BSPE 97636-00-1051-7

한국 심해환경 연구해역의 미생물 생체량 및 생산력 연구

Biomass and Productivity of Bacterioplankton in the Northeast Equatorial Pacific: Potential Environmental Implication of Deep-Sea Manganese Nodule Mining

1998. 2.

한 국 해 양 연 구 소

제 출 문

한국해양연구소장 귀하

본 보고서를 "한국 심해환경 연구해역의 미생물 생체량 및 생산력 연구" 사업의 최종보고서로 제출합니다.

1998 년 2 월

연구책임자 : 현 정 호

연 구 원 : 이 경 용
정 갑 식
정 회 수
지 상 범
김 현 섭
김 경 홍
박 승 현
박 정 희
최 윤 지
이 근 창
조 광 식
김 연 주

요 약 문

I. 제 목

한국 심해환경 연구해역의 미생물 생체량 및 생산력 연구

II. 연구개발의 내용 및 결과

심해저 망간단괴 채광에 따른 잠재적인 환경충격을 평가하기 위해서는 광범위한 환경기초 연구가 필요하다. 한국 심해환경연구의 일환으로 1996년과 1997년에 북적도 태평양의 한국 심해환경 연구해역에서 수층의 물리, 화학 및 생물학적 환경요인과 함께 박테리아의 생체량 및 생산력을 측정하였다. 수온, 염분도, 광 투과도 및 엽록소 농도의 수직적 분포특성이 강한 계절적 수온약층의 형성에 의해 결정되는 것으로 나타났다. 박테리아의 생체량 및 생산력도 이러한 물리, 화학 및 생물학적 요인들에 의해 영향을 받아 표층하 엽록소 최대층이 존재하며, 생장제한 영양염이 증가하기 시작하는 수온약층 부근에서 최대로 나타났다. 수평적으로는 표층수의 용승이 일어나는 북적도 해류(서향류)와 북적도반류 (동향류)의 경계면에서 높은 박테리아의 생체량과 생산력을 나타냈다.

조사해역 박테리아의 생장은 일차적으로는 이용 가능한 유기탄소에 의해서, 그리고 미량영양원들 (예: 철이온)에 의해서 일정한 정도가 제한되는 것으로 나타났다. 영양염과 각종 미량원소들이 풍부한 저층수와 퇴적물 및 망간단괴 조각들을 첨가한 표층수내의 박테리아 생장이 현저히 증가하는 것으로 나타났다. 이상의 결과들로 미루어 심해저 망간단괴 채광시 저층에서 표층으로 유입되는 저층수 및 퇴적물들이 박테리아 군집 및 수서생태계에 미치는 환경충격을 고려한 채광 시스템이 개발되어야 할 것이다.

SUMMARY

I. Title

Biomass and Productivity of Bacterioplankton in the Northeast Equatorial Pacific: Potential Environmental Implication of Deep-Sea Manganese Nodule Mining

II. Abstract

In order to assess potential environmental impact by manganese nodule mining, extensive environmental baseline researches are necessary. As a part of environmental studies in the KODOS (Korea Deep Ocean Study) project, bacterial biomass and productivity together with physico-chemical and biological environmental parameters in the water column of the northeast equatorial Pacific environment have been investigated in 1996 and 1997. A strong seasonal thermocline influenced the vertical distribution patterns of water temperature, salinity, light transmission and the concentrations of chlorophyll-*a*. Distribution patterns of bacterial biomass and productivity were mainly determined by the physico-chemical and biological parameters in the water column, i.e., high biomass and productivity at the strong seasonal thermocline where chlorophyll maximum layer occurs. Horizontally, high biomass and productivity were appeared around the boundary of westward north equatorial current and eastward north equatorial counter current where surface divergence occurs.

Heterotrophic bacterial growth was limited by the availability of organic carbon (and trace nutrients to some extent) in the nutrient depleted surface water column. Addition of nutrient- and trace metal-enriched bottom water-sediment slurry remarkably enhanced the bacterial growth. The results suggested that the potential environmental impact by the intrusion of benthic plume on the surface water column ecosystem, including bacterial community, should be carefully considered in designing and constructing the mining system in the future.

CONTENTS

표 목 차
(List of Tables)

그 림 목 차
(List of Figures)

Introduction

As land based natural resources are exhausted, the mineral resources in the deep seabed, such as ferromanganese nodules, cobalt-rich manganese crusts, and polymetallic sulfide deposits in the hydrothermal vents, have received a great attention. (Earney, 1990; Emery and Broadus, 1989; Emery and Skinner, 1977). Those deep sea mineral resources are abundant in rare strategic metals such as Ni, Co, Cu and Mn (Amsbaugh and Van der Voort, 1982), and manganese nodules that are ubiquitous on the deep-sea floor have been explored the most extensively for the purpose of commercial exploitation during the past 30 years (Andrews and Friedrich, 1979; Padan, 1990).

It should be noted, however, that several environmental impacts in the water column and the benthic environments may occur by the mining activity (Curtis, 1982). The surface water column of the northeast equatorial Pacific over the manganese nodule field is a typical oligotrophic environment. Major inorganic nutrients such as nitrogen, phosphorus, and silicate are biolimiting in the surface mixed layer (Riley and Skirrow, 1965). Trace metals, such as Fe, Zn, Cd, and Mo, that are essential for the phytoplankton growth are also biolimiting in the surface water of oligotrophic open ocean, while both bottom water and sediments are abundant in those biolimiting trace elements (Martin and Gordon, 1988; Bruland and Frank, 1983). During the commercial mining of deep seabed manganese nodules, sediments and nutrient rich bottom water will be hydraulically drawn to the mining vessel together with the nodules. After separating nodules, the remaining mixture of bottom water, sediment and nodule fragments that may be discharged back into the water column is a primary environmental concern. The intrusion of nutrient- and metal-enriched bottom water into the surface water column may influence the community structure of microbial populations (i.e., by causing either surface blooming or toxic metal accumulation) must be resolved first before any commercial exploitation of manganese nodules.

In the oligotrophic open ocean, heterotrophic bacteria play a significant role as a major consumer of the photosynthetically fixed organic carbon and as a trophic link in the microbial food web processes. Bacteria consume up to 50% of total primary production (Cole *et al.*, 1988). Bacterial biomass dominates phytoplankton in the oligotrophic open ocean, indicating the system mostly maintained by heterotrophs with respect to biomass distribution (Cho and Azam, 1990; Dortch and Packard, 1989;

Fuhrman *et al.*, 1989). Both autotrophic picoplankton and heterotrophic bacterioplankton serve as a food for small heterotrophic nanoflagellates and ciliates that are then consumed by metazoan microzooplanktons (Azam *et al.*, 1983; Sherr *et al.*, 1986). Bacteria also play a remineralizer recycling dissolved organic carbon from non-sinking particles (Cho and Azam, 1988; Karl *et al.*, 1988). In order to evaluate and discuss any potential environmental impacts of Mn nodule mining on the water column ecosystem, it is essential to investigate the natural state of bacterial biomass and productivity and major natural factors controlling bacterial growth before any artificial impact on the planktonic communities.

Several results were reported in relation to the plume effect on the photoautotrophic plankton communities during DOMES (Deep Ocean Mining Environmental Study) project (Fryxell *et al.*, 1979; Chan and Anderson, 1981; Hirota, 1981), and recently during KODOS (Korea Deep Ocean Study) protect (Hyun *et al.*, 1998). In spite of the ecological significance in the oligotrophic open ocean, however, experiments designed for heterotrophic bacteria have not been conducted so far.

During the KODOS 96-1 and 97-2 cruises, designed for the environmental baseline research on the water column and benthic ecosystem on the manganese nodule field, we collected physico-chemical and biological environmental parameters, and performed shipboard-enrichment experiments in order to (1) investigate the bacterial biomass and productivity, (2) find out the major limiting nutrient for the growth of heterotrophic bacteria in the northeast equatorial Pacific, and (3) the potential effects of nutrient and metal-rich bottom water-sediment slurry on the growth of heterotrophic bacteria. The results discussed in this report can be used as a basic information in designing and constructing environmentally-affiliated mining system in the future.

Methods

Study area

The northeast equatorial Pacific is characterized by a physically dynamic oceanic circulation pattern which generates complex physico-chemical environment. Westward north equatorial current (NEC; 8 - 20°N) and eastward north equatorial counter current (NECC; 3 - 8°N) induce regional divergence along the boundary of NEC and NECC. The divergence results in the formation of nutrient richer surface water than that of the displaced surface water, and therefore results in enhanced biological

productivity (Pickard and Emery, 1982).

