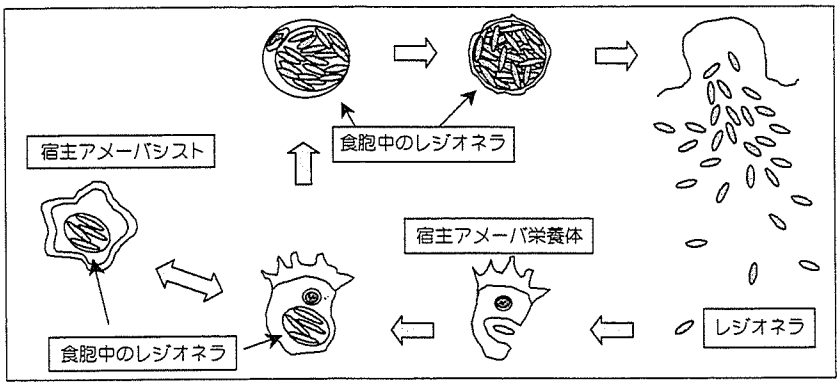

図1　環境中でのレジオネラの増殖様式
レジオネラは細胞寄生性の細菌で，ヒトに感染した場合にはマクロファージなどに寄生して増殖する。
（八木田ら原図より引用）

原生動物を宿主としている[1]。したがって，生活用水のレジオネラ汚染の背景には必ずアメーバなどの原生動物とその餌である微生物の繁殖がある。レジオネラの宿主として，42℃前後の温水に棲息するアカンソアメーバ(*Acanthamoeba*)をはじめとする多様なアメーバや繊毛虫のテトラヒメナ(*Tetrahymena*)が報告されている[2]。アカンソアメーバを宿主とした場合，感染後2日程度でレジオネラはアメーバをほぼ埋め尽くすほどに増殖し，その数は1,000個以上に達する。やがてレジオネラは運動性を獲得し，新たな宿主を求めて水中に遊出する(**図1**)。これまでの集団感染事例をみると，レジオネラは浴槽水中で10^3～10^6cfu/100mLの範囲(ほぼ10^4cfu/100mL弱に収斂)に達していた。単純計算すると浴槽水には1～10^3個/100mL程度の感染アメーバが必要となる。これを10トン程度の営業用の浴槽にてあてはめると10^4～10^7オーダーの感染アメーバが必要となる。仮にアメーバの1％程度が感染しているものとすれば，アメーバ数はさらにその100倍以上にのぼることになる。そのアメーバを支える餌の細菌類にいたっては膨大なものになることは容易に想像されよう。レジオネラ汚染はかくもはなはだしい微生物汚染が背景にあってのことである。

ところで，循環式浴槽は浴槽水をろ過循環させて長期にわたり継続使用することから，微生物，ひいてはレジオネラ汚染の極めて起きやすい構造となっている。そればかりか本装置では積極的に微生物を定着させて入浴者が持ち込む汚れ（有機物汚染）の処理に活用している。一見すると水の使用部分と汚染水の処理装置(活性汚泥槽)を同居させており，これらが有効に機能すれば極めて利用価値の高い装置といえる。しかしながら，致命的なことに活性汚泥こそがレジオネラの増殖の場となっている。また，そこからレジオネラを選択的に取り除く有効な手段はない。厚生労働省は先の公衆浴場法ならびに旅館業法に係る通達（健発第1029004号，平成14年10月)で遊離残留塩素（1日2時間以上0.2～0.4mg/Lを保つこと）による浴槽水の衛生管理を勧奨している。本措置は有効ではあるが，同時にシステムの心臓部ともいえる活性汚泥を排除することを意味し，この時点で循環式浴槽システムは理論的に破綻したと言える。さらに，やむを得ず用いている塩素消毒に問題がないわけではない。塩素の使用ではトリハロメタン等に代表される消毒副生成物の発生が避けがたく，入浴により経皮的，あるいは経気道的暴露が免れない。ちなみに，浴用水における微生物対策は0.2mg/L以上の有効遊離残留塩素濃度が効果的であることが示されている（**図2**）[3]。

レジオネラの感染様式は汚染された水から発生したエアロゾルを吸引することによるもので，その際のエアロゾルの粒径は2～5μm程度とされている[3]。我々の生活環境にあって，入浴施設の他にエアロゾルの発生につながる装置としては空調設備の冷却塔，加湿器や噴霧器あるいは，水を用いる歯科・医療器具など様々なものがある。これらの装置に共通する点は温水の貯留である。現に，レジオネラ症は冷却塔や修景用噴水，超音波式ネブライザー，加湿器や温泉浴槽，場合によってはシャワーや呼吸補助装置などを介した発生が知られている[4]。また，例外的にではあるがプラン

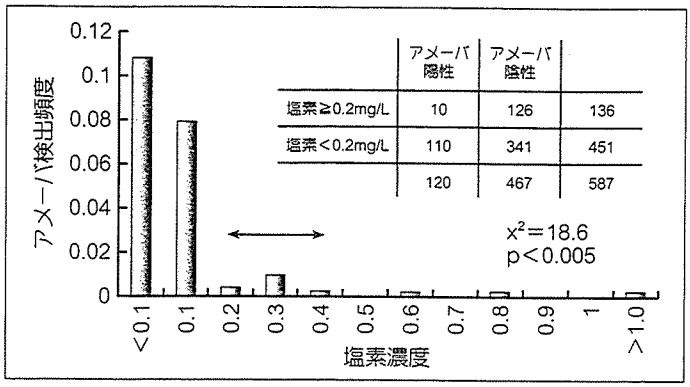

図2　遊離残留塩素濃度とアメーバ検出頻度 (n = 587)

