
7 Jul 03	34															
8 Jul 03	35	10087	7.57	8529	3.20	4.37	15	11,433	12,500	0.93	870	76	2.7	309	26	11,125

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