Cruises for the environmental studies were carried out as part of KODOS project during May, 1996 and June-July, 1998. The KODOS area is located at 9°54' - 10°27'N and 131°13' - 131°53'W within Clarion-Clipperton Fracture Zone (C-C Zone) in the northeast equatorial Pacific (Fig. 1). This area was selected based on the bottom conditions including manganese nodule abundance, sediment softness, and topography which are major factors determining future manganese nodule mining. Westward north equatorial current (NEC) prevails in the KODOS area (Stn. 1 and 5) in 1996. Stn. 29 (9°N) and Stn. 26 (7°N) are located in the south edge of NEC, and in the eastward NECC, respectively.

Physico-chemical parameters

Water temperature and salinity were measured using CTD meter (SBE 911 plus, Seabird Electronics Co.). Seawater samples for chemical analysis were collected in the acid-washed Niskin bottles attached on the rosette sampler. Samples for the nutrient analysis were stored at - 20°C until ready to process in the lab (Parsons *et al.*, 1984). Dissolved oxygen (DO) was measured using DO sensor attached on the CTD meter, and was corrected by the titration method (Parsons *et al.*, 1984). Light transmission was recorded by transmissometer (Seatech Co., PN24064; Light path 25 cm) attached on the CTD meter.

Chlorophyll-a

Concentrations of Chl-*a* were measured fluorometrically (Parsons *et al.*, 1984). In order to analyze the size distribution of Chl-*a* containing cells, water samples were fractionated by passing it through either 20-μm nylon mesh for nano fraction or 3-μm polycarbonate mambrane filters for the pico fractions (Poretics Co., Livermore, Ca.).

Microscopic enumeration of bacteria

For the enumeration of bacterial abundance, water samples were preserved with glutaldehyde (final conc. of 1%), and stored in the freezer at - 20°C until ready to process. Bacterial water samples were filtered onto Nuclepore filters (0.2 μm pore size, black), stained with DAPI (Porter and Feig, 1980) and mounted with immersion oil (Cargille type A). Samples were enumerated using an epifluorescence microscope (Nikon) equipped with mercury lamp (HB-10101 AF), UV excitation filter and BA 420 barrier filter.

Heterotrophic bacterial production

Bacterial production was estimated from the incorporation rate of ^{3}H-thymidine (Fuhrman and Azam, 1980, 1982). Briefly, samples were incubated with ^{3}H-TdR (final conc. of 5 nM thymidine, New England Nuclear Co., NET 027X) for 30 min. in disposable plastic centrifuge tubes. Samples were poured into a 50% ice-cold TCA (final conc. of 5%), and TCA insolubles were extracted for 15 min. Samples were collected by vacuum filtration on 0.2 μm cellulose nitrate membrane filters (MFS), and rinsed with ice-cold 80% ethanol. Filters were placed in scintillation vials, and the radioactivity in TCA-insoluble macromolecules were counted using liquid scintillation counter (LKB, RackBeta II) after the addition of 10 mL scintillation fluor (Lumagel Safe, Lumac-LSC).

Conversion factors

Phytoplankton biomass was estimated from Chl-*a* measurement using a C:Chl-*a* ratio of 35 for samples above the pycnocline and 17 for those below the pycnocline. The values were selected from Vedernikov's results used at the equatorial Pacific (reviews of Longhurst and Harrison, 1989; Li *et al.*, 1992).

Bacterial carbon biomass was estimated from bacterial cell number and conversion factor of 20 fg carbon per cell (Lee and Fuhrman, 1987). Conversion factor used for converting the TdR into bacterial cell production was 2.15 x 10^{18} cells mol^{-1} which was used in the JGOFS EqPac studies (Kirchman *et al.*, 1995).

Limiting resources

Time-course experiments were performed in order to determine the most limiting resources (organic carbon or nitrogen) for the heterotrophic bacterial growth in the KODOS area. Water samples were collected at Stn. 5 in 1996, and dispensed to acid washed 4 L Cubitainers (Hedwin Co.). The samples were amended with: (1) amino acids mixture (final conc.; 0.5 μM of each of Asp, Glu, Ser, Thr, His, Gly, Ala, Tyr, Val, Met, Phe, Ile, Leu, and Lys); (2) NH_4Cl (final conc.; 1 μM of N); (3) Glucose (final conc. of 2 μM, Simga Co.); (4) amino acid mixture and ammonia; and (5) unamended control.. After amended, 50 mL water samples were withdrawn from the Cubitainers, and were incubated for 1 hour after the addition of 4 μM of ^{3}H-TdR (Specific activity: 20 Ci $mmole^{-1}$). Radioactivities were counted after the treatment of radiolabeled extracts as described in the method of bacterial production.

Iron effect

In order to determine the effect of Fe for the heterotrophic bacterial growth in the KODOS area water samples were collected at Stn. I-5 in 1997, and dispensed to acid washed 4 L Cubitainers (Hedwin Co.). The samples were amended with: (1) amino acids mixture (final conc.; 2.5 nM of each of amino acid, Sigma AA-S-18); (2) $FeCl_3$ $6H_2O$ (final conc.; 50 nM of Fe); (3) amino acid mixture plus Fe; and (4) untreated control. After amended, 40 mL water samples were withdrawn periodically from the Cubitainers, and were incubated for 30 min after the addition of 3 μCi of ^{3}H-TdR (Specific activity: 6.7 Ci mmole^{-1}). Variations of radioactivities incorporated into TCA insolubles were counted after the treatment of radiolabeled extracts as described in the method of bacterial production.

Slurry effect

Time-course experiments were designed in order to determine the impact of bottom water and sediment slurry on the heterotrophic bacterial growth in the KODOS area. Water samples were collected at Stn. P-21 during KODOS97-2, and dispensed to acid washed 4 L Cubitainers (Hedwin Co.). Slurries were prepared by mixing surface sediment in the 0 – 3 cm depth of the multiple corer and Mn nodules fragments with bottom water (250 mL). The slurries were thoroughly mixed, and divided into 2 portions. One portion (Slurry I) was added to surface water sample, and one other portion was sonicated for 120 min (Slurry II) before mixed with the surface water samples. It was speculated that the sonication may alter the physical properties of particles in the slurry, and may release more trace metals. The final concentration of the slurries in the water sample was 0.01%. After amended, 40 mL water samples were withdrawn from the Cubitainers, and were incubated for 30 min. after the addition of 3 μCi of ^{3}H-TdR (Specific activity: 6.7 Ci mmole^{-1}). Radioactivities were counted after the treatment of radiolabeled extracts as described in the method of bacterial production.

Results and Discussion

Environmental parameters

Water temperature varied from 26.53 to 27.61°C (average 27°C) above the thermocline, and ranged from 10.4 to 15.3°C (average 12°C) below the thermocline to 200 m depth (Fig. 2). In the KODOS area (Stn. 1 and 5), a seasonal thermocline was formed at about 75 m depth while it was

at 50 m depth at Stns. in the lower latitude (Stn. 26, 27 and 29). Divergence of water mass by Coriolis force occurred at Stn. 27 (8°N) where westward north equatorial current (NEC) and eastward north equatorial counter current (NECC) formed a front. This divergence caused a formation of a shallow thermocline depth of 30 m at Stn. 27 (Fig. 2). Salinity ranged from 33.6 to 34.6 ‰ (average 34.2 ‰) above the thermocline, and ranged from 34.57 to 34.73 ‰ (average 34.70 ‰) below the thermocline (Fig. 2). Lower salinity in the surface layer of the lower latitude (Stns. 26, 27 and 29; average 33.8 ‰) is due to higher precipitation compared to that of the KODOS Stns (average 34.4 ‰). DO measurement showed a uniform distribution (average 4.07 mL L^{-1}) above the thermocline, but showed a sharp decrease from BDL (below detection level) to 1.29 mL L^{-1} (average 0.37 mL L^{-1}) below the thermocline due to the intense microbial respiration and the decreased supply from surface (Fig. 3). Light transmission ranged from 87.7 to 90.5% (average 89.4%) in the surface mixed layer, but increased with the range from 89.42 to 91.36 (average 90.6%) below the thermocline (Fig. 3). Low transmission above the thermocline is influenced by the high biomass in the mixed layer. The lowest transmission appeared around the thermocline where chlorophyll maximum layer exists with average from 87.5 % (30 m depth in Stn. 26) to 89.6 % (72 m depth in Stn. 5) indicating that the biomass is the major factor controlling the light transmission in the water column of the study area. Inorganic nutrients (nitrate, phosphate and silicate) were appeared to be depleted in the surface mixed layer by biological uptake, and increased gradually from the bottom of the thermocline by the diffusion from below the thermocline (Fig. 4). In the surface mixed layer, nitrate, phosphate and silicate ranged from 0.03 to 1.47 μM (average 0.33 μM), from 0.02 to 0.68 μM (average 0.25 μM), and from 0.43 to 2.81 μM (average 1.26 μM), respectively. The inorganic nutrients concentrations from the thermocline to 200 m depth were then increased, and ranged from 9.64 to 37.43 μM (average 22.79 μM), from 0.76 to 3.36 μM (average 1.78 μM), and from 8.12 to 49.72 μM (average 19.51 μM), respectively. The concentrations in the surface mixed layer were higher in the Stns. 26 and 29, which is associated with the surface water divergence occurring at the Stn. 27 (Fig. 4). It is, therefore, that the distribution of physico-chemical parameters was closely associated with the vertical and horizontal distribution patterns of water mass.