浴用水における微生物対策は0.2～0.4mg/Lの有効遊離残留塩素濃度が効果的であることが示されている。

ターの土や腐葉土で増殖していたレジオネラ（*L. longbeachae*）が原因と考えられる感染事例の報告もある[5, 6]。加湿器や噴霧器あるいは，水を用いる歯科・医療器具などにおいては定期的な高濃度の薬品による消毒，水の滞留を防ぐ工夫と，洗浄し易い単純な構造であることが求められる。あわせて，ホースなどの交換を頻繁に行うことが汚染防止につながる。ちなみに，歴史的には冷却塔水のレジオネラ汚染は有名で，かつての集団感染はほとんど冷却塔水を汚染源としていた。ところが，近年では空調施設自体の維持のために冷却塔水の水質管理が徹底されていること，エアロゾルの飛散を抑える構造が推奨されていること，さらに冷却塔の設置場所の規制などが義務付けられたことから，わが国では冷却塔を介したレジオネラ症の報告が聞かれなくなっている。

Ⅲ　病原性を有する自由生活性アメーバ類

自然界，特に温水に生息するアメーバ類の中にはそれ自体が強い病原性を有する種類が知られており，稀な疾患であるがわが国にも存在する。余談であるが，筆者らが病原アメーバに着目したのは温泉をこよなく愛するわが国において入浴施設を介したアメーバ性脳炎の発症を危惧したことによる。

アメーバ性髄膜脳炎の病形はネグレリア（*Naegleria fowleri*）を病原体とした原発性アメーバ性髄膜脳炎(primary amoebic meningoencephalitis: PAM）と肉芽腫性アメーバ性脳炎（granulomatous amoebic encephalitis：GAE）の2型に大別される。前者は，鼻腔の嗅神経末端から侵入し，嗅神経沿いに中枢神経へ到達し，感染から5～10日のうちに死に至らしめる。わが国では1996年に佐賀県在住の女性が本症に罹患・死亡している。後者の亜急性，慢性の肉芽腫性アメーバ性脳炎は免疫不全者における日和見感染とされ，1週間から数カ月を経て死に至る。これまで，肉芽腫性アメーバ性脳炎はもっぱらアカンソアメーバに起因するものとされてきたが，1980年代になってバラムーティア（*Balamuthia mandrillaris*）という新たな病原アメーバが知られるに至っている。バラムーティアは免疫不全者における日和見感染に限らないことが明らかになっている。筆者らはわが国で報告された6例のアメーバ性脳炎の病原体について再検証を行い，*N. fowleri* および *Acanthamoeba* spp. に起因する脳炎が各1例，*B. mandrillaris* の感染による脳炎が4例であることを確認した。後者のバラムーティアの生息場所，感染経路等は未だに不明である。病理標本の観察からは明らかに血行性に病巣の広がりがみられている。

筆者らは全国14地域の地方衛生研究所の協力を得て各地域の温水利用施設を対象にアメーバの分離・同定ならびに汚染実態の把握に努めた。その結果，浴用水およびその排水などからは高率にアメーバが分離され，中でもネグレリア属が優占種の一角を占めていた。さらに，日本各地で *N.*

PAM (primary amoebic meningoencephalitis；原発性アメーバ性髄膜脳炎)
GAE (granulomatous amoebic encephalitis；肉芽腫性アメーバ性脳炎)

australiensis および *N. philippinensis* の棲息が確認され，マウスに対する致死的な障害性を持つことが再確認された。幸い，これらのアメーバ種は体表の傷口や粘膜からの侵入，あるいは経鼻感染の証拠はなく，感染力は *N. fowleri* に比べてはるかに劣ることが示されている。しかしながら，入浴者への不測の感染事故を回避する上で汚染防止は必須と考える。

このほか，アカンソアメーバはコンタクトレンズ装用者に角膜炎（Acanthamoeba keratitis）を起こすことが知られており，これまでもレンズの衛生管理の重要性が指摘されてきた。本症は1988年にわが国で初めて確認され，1993年までの6年間におよそ40例が報告された。その後も引き続き患者の発生がみられている。本症は早期診断が極めて重要で，早期の治療が予後に大きく影響することが知られている。アカンソアメーバは浴槽水からも多く分離されることから，コンタクトレンズを装用したままの入浴は厳に慎むべきである。

Ⅳ　利便性の追求とリスク

直面しているレジオネラ問題は人が作り出した温水環境を介して起きている。いわば，便利さや快適さを求める過程で発生した人災といえる。理論的にはこの種の問題の解決は極めて容易である。設備を使用しなければ本件は解決する。しかしながら，使用を前提とした現実対応は極めて難しく，その衛生管理に要する労力は計り知れない。