Chl-a and phytoplankton biomass

Concentrations of Chl-*a* ranged from 0.11 to 0.67 mg m^{-3}, and

appeared to be regulated by the physico-chemical conditions of water mass. Subsurface Chl-*a* maximum (SCM) layer generally appeared at the seasonal thermocline where both light and nutrients conditions for the growth of phytoplankton are available (Fig. 5). The SCM in the KODOS area appeared at the depth of about 75 m, while it was about 50 m depth in Stn. 26. Size fractionation of Chl-*a* revealed that nano- (passing through 20 μm screen) and pico fractions (passing through 3 μm filters) accounted for approximately 90% and 57% of total Chl-*a*, indicating that the small-sized phytoplankton is the major component for primary production (Table 1). Carbon biomass, estimated from the Chl-*a* concentrations integrated to 120 m depth, ranged form 85.7 to 111.5 mmole C m^{-2} (average 97.8). Chl-*a* biomass was higher at Stn. 26 than that in the KODOS area because of the high concentrations of nutrients in the surface mixed layer by divergence around Stn. 27 (Table 1).

Bacterial biomass and productivity

Bacterial biomass ranged from 56.7 to 77.5 mmole C m^{-2} (average 65.2) (Table 1). Integrated biomass, down to 120 m in the Stns. 26 and 29, were lower than those in the KODOS area. It seems to be related to the shallow thermocline depth, since bacterial biomass decreased sharply below the thermocline (biomass integrated to 60 m depth was similar among the Stns. ranging from 35 to 43 mmole C m^{-2}). The biomass presented in this study is generally lower than those of Kirchman *et al.* (1995) during February-March (El Nino) and August - September, and those of Ducklow *et al.* (1995) during March-April, and October. Seasonal and spatial variations of bacterial biomass should be investigated further.

Heterotrophic bacterial production ranged from 11.0 to 19.8 mmole C m^{-2} d^{-1} (average 14.7). Bacterial productions in Stns. 26 and 29 were higher than those in the KODOS area (Table 1), which were associated with the higher chlorophyll and nutrients concentration resulting from the divergence. Vertical profile of bacterial cell number and production was associated with the distribution patterns of physico-chemical and biological parameters (Fig. 6). Bacterial production was generally the highest at the SCM layer which was closely related to the thermocline depth and vertical distribution of nutrients. Maximum bacterial productivity was observed between 50 - 75 m depth in the KODOS area while it was observed between 10 - 30 m depth in Stn. 26 and between 10 - 50 m depth in Stn. 29 (Fig. 6). This maximum bacterial biomass and productivity was prominent when the water samples were carefully collected in association with the seasonal thermocline at Stn. I-21 and I-25 in 1997 (Fig. 7). Overall results

indicated that the distribution patterns of bacterial biomass and production in the northeast equatorial Pacific were mainly determined by the combined effects of physico-chemical and biological parameters.

Biomass turnover rates

Bacterial turnover rates in the upper mixed layer were estimated by dividing bacterial production by bacterial biomass (Table 1). Turnover rates of the bacterial biomass showed an average of 0.16 d^{-1} in the KODOS area (Stns. 1 and 5), while 0.30 d^{-1} for Stn. 29 and 0.35 d^{-1} for Stn. 26. The higher turnover rates in Stns 26 and 29 were characterized by higher productivity and a little lower biomass compared with those of KODOS area (Stn. 1 and 5) located in the middle of NEC.

Higher bacterial growth in the Stns. 26 and 29 seems to be associated with enhanced phytoplankton biomass. The depth integrated biomass of Chl-*a* in Stn. 26 (111.5 mmole C m^{-2}) is higher than the mean biomass in the KODOS Stns. (90.4 mmole C m^{-2}) (Table 1). Stn. 26 and 29 are located in south and north of the surface-water divergence occurring at the boundary of NEC and NECC in Stn. 27, and showed higher nutrient concentrations (Fig. 4), resulting in the higher phytoplankton biomass in Stn. 26 and 29. Another indication explaining that the KODOS area is relatively more oligotrophic environment (lower Chl-a biomass) is that the pico fraction in Stn. 26 (40%) is lower than those in the KODOS Stns. (average 65%). Smaller cells are more abundant in the oligotrophic environment (Herbland *et al.*, 1985; Le Bouteiller *et al.*, 1992). It is also known that the ratio of bacteria to phytoplankton biomass increased from eutrophic to oligotrophic environments (Cho and Azam, 1990; Cole *et al.*, 1988). The ratio in this study also follows the pattern in which bacterial biomass in Stn. 26 was 51% while it was average 82% in KODOS area.

The enhanced bacterial growth rates in Stns. 26 and 29, therefore, are related to the enhanced phytoplankton biomass in the diverging region showing higher nutrient concentrations in surface water. Several plausible mechanisms for the resource supply for the bacterial growth are well reviewed by other scientists (i.e., extracellular release of photosynthetically fixed carbon of phytoplankton, zooplankton sloppy feeding and excretion by zooplankton) (Azam and Cho, 1988; Ducklow and Carlson, 1992).

Limiting resources

Bacterial growth in a sample amended with amino acids mixture (DFAA) showed the highest growth, followed by glucose- and ammonium-

amended samples, respectively. The results indicated that (1) heterotrophic bacterial growth in the northeast equatorial Pacific is limited by the availability of dissolved organic carbon, and (2) the higher incorporation rate in DFAA-amended samples over glucose-amended samples suggested that the quality of dissolved organic carbon was significant in determining the efficiency of bacterial growth in the energy-limited environment because cells can save energy by avoiding amino acid biosynthesis. In the subarctic Pacific, Kirchman (1990) found that the addition of dissolved free amino acids (DFAA) consistently stimulated ^{3}H-thymidine incorporation from 31 to 393% when compared with unamended controls. The effect of glucose or glucose plus ammonium on the heterotrophic bacterial growth was always less than that of DFAA-amended samples. Kirchman *et al.* (1995) also concluded that the supply of dissolved organic matter regulated the large scale variations in heterotrophic bacterial growth in the equatorial Pacific.

As for the energy limitation, Fe limitation for the heterotrophic bacterial growth has been proposed (Tortell *et al.,* 1996). Higher Fe:C ratio in the heterotrophic bacterial cell than that in the eukaryotic phytoplankton suggested that bacterial growth in the open ocean may be limited by the Fe deficits causing low electron transport system and low growth efficiency (Pakulski *et al.,* 1996; Tortell *et al.,* 1996). Our amino acid and Fe enriched experiments (Fig. 9) showed that bacterial growth was the most strikingly stimulated by the addition of amino acids. In the Fe amended samples, bacterial growth showed certain increase in the 10 m depth. In sample collected at 60 m depth where strong seasonal thermocline, chlorophyll maximum layer, and sharp concentration gradients of bio-limiting nutrients occurs, Fe-addition did not stimulate the bacterial growth. The results indicate that high energy-yielding organic substrates are primary factors controlling heterotrophic bacterial growth as was appeared in Fig. 8. On the other hand, Fe in the nutrient-depleted surface mixed layer (i.e., 10 m depth) may play as a contributing factor enhancing growth efficiency in generating energy from small amount of available organic substrates. Because organic substrate (and probably other bio-limiting major and trace nutrients) for the heterotrophic bacterial growth are relatively more abundant in 60 m depth (i.e., chlorophyll maximum layer), higher bacterial thymidine incorporation was appeared (Fig. 9).

Environmental implication of future mining activity

The facts that heterotrophic bacterial growth is limited by dissolved organic carbon (and by Fe to some extents) have a significant environmental

implication associated with the deep seabed mining in the future. After separating the nodules, the remaining mixture of bottom water, sediment and nodule fragments may be discharged into water column. Several potential environmental impacts of the mining-induced sediment plume on the deep seabed benthic community and the plankton populations in the water column have been speculated (Curtis, 1982; Amann, 1992). As for the impact of surface plume on the photoautotrophic plankton: (1) it may increase turbidity, reduce light penetration into water column, and thus affect the primary production; (2) abundant dissolved metals may be introduced into the surface water column, which would be either inhibitor or stimulator for the phytoplankton growth; and (3) introduction of nutrient-rich bottom water or pore water into nutrient-depleted surface water column may enhance the primary productivity.

In ecological point of view, the equatorial Pacific, together with the Antarctic and subarctic Pacific, is one of the open oceans characterized as high nitrate but low chlorophyll (HNLC) environments (Cullen, 1991). Fe limited primary production and grazing impact on the phytoplankton community are attributed to the reasons for HNLC condition (Banse, 1995; Martin *et al.*, 1994; Frost, 1996; Price *et al.*, 1994; Cullen, 1995). The low Chl-*a* condition is associated with Fe limitation for the phytoplankton growth (Martin *et al.*, 1994), grazing control (Frost, 1991), or both Fe limited grazing control (Price *et al.*, 1994). The sediment and pore water of the Clarion-Clipperton fracture zone in the northeast equatorial Pacific contain large amount of numerous trace metals such as Fe, Mn, Cu, Ni, and Cd (Jung *et al.*, 1991; Klinkhammer *et al.*, 1982). Those metals are essential for the growth of phytoplankton (Howarth and Cole, 1985; Huntsman and Sunda, 1980; Lee *et al.*, 1995). Because Fe (and possibly other trace metals such as Zn, Ni and Co, etc.) is substantially limiting the growth of phytoplankton (Martin *et al.*, 1994; Frost, 1996), the addition of chemical substrates such as nutrient- and trace metal-enriched bottom water-sediment plume will be a direct and significant disturbance affecting the microbial community structure in the surface water column. Hyun *et al.* (1998) found that the addition of Fe and bottom water-sediment slurry simultaneously stimulated the growth of *Synechococcus*, however the stimulated growth in the slurry-amended sample lasted longer than only Fe-treated sample, probably due to the additional supply of inorganic nutrients. The results suggested that the discharge of bottom water and sediment may cause substantial increase of the phytoplankton biomass (Hyun *et al.*, 1998). Bacterial growth in the area is presumably controlled by the availability of organic carbon and Fe (or possibily by any other bio-limiting trace metals)

(Fig. 8 and 9). In the oligotrophic open ocean, photosynthetically fixed organic carbon is the major source for the heterotrophic bacterial growth (Ducklow and Carlson, 1992). Therefore, the enhanced primary production stimulated by the addition of nutrient- and trace metal-rich bottom water, pore water, sediments and ferromanganese nodule fragment can be a direct sources of the organic carbon for the heterotrophic bacterial growth. The bottom water and sediment may support bacterial growth by directly supplying those trace nutrients such as Fe, Mn, Co, Zn and Mo that are abundant in the bottom water, sediment and nodule fragments. We have tested the potential impact of slurry (mixture of bottom water, sediment and nodule fragment) on the heterotrophic bacterial incorporation of thymidine (Fig. 10). Compared to the untreated control after 24 hours, bacterial incorporation of ^{3}H-thymidine in samples collected at 10 m depth showed a 2 fold increase in slurry-amended sample (Slurry I), and more than 5 fold increase in a sample amended with sonicated slurry (Slurry II). Higher incorporation in a slurry II suggested that the sonication altered the physical properties of nodule fragments, and may have released more trace elements, which enhance the efficiency of energy-yielding process. The samples collected at 60 m depth, where seasonal thermocline, chlorophyll maximum layer and sharp increase of bio-limiting elements occurs, did not show any remarkable increase in thymidine incorporation compared to that in unamended control (Fig. 10). The results appeared in this experiment indicate that the slurry introduced into the surface water column affect the heterotrophic bacterial growth by either supplying organic carbon via phytoplankton growth utilizing those inorganic and trace nutrients or directly enhancing the efficiency of bacterial growth. Based on the recent results regarding phytoplankton blooming in the iron dumping area during IronEx (Martin *et al.,* 1994; Coale *et al.,* 1996) and Fe limitation for the heterotrophic bacterial growth (Tortell et al., 1996; Pakulski *et al.,* 1996), the mining activity in the C-C zone may be regarded as a long-term fertilization for the growth of phytoplankton and bacteria. Overall, our results imply that the benthic plume introduced into nutrient depleted surface water column during mining operation may affect the heterotrohic bacterial growth. Any potential impacts of bottom water-sediment slurry on the primary productivity and heterotrophic bacterial growth have not been reported in a long-term basis. Those researches are important in evaluating the environmental impact of Mn mining on the whole ecosystem of the C-C zone in the northeast equatorial Pacific, and in designing and developing technologies for the environmentally acceptable mining systems.

Acknowledgments

This study was supported by the Ministry of Maritime Affairs and Fisheries, the Government of the Republic of Korea and Basic Research Fund of Korea Ocean Research and Development Institute (PN97636). We thank captain and crews of the R/V Onnuri, and all the members in the KODOS project for their professional assistance during the cruises and in the lab. Thanks also go to C. Lee for her critical review of the manuscript.

References

Amann, H (1992) Deep sea mining and its impact. In: *The environmental impact of deep sea mining,* Section III. Thetis technologie GMBH, Hannover, 283 pp.

Andrews, J. E. and G. H. W. Friedrich. (1979) Distribution patterns of manganese nodule deposits in the northeast equatorial Pacific. *Marine mining,* **2,** 1-43.

Azam, F. and B. C. Cho (1988) Bacterial utilization of organic matter in the sea, In: *Ecology of microbial communities*, M. Fletcher, T. R. G. Gray, and J. G. Jones, editors, Cambridge University Press, Cambridge, pp. 261-281.

Banse, K. (1995) Community response to IRONEX. *Nature*, **375**, 112.

Cho, B. C. and F. Azam (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature,* **332,** 441-443.

Cho, B. C. and F. Azam (1990) Biogeochemical significance of bacterial biomass in the ocean's euphonic zone. *Marine Ecology Progress Series,* **63,** 253-259.

Cole, J. J., M. L. Pace and S. Findlay (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Marine Ecology Progress Series,* **43,** 1-10.

Coale, K. H., K. S. Johnson, S. E. Fitzwater, and 16 others (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean, *Nature,* **383,** 495-501.

Cullen, J. J. (1991) Hypotheses to explain high-nutrient conditions in the open sea. *Limnology and Oceanography*, **36**, 1578-1599.

Cullen, J. J. (1995) Status of the iron hypothesis after the open-ocean enrichment experiment. *Limnology and Oceanography*, **40**, 1336-1343.

Curtis, C. (1982) The environmental aspects of deep ocean mining. *Oceanus,* **25**, 31-36.

Ducklow, H. W., H. L. Quinby and C. A. Carlson (1995) Bacterioplankton dynamics in the equatorial Pacific during the 1992 El Nino, *Deep-Sea Research*, **42**, 621-638.

Ducklow, H. W. and C. A. Carlson (1992) Oceanic bacterial production. *Advances in Microbial Ecology*, **12**, 113-181.

Frost, B (1991) The role of grazing in nutrient-rich areas of the open sea, *Limnology and Oceanography*, **36**, 1616-1630.

Frost, B (1996) Phytoplankton bloom on iron rations. *Nature*, **383**, 475-476.

Fuhrman, J. A. and F. Azam (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California, *Applied and Environmental Microbiology*, **39**, 1085-1095.

Fuhrman, J. A. and F. Azam (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Marine Biology*, **66**, 109-122.

Herbland, A., A. Le Bouteiller and P. Raimbault (1985) Size structure of phytoplankton biomass in the equatorial Atlantic Ocean, *Deep-Sea Research*, **32**, 819-836.

Howarth, R. W. and J. J. Cole (1985) Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. *Science,* **229,** 653-655.

Huntsman, S. A. and W. G. Sunda (1980) The role of trace metals in regulating phytoplankton growth with emphasis on Fe, Mn and Cu. In: *The physiological ecology of phytoplankton,* I. Morris, editors, Blackwell Scientific Publications, Oxford, pp. 285-328.