以下のような試算もある。公衆浴場法では浴槽水の有機物汚染に係る水質基準として過マンガン酸カリ消費量が25mg/L以下であることと規定されている。また，浴槽原水の基準値は過マンガン酸カリ消費量10mg/L以下とされている。不勉強にしてこの基準値が何を根拠として定められたのか知らないが，原水の水質を考慮すると15～25mg/Lの範囲の持ち込みが上限となる。我々の調査によると入浴者一人が持ち込む有機物量は過マンガン酸カリ消費量に換算しておよそ0.5gであった。仮に入浴者の持ち込む汚れ（有機物）を通して入浴者数を規制するとすれば，たかだか10トン程度の浴槽水に延べ300～500人が入浴できる計算となる。200L程度の家庭用の浴槽に当てはめると延べ6～10人の入浴者に相当する。この基準の是非についても合わせて議論していくべきものと考えている。

循環式浴槽に関しては，原点に立ち返って浴槽水の繰り返し使用そのものの是非を論ずべき時期に来ていると考える。いずれにせよ，利便性を得るのに生活環境の悪化を交換条件とするのであれば，人知もあまり大したものではない。

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LOCUS ON CHROMOSOME 13 IN MICE INVOLVED IN CLEARANCE OF *LEGIONELLA PNEUMOPHILA* FROM THE LUNGS

Seiji Kobayashi, Fumiaki Kura, Junko Amemura-Maekawa, Bin Chang, Naoki Yamamoto, and Haruo Watanabe

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Legionella pneumophila is a causative agent of pneumonia in humans. Until now, the mechanisms that control the *L. pneumophila* infection in the lungs have not yet been thoroughly assessed. We found that the mouse strain B10.A/SgSnSlcNiid (B10.A/Niid) was highly susceptible to intranasal infection by *L. pneumophila* 80-045 serogroup 1, while the mouse strain C57BL/10 was resistant to it (Fig. 1). The purpose of the present study is to identify the loci responsible for regulating *L. pneumophila* replication in the lungs, by comparing susceptible B10.A/Niid with other resistant inbred strains.

First, the susceptibility of B10.A/Niid mice might be a general response against the *L. pneumophila* species. Alternatively, the response could be strain dependent. Therefore, we tested the susceptibility to five kinds of laboratory and clinical strains of *L. pneumophila* serogroup 1. We observed that B10.A/Niid mice were susceptible to all the *L. pneumophila* serogroup 1 strains used in the experiment, whereas the C57BL/10 mice were not. In contrast, B10.A/Niid mice showed similar clearance of the *L. pneumophila* Chicago (serogroup 6) and *L. bozemanii* WIGA strains that was similar to the clearance observed in C57BL/10 mice. Thus, the susceptibility of B10.A/Niid mice could be partly attributed to their inability to sufficiently clear *L. pneumophila* serogroup 1 strains.

Second, in order to identify the locus that is responsible for the susceptibility phenotype on the major histocompatibility complex (MHC) region of the H-2^a haplotype, we analyzed the influence of the MHC haplotypes on *L. pneumophila* infection by using three types of B10.A intra-MHC-congenic mice, i.e., B10.A (2R), B10.A (3R), and B10.A (5R), as well as the parental mice, B10.A/SgSnJ, obtained from the Jackson laboratory. Surprisingly, *L. pneumophila* could not replicate in the lungs of any of these mice, indicating that the H-2 region might not be involved in the susceptibility of B10.A/Niid mice to *L. pneumophila* and that B10.A/Niid mice might possess spontaneous defects. Furthermore, a series of mating experiments were performed to characterize the genetic basis of the B10.A/Niid susceptibility phenotype. B10.A/Niid mice were mated with C57BL/10 mice, and the susceptibility of the F_1 progeny was assessed. All the (B10.A/Niid × C57BL/10) F_1 animals were resistant to *L. pneumophila*

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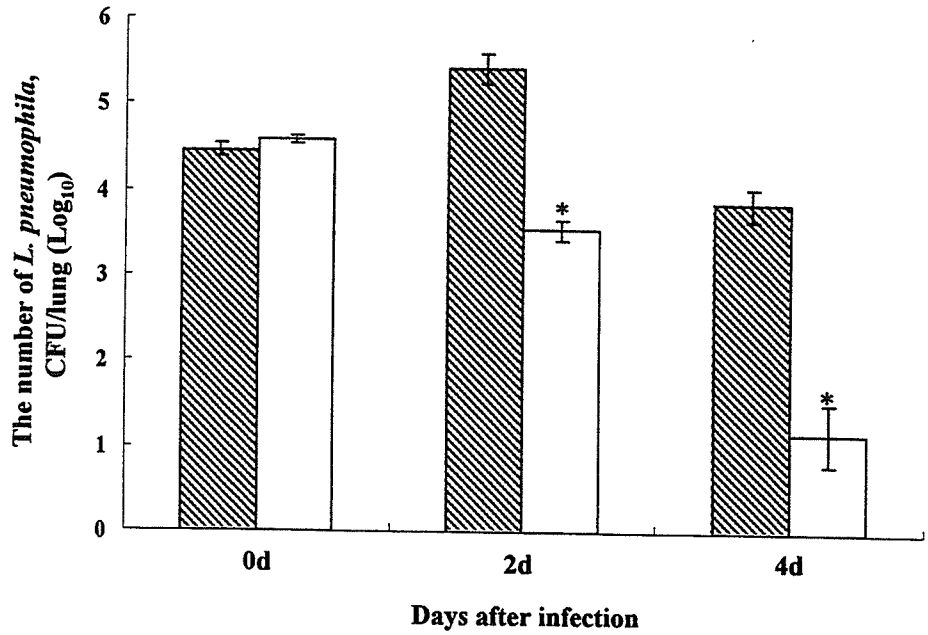

FIGURE 1 The mouse strain B10.A/Niid is susceptible to pulmonary *L. pneumophila* infection. B10.A/Niid (striped bar) and C57BL/10 strains (white bar) were infected with 3×10^4 CFU of *L. pneumophila* 80-045 (serogroup 1) per lung by intranasal infection. Data were mean ± standard deviation of 6 to 8 mice. The data shown are the combined data from two experiments. The asterisk (*) indicates a significant difference ($P < 0.05$) between B10.A/Niid and C57BL/10 strains.

infection in the lungs. The ratio of resistant individuals to susceptible individuals was 17:18 for B10.A/Niid × F_1 and F_1 × B10.A/Niid backcross progeny. Thus, the susceptibility of B10.A/Niid mice appears to be due to a major recessive gene mutation, although the susceptibility pattern of the F_2 progeny showed a somewhat continuous distribution between the resistant C57BL/10 and susceptible B10.A/Niid parents.

Third, to identify the responsible locus, we performed a whole-genome single-point linkage analysis on 280 (AKR/N × B10.A/Niid)F_2 mice using 81 simple sequence-length markers that are polymorphic for B10.A/Niid and AKR/N. On the basis of this analysis, an *L. pneumophila* susceptibility locus with significant linkage was mapped between *D13Die26* and *D13Mit287* (mapped position 54 to 57 cM) on chromosome 13 ($P < 0.00001$) (Fig. 2); no additional significant linkages were observed. The mapped locus was near the *Lgn1* locus that determines the ability of macrophages to restrict *L. pneumophila* growth (1, 2). A/J mouse macrophages are permissive for *L. pneumophila* growth (*Lgn1*^{s}).

Last, we compared the replication in thioglycolate-elicited macrophages obtained from B10.A/Niid mice and the B10.A/Niid-*Lgn1*^{s} congenic mouse strain that was constructed by introgressively backcrossing (B10.A/Niid × A/J) F_1 mice to B10.A/Niid mice. We found that thioglycolate-elicited macrophages from B10.A/Niid mice could restrict *L. pneumophila* growth, while those from B10.A/Niid-*Lgn1*^{s} mice could not. This result suggests that the susceptibility locus in B10.A/Niid mice is different from *Lgn1*.

In conclusion, our present study indicates that B10.A/Niid mice have a genetic defect that affects *L. pneumophila* serogroup 1 growth in the lungs. Furthermore, we also found that the responsible locus was located near *Lgn1* but was phenotypically distinct from it. We propose to designate this locus as *Lgn2*.

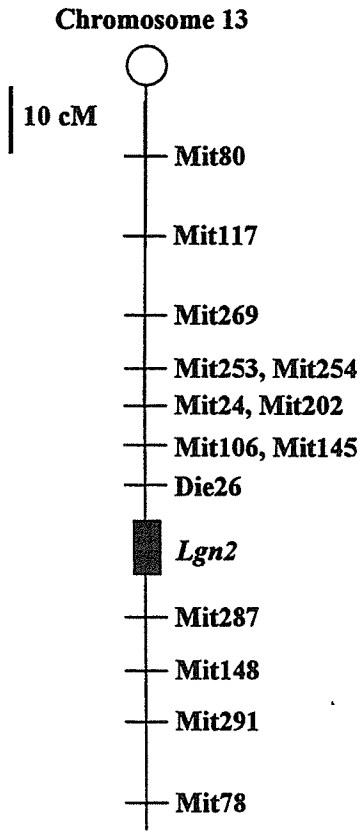

FIGURE 2 Schematic representation of chromosome 13. Markers shown were used to genotype mice of the F_2 cross. Approximate location of the markers was obtained from the mouse genome database. The centromere is indicated by a large circle.

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GROWTH OF *LEGIONELLA* IN NONSTERILIZED, NATURALLY CONTAMINATED BATHING WATER IN A SYSTEM THAT CIRCULATES THE WATER

Katsuhiko Ohata, Kanji Sugiyama, Mitsuaki Suzuki, Rieko Shimogawara, Shinji Izumiyama, Kenji Yagita, and Takuro Endo

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For a long time in Japan, taking a bath in the hot springs has been a popular activity. In recent years, many public bathhouses introduced bathing water circulating systems for extended use, in which a sand filtration unit was installed. However, this resulted in several large-scale outbreaks of legionellosis (1) due to the microbiologically insufficient maintenance of the bathing facilities. Occurrence of *Legionella* in bathing water circulating systems, appears to be common and is a serious public health concern in Japan.

We constructed a life-size model plant of a bathing water circulating system for the simulation experiment. The model plant is composed of a filter (a sand filtration unit filled with three layers of ceramic sand [about 100 kg], with a linear velocity of 25.5m/h), two bathtubs (1 m^3 × 2), an automatic chlorine (in the form of sodium hypochlorite) injector, a hair catcher, a circulating pump, a heater, and a UV light lamp. The water temperature is kept at about 40°C (104°F) and circulated at about 5 m^3/h (Fig. 1).