Hyun, J.-H., K.-H. Kim, H.-S. Jung and K.-Y. Lee (1998) Potential environmental impact by deep-seabed manganese nodule mining on the Synechococcus (cyanobacteria) in the northeast equatorial Pacific:

effect of bottom water-sediment slurry. *Marine Georesources and Geotechnology,* **In review** (revised version).

Jung, H. S., J. K. Kang, K. S. Jeong and D. H. Shin (1991) Geochemical characteristics of light yellow brown surface sediments and dark brown colored subsurface sediments in KODOS-89 area, western part of Clarion-Clipperton fracture zone in north Pacific. *Journal of the Oceanological Society of Korea,* **26,** 193-203.

Karl, D. M., G. A. Knauer, and J. H. Martin (1988) Downward flux of particulate organic matter in the ocean: a particle decomposition paradox. *Nature,* **332,** 438-441.

Kirchman, D. L. (1990) Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. *Marine Ecology Progress Series,* **62,** 47-54.

Kirchman, D. L., J. H. Rich, and R. T. Barber (1995) Biomass and biomass production of heterotrophic bacteria along 140°W in the equatorial Pacific: effect of temperature on the microbial loop, *Deep-Sea Research II,* **42,** 603-619.

Klinkhammer, G., D. T. Heggie and D. W. Graham (1982) Metal diagenesis in oxic marine sediments. *Earth and Planetary Science Letters,* **61,** 211-219.

Lee, S., and F. A. Fuhrman (1987) Relationship between biovolume and biomass of naturally derived marine bacterioplankton. *Applied and Environmental Microbiology*, **53**, 1298-1303.

Lee, J. G., S. B. Roberts and F. M. M. Morel (1995) Cadmium: A nutrient for marine diatom *Thalassiosira weissfloggi. Limnology and Oceanography,* **40,** 1056-1063.

Le Bouteiller, A., J. Blanchot and M. Rodier (1992) Size distribution patterns of phytoplankton in the western Pacific: towards a generalization for the tropical open ocean, *Deep-Sea Research,* **39,** 805-823.

Li, W. K. W., P. M. Dickie, B. D. Irwin, and A. M. Wood (1992) Biomass

of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea, *Deep-Sea Research,* **39**, 501-519.

Longhurst, A. R., and W. G. Harrison (1989) The biological pump: profiles of plankton production and consumption in the upper ocean. *Progress in Oceanography*, **22**, 47-123.

Martin, J. H., K. H. Coale, K. S. Johnson, and 41 others (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature,* **371,** 123-129.

Padan, J. W. (1990) Commercial recovery of deep seabed manganese nodules: Twenty years of accomplishments. *Marine mining,* **9,** 87-103.

Pakulski, J. D., R. B. Coffin, C. A. Kelley, S. L. Holder, P. Aas, M. M. Lyons and W. H. Jeffrey (1996) Iron stimulation of Antarctic bacteria. *Nature,* **383,** 133-134..

Parsons, T. R., Y. Maita and C. M. Lalli (1984) *A manual of chemical and biological methods for seawater analysis.* Pergamon Press, 177 pp.

Pickard, G. L., and W. J. Emery (1982) *Descriptive physical oceanography.* Pergamon Press, 249pp.

Porter, K. G. and Y. S. Feig (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography,* **25,** 943-948.

Price, N. M., B. A. Ahner and M. M. Morel (1994) The equatorial Pacific ocean: grazer-controlled phytoplankton populations in an iron-limited ecosystem. *Limnology and Oceanography*, **39**, 520-534.

Tortell, P. D., M. T. Maldonado and N. M. Price (1996) The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature,* **383,** 330-332.

Table 1. Heterotrophic bacterial carbon biomass (BCB, mmole C m^{-2}) and production (BCP, mmole C m^{-1} d^{-1}), and chlorophyll carbon biomass (Chl-C, mmole C m^{-2}) and the ratio of BCB to Chl-C integrated to 120 m depth.

Month/ Year	Stn.	Location	BCP	BCB	Chl-C			BCB / Chl-C
					Total	Nano	Pico	
May/1996	1	10°27'N; 131°53'W	11.2	77.5	85.7	80.3	61.5	0.90
	5	09°54'N; 131°53'W	11.0	69.2	95.1	84.3	55.2	0.73
	29	09°00'N; 131°43'W	16.8	56.7	-	-	-	-
	26	07°00'N; 131°43'W	19.8	57.3	111.5	93.0	44.0	0.51
		Mean:	13.6	65.2	101.3	88.7	55.6	0.72
		(± SD)	(± 4.4)	(± 10.0)	(± 12.7)	(± 9.4)	(± 9.4)	(± 0.19)

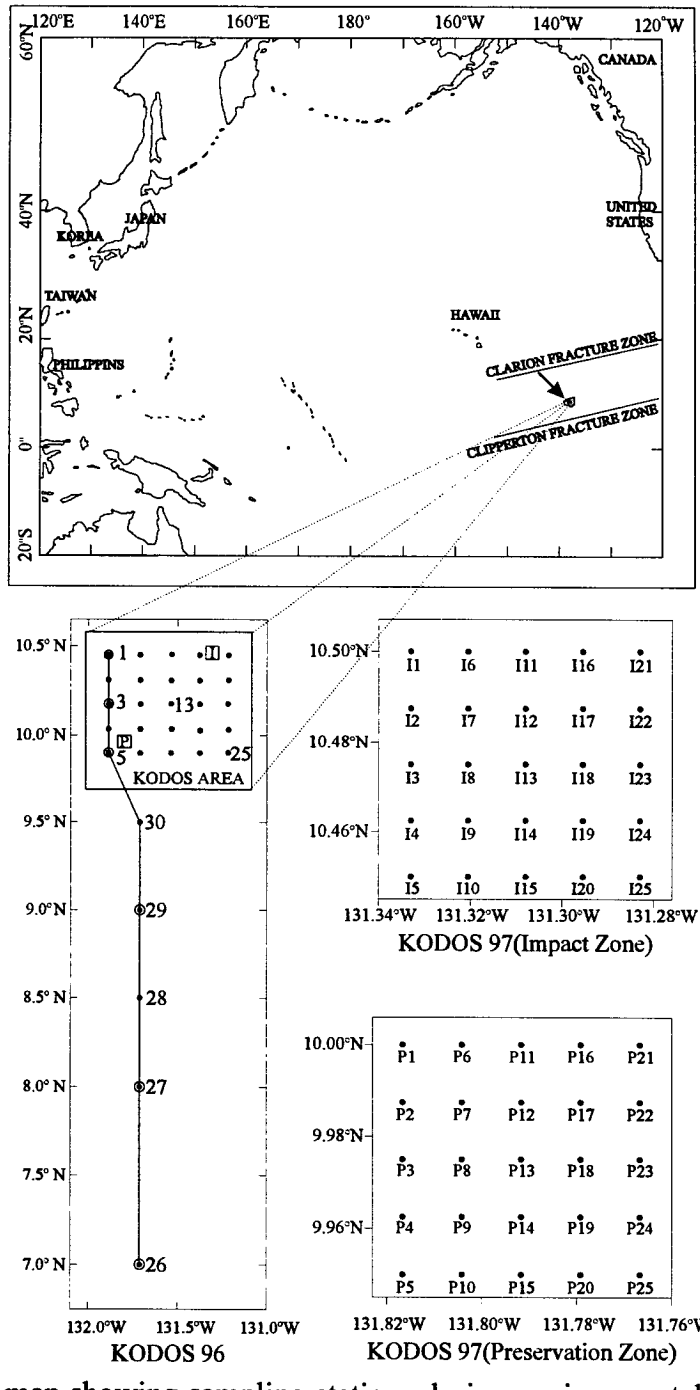

Fig. 1 A map showing sampling stations during environmental cruises of the KODOS 96 and KODOS 97.

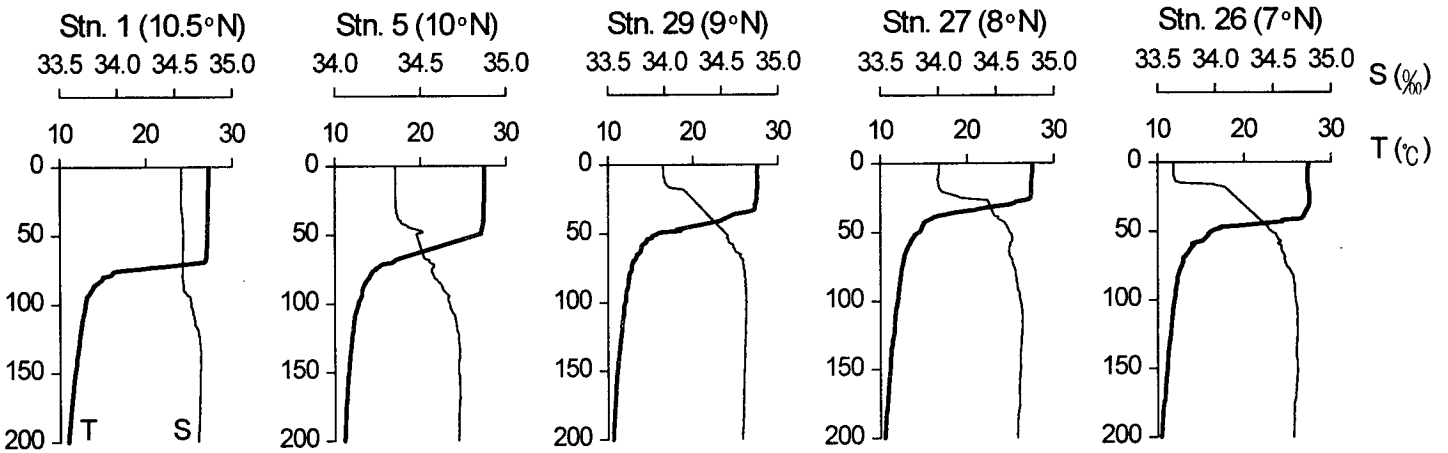

Fig. 2 Vertical profiles of the water temperature (T) and salinity (S) in the study area.

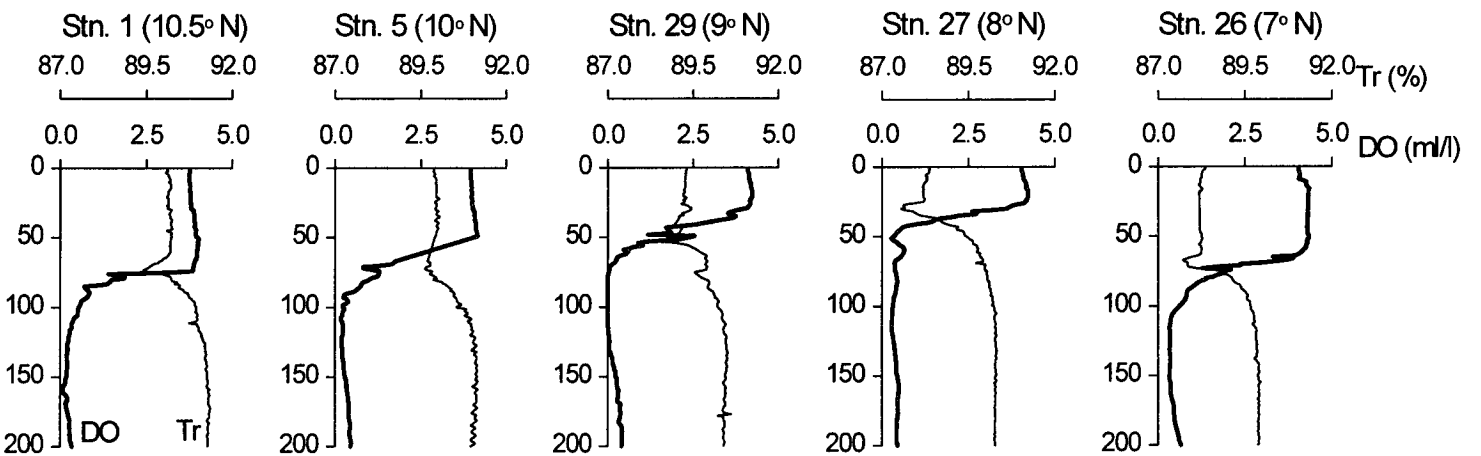

Fig. 3 Vertical profiles of dissolved oxygen (DO) and light transmission (Tr) in the study area.

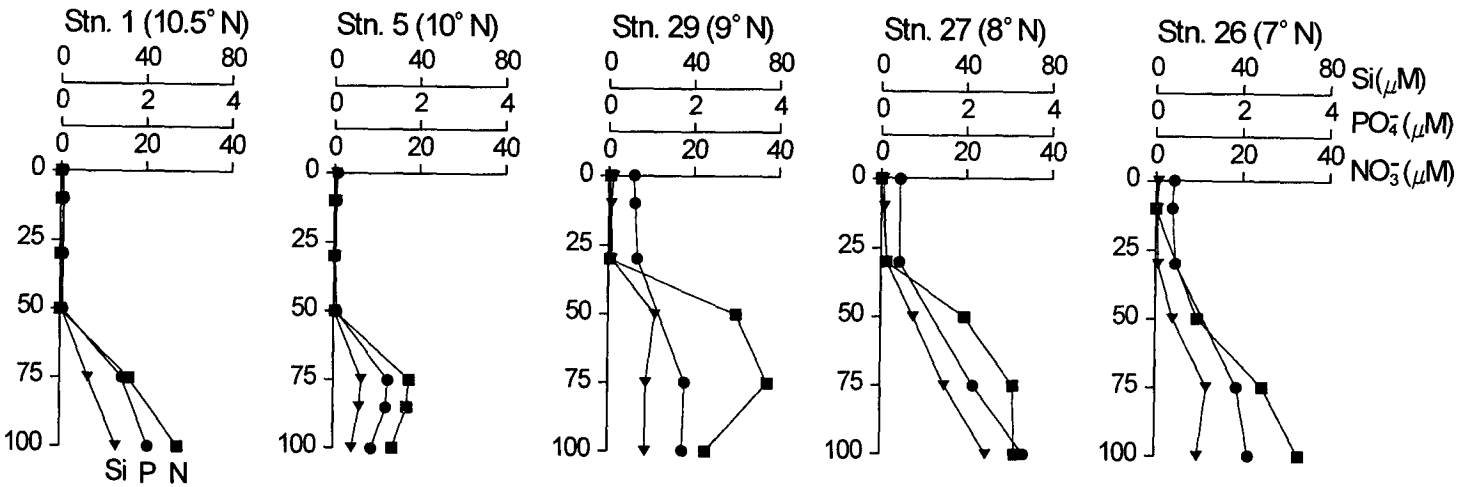

Fig. 4 Vertical profiles of nitrate (N), phosphate (P) and silicate (Si) in the study area.