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These experiments are aimed at monitoring changes in the microbial constituents, especially a possible occurrence of *Legionella* in a bathing water circulating system, and developing preventive measures and intervention strategies. Prior to microbial monitoring, a total of either 16 or 40 volunteers were asked to take baths for 10 days (experiment 1) or for 14 days (experiment 2), respectively. Chlorine was added at concentrations ranging from 0.2 to 1.5 mg/liter into the bathing water. After that, in experiment 1, the chlorine injector was turned off and the residual chlorine was degraded completely with the ultraviolet irradiation. Again, five volunteers took a bath, and the ultraviolet irradiation was turned off (day 0). The bathing water was circulated under nonsterilization (1st to 23rd days) and the circulating system was sterilized by 10 ppm chlorine at the end of the experiment (23rd day). In experiment 2, after the residual chlorine was totally degraded with the ultraviolet irradiation, three volunteers took a bath (0 to 1st days). After that, the ultraviolet irradiation was turned off (2nd day) and the bathing water was circulated under nonsterilization (3rd to 31st days). After the bathing water was exchanged (31st day), it was recirculated (31st to 36th days). At the end of the experiment, the system was sterilized by 6% H_2O_2 (36th day).

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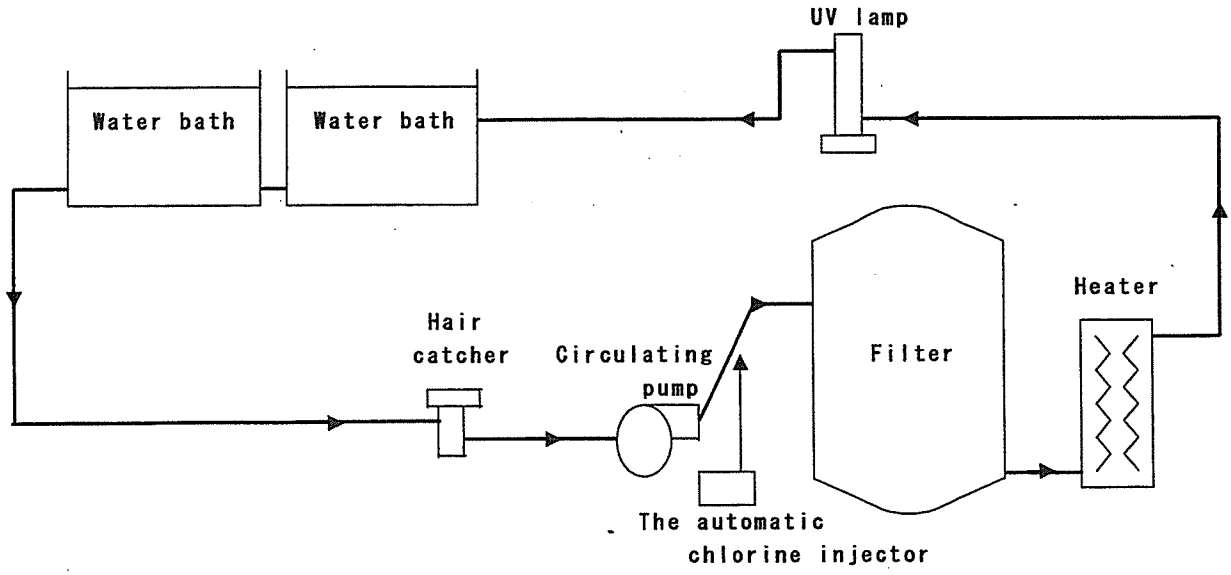
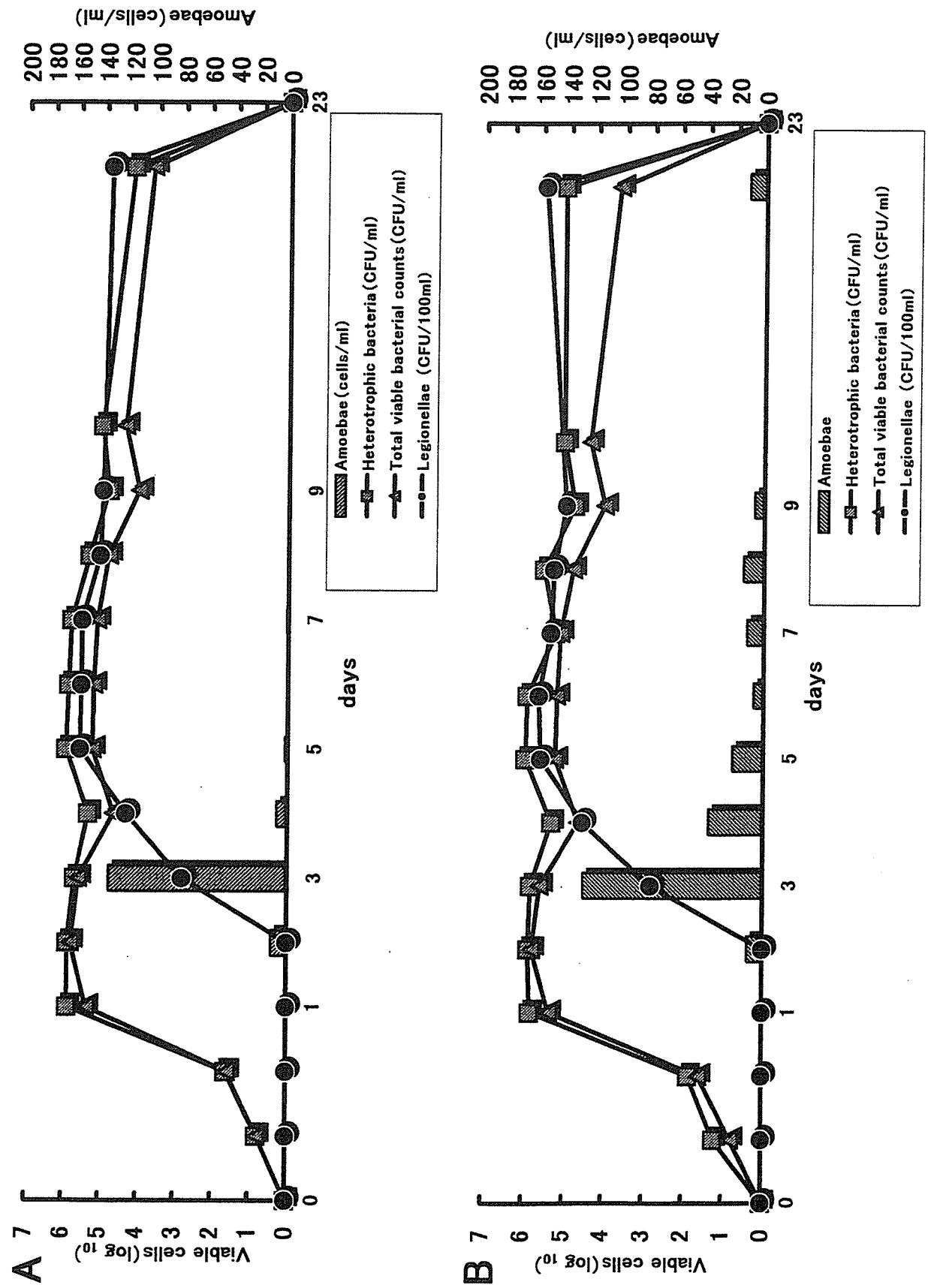