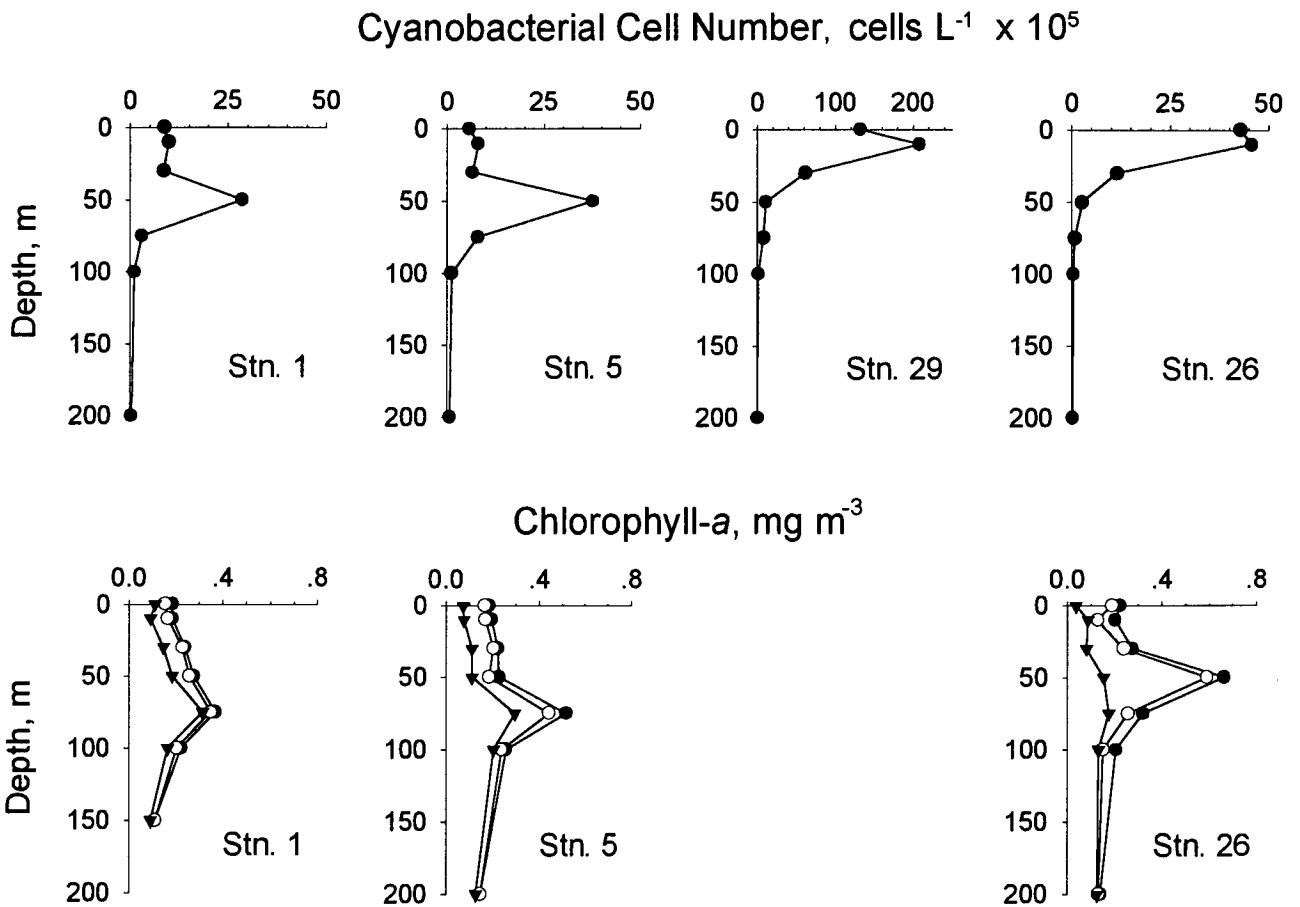

Fig. 5 Vertical profiles of cyanobacterial cell number and chlorophyll-*a* in the study area.

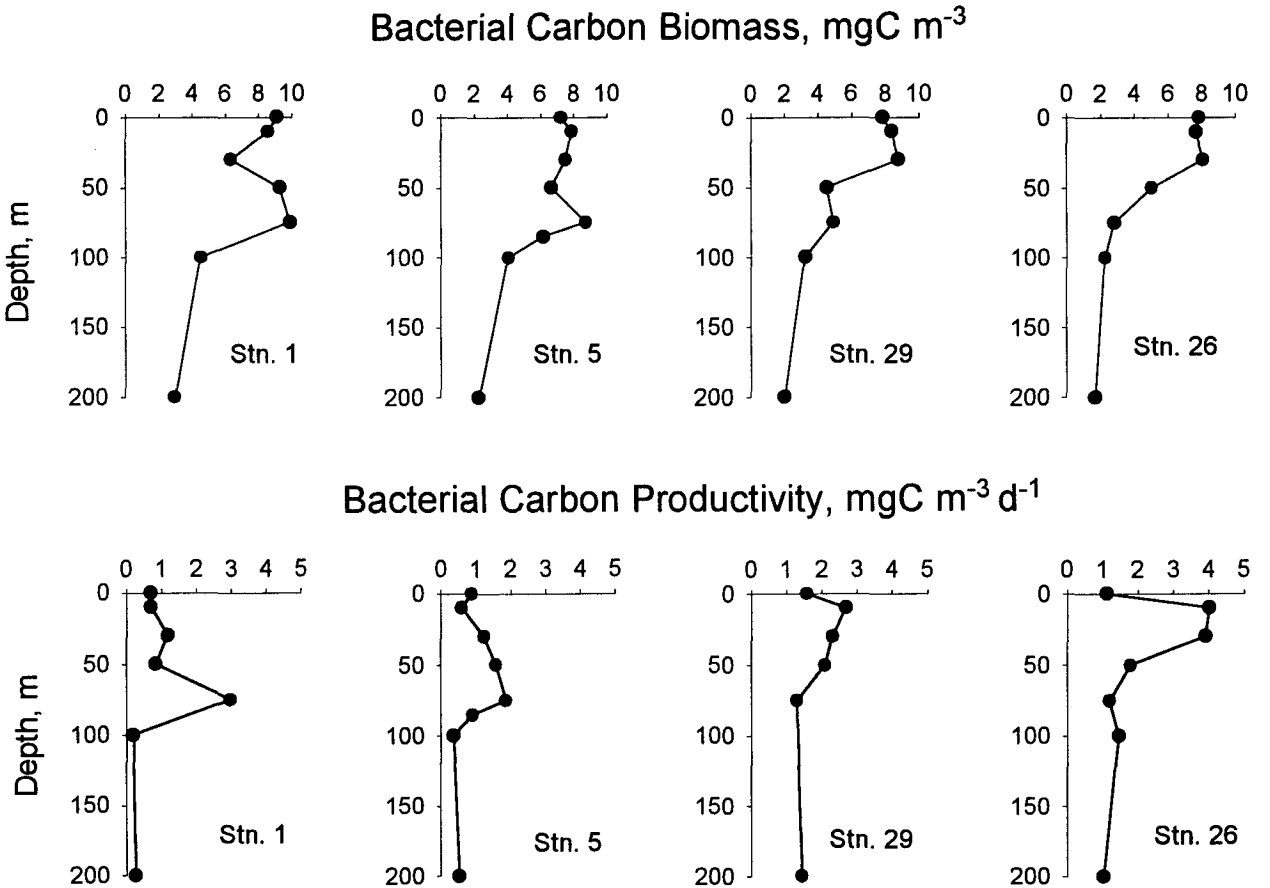

Fig. 6 Vertical profiles of bacterial carbon biomass and bacterial carbon productivity in the study area.

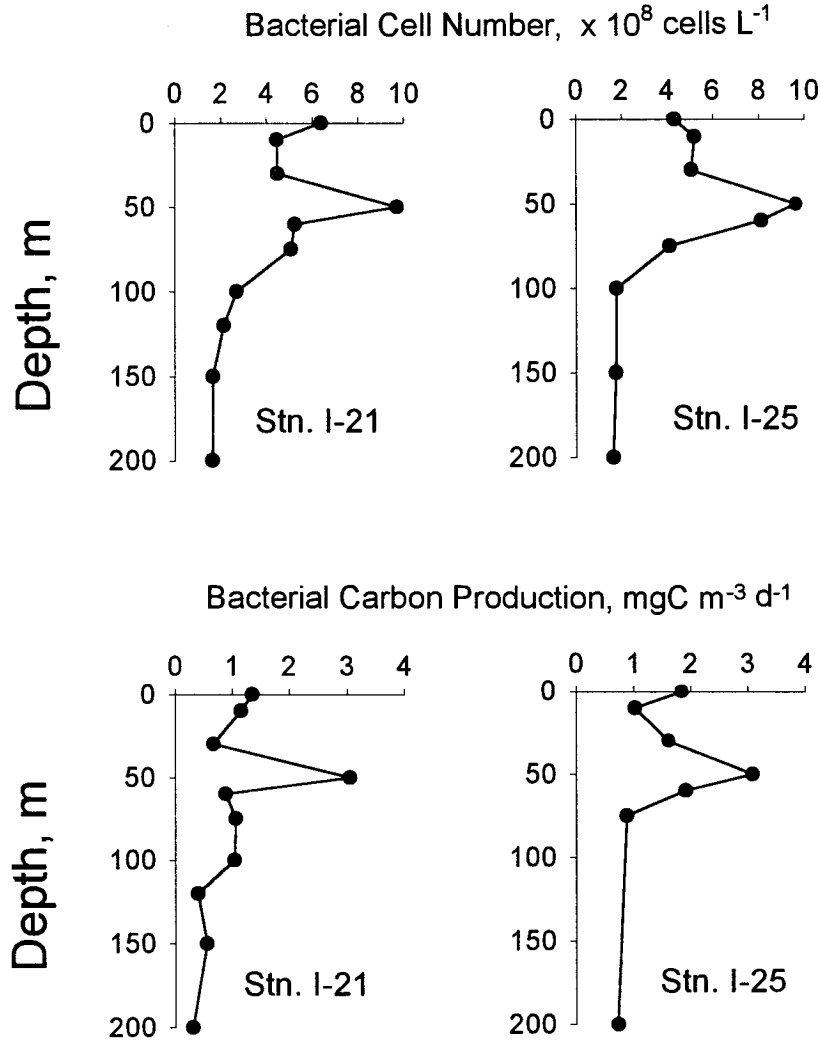

Fig. 7 Vertical profiles of bacterial cell number and carbon productivity in Stns. I-21 and I-25.