FIGURE 1 A system that circulates bathing water of the model plant. The pointed arrows show the flow of the circulating water.

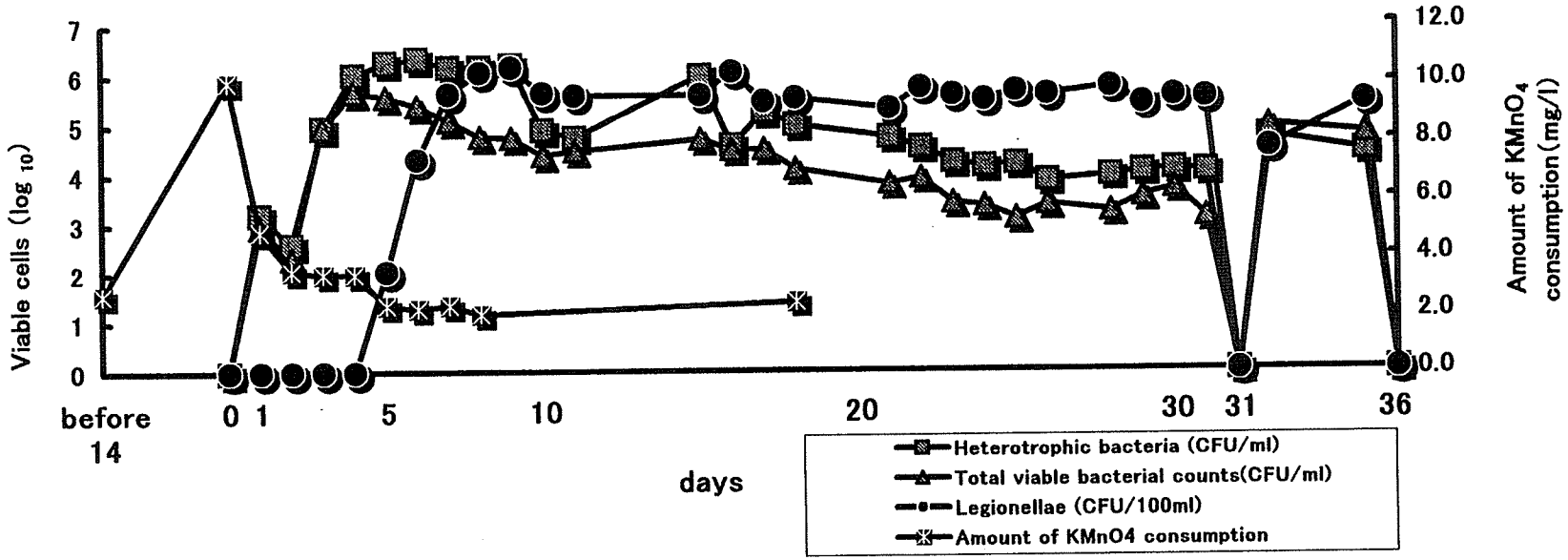
In these experiments, from the day when the chlorine injector was turned off, microbial monitoring occurred for the number of *Legionella*, total viable bacterial counts, heterotrophic bacteria, and free-living amoebae in the bathing water. This microbial monitoring occurred in the water, in the filter, and in other parts of the model plant, either almost daily or at certain intervals. GVPC Agar (bioMerieux, France) was used for detection of *Legionella*, Standard Method Agar (Nissui Pharmaceutical, Tokyo) for total viable bacterial counts, R2A Agar (Becton Dickinson, USA) for heterotrophic bacteria, and Bacto-Agar (Difico, USA), on which a monolayer of *Escherichia coli* was deposited, for free-living amebae. The $KMnO_4$ consumption test was also applied continuously to the bathing water in experiment 2.

As a result of experiment 1 (Fig. 2), *Legionella* was detected in both the bathing water and the filter water at concentrations of 6.6×10^2 CFU/100 ml on the 3rd day after residual chlorine disappeared. The number of *Legionella* in the bathing water and the filter water increased to 10^5 CFU/100 ml on the 5th day and remained 10^4 to 10^5 CFU/100 ml throughout the experiment. A positive bacterial culture was obtained in the bathing water and the filter water immediately after residual chlorine disappeared, and the number of total bacterial counts reached 10^5 CFU/ml within a day. Then it decreased gradually to 10^3 to 10^4 CFU/ml at the end of the experiment. The number of heterotrophic bacteria was almost the same as the number of total bacterial counts. Free-living amoebae, known to be the hosts of *Legionella* in this environment, were detected 2 days after residual chlorine disappeared. They were calculated to be 12 cells/ml in the bathing water and 11 cells/ml in the filter water. On the 3rd day, the number of amoebae increased to 136 cells/ml and 129

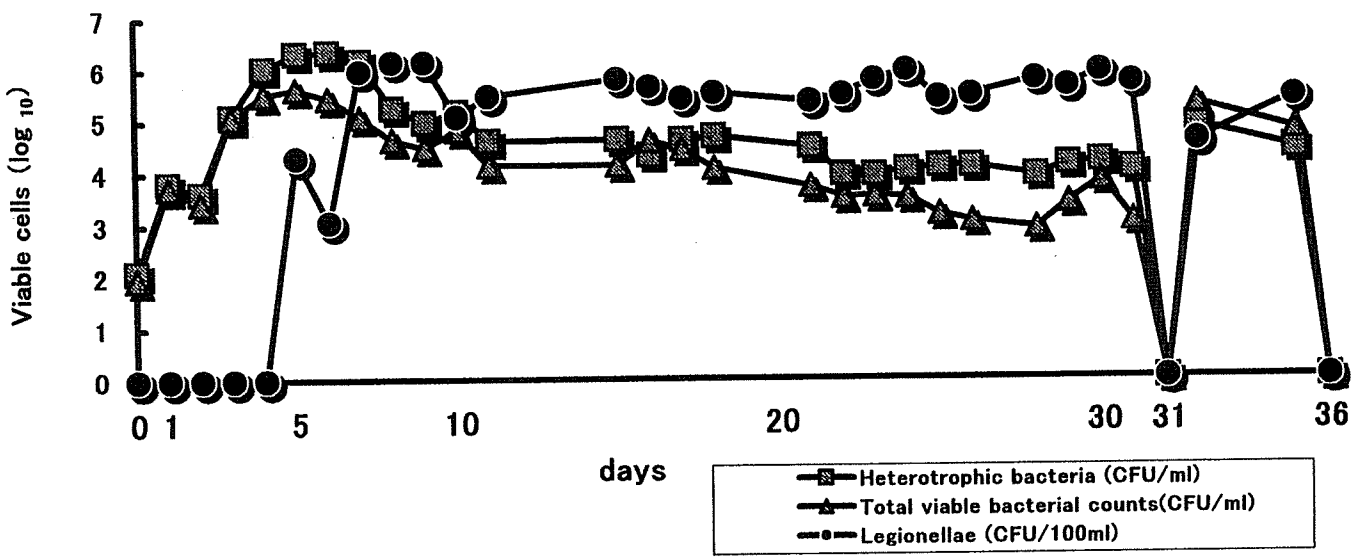
FIGURE 2 The growth of *Legionella*, etc. in the bathing water (A) and the filter water (B) in experiment 1. For 10 days about 16 volunteers took baths under the system with chlorine control, in which the chlorine injector was turned off. After that, five volunteers took baths, and the ultraviolet irradiation was turned off (day 0). The bathing water was circulated under nonsterilization (1st to 23rd days). At the end of the experiment (23rd day) the whole system was sterilized by 10 ppm sodium hypochloride solution. From the day when the chlorine injector was turned off, there was microbial monitoring for the number of *Legionella*, total viable bacterial counts, heterotrophic bacteria, and free-living amoebae.


A
B
Viable cells (log 10)
Amoebae (cells/ml)
days
Amoebae (cells/ml)
Heterotrophic bacteria (CFU/ml)
Total viable bacterial counts (CFU/ml)
Legionellae (CFU/100ml)

A


Viable cells (log 10)
Amount of KMnO4 consumption (mg/l)
before 14
0 1
5
10
20
30
31
36
days
Heterotrophic bacteria (CFU/ml)
Total viable bacterial counts(CFU/ml)
Legionellae (CFU/100ml)
Amount of KMnO4 consumption

B


Viable cells (log 10)
0 1
5
10
20
30
31
36
days
Heterotrophic bacteria (CFU/ml)
Total viable bacterial counts(CFU/ml)
Legionellae (CFU/100ml)

cells/ml, followed by a rapid decrease from the bathing water. They disappeared within 5 days after that, for unknown reasons. The number of amoebae in the filter water fluctuated and amounted to 12 cells/ml at the end of the experiment.