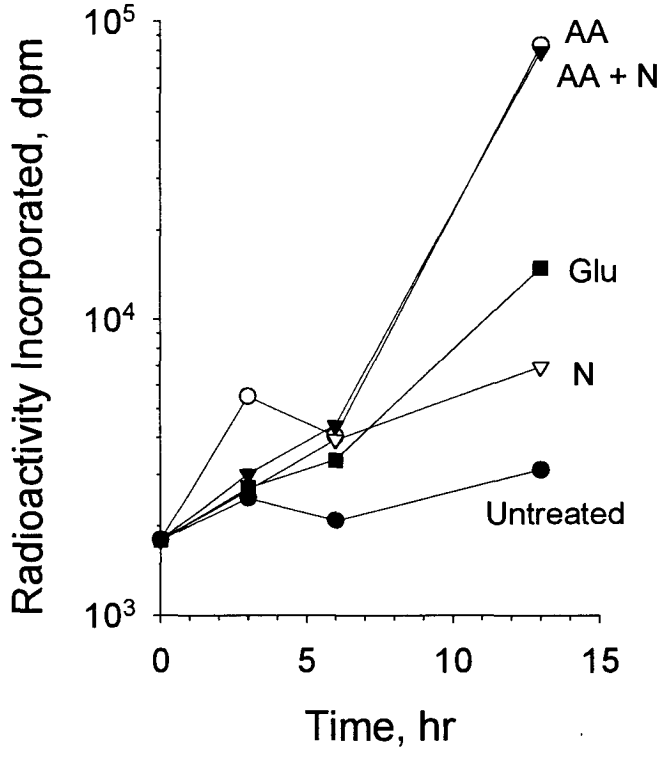

Fig. 8 Limiting resources assay for the heterotrophic bacterial growth. Note that the sample amended with amino acid (AA) showed higher bacterial growth than those amended with glucose (Glu) and ammonia (N).

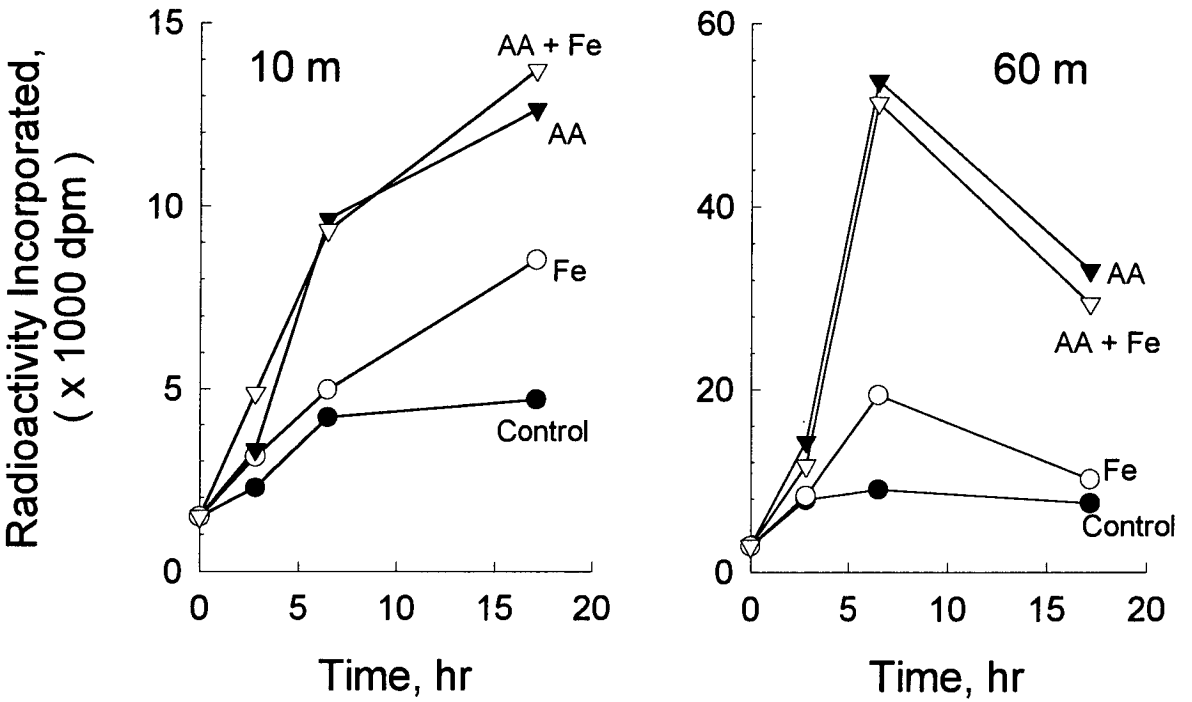

Fig. 9 Effect of Fe and amino acids (AA) on the heterotrophic bacterial growth. Note that the bacterial growth enhanced the most by the addition of AA. Bacterial growth in Fe-amended samples showed a substantial increase in a sample collected at 10 m depth where bio-limiting nutrients are depleted), while Fe effect was negligible in a sample collected at 60 m depth where bio-limiting elements start a sharp increase with depth.

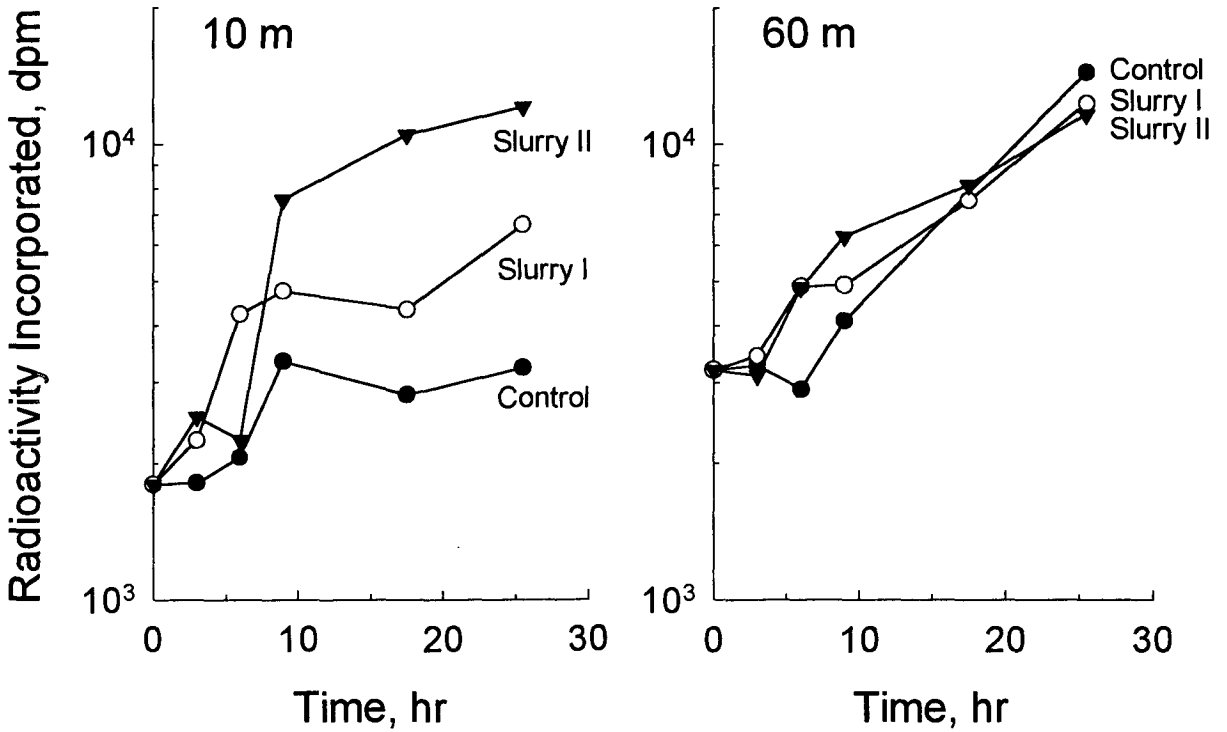

Fig. 10 Effect of bottom water-sediment slurry on the heterotrophic bacterial growth. Slurry effect was remarkable in a sample collected at 10 m depth. The sonicated slurry (Slurry II) showed more prominent effect for the bacterial growth than untreated slurry (Slurry I).