In experiment 2 (Fig. 3), *Legionella* in the bathing water and the filter water became detectable from 3 days after stopping ultraviolet irradiation, which was 5 days after residual chlorine disappeared and the number of *Legionella* detected in the bathing water and the filter water was 1.1×10^2 CFU/100 ml and 2.0×10^4 CFU/100 ml, respectively. The number of *Legionella* had increased to 10^6 CFU/100 ml within 5 days and was 10^5 to 10^6 CFU/100 ml at the end of the experiment. The total viable bacterial counts reached 10^4 to 10^5 CFU/ml in 5 days after the residual chlorine disappeared. The number of heterotrophic bacteria was almost the same as the number of total bacterial counts. A large number of *Legionella*, namely, 1.6×10^7 CFU/g, 3.1×10^5 CFU/g, and 1.8×10^5 CFU/g, was detected from the sand collected from the upper, middle, and lower parts of the filter, respectively. Similarly, 9.1×10^3 cells/g, 1.5×10^3 cells/g, and 1.6×10^3 cells/g of amoebae were detected from the filter. In addition, it is noteworthy that *Legionella* was detected from the hair catcher in the model plant at concentrations around 3.1×10^4 CFU/swab in experiment 1 and, 1.2×10^5 CFU/swab in experiment 2. The amount of $KMnO_4$ consumption in the bathing water was 2.7 mg/liter before use and was calculated to be around 10.1 mg/liter after the use by 40 volunteers under the presence of chlorine residues.

In the present experiments, it was clearly demonstrated that *Legionella* occurred in the bathing water circulating system within a short period in a sequential manner of microbial growth. Namely, concentration of organic matter (dirt) in the bathing water that can be monitored as the $KMnO_4$ consumption value increased in correlation to the number of bathers. The deposited dirt allows bacteria to rapidly undergo multiplication in the bathing water, which consequently supports the occurrence of a large number of host amoebae. The growth of *Legionella* is a manifestation of the extended use of bathing water under inadequate hygienic maintenance. It also turned out that the filter acts as the main hotbed for *Legionella* multiplication (2).

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FIGURE 3 The growth of *Legionella*, etc. in the bathing water (A) and the filter water (B) in experiment 2. For 14 days about 40 volunteers took baths under the system with chlorine control, in which the chlorine injector was turned off. After that, three volunteers took baths (0 to 1st days) and the ultraviolet irradiation was turned off (2nd day). The bathing water was circulated under nonsterilization (3rd to 31st days). After bathing water was exchanged (31st day), it was recirculated (31st to 36th days). At the end of the experiment (36th day) the whole system was sterilized with 6% H_2O_2. From the day when the chlorine injector was turned off, there was microbial monitoring for the number of *Legionella*, total viable bacterial counts, heterotrophic bacteria, and the amount of $KMnO_4$ consumption.

INHIBITION OF *LEGIONELLA* GROWTH IN CIRCULATING BATHING WATER BY A FILTER REFRESHMENT METHOD USING A HIGH CONCENTRATION OF CHLORINE

Kanji Sugiyama, Katsuhiko Ohata, Mitsuaki Suzuki, Rieko Shimogawara, Shinji Izumiyama, Kenji Yagita, and Takuro Endo

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In most of hot spring baths in Japan, bathing water is circulated for extended use to conserve hot spring water. In recent years, massive outbreaks of Legionnaires' disease among hot spring bath users have been reported in many districts in Japan (1–3).

Through experiments using a model bathing facility (consisting of a bathtub (2 m^3), a hair catcher, a circulating pump, a filter (ceramic filtering medium, 100 kg, LV: 25.5 m/h), a water heater (40°C), and pipe), we could reproduce the growth of *Legionella* (10^5 to 10^6 CFU/100 ml) naturally under a circulating condition when chlorine was not added into the water (see chapter 102). Furthermore, we found that the filtering medium was the most highly contaminated by *Legionella* among the parts of the circulating system. Thus, the filtering medium itself became a new source resulting in continuous contamination of bathing water by *Legionella* when disinfection of the filtering medium was inadequate, even though the bathing water had been replaced (see chapter 102).

In the present study, we used a bath model to investigate the effectiveness of backwashing the filtering medium using a high concentration of chlorine for disinfection and growth inhibition of *Legionella*. We then assessed the usefulness of this method from the perspective of hygiene control of circulating bathing water.

The procedure for assessing short-term and long-term effectiveness of backwashing using a model bath is as follows.

Short-term effectiveness: After proliferation of *Legionella* in the model bath, backwashing with chlorinated water at concentrations ranging from 5 to 10 mg/liter was performed for 5 min. We called this "the filter refreshment method." The water samples and ceramic sand were collected from the filter unit and analyzed both for *Legionella* and for host amoebae. Bacto-Agar (DIFCO), a monolayer that heat-inactivated *Escherichia coli* is deposited on, was used for isolating amoebae. The effectiveness of backwashing with tap water (0.2 mg/liter of chlorine concentration) was also assessed.

Long-term effectiveness: After depositions of organic substances by bathing in the presence of chlorine in the model bath, the addition of chlorine was stopped while the bath was in use until the chlorine was disappeared.

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Chlorine backwashing by the filter refreshment method (5 to 10 mg/liter) was performed once a day for 9 days, and the bathing water and the water from the filter unit were collected every day prior to backwashing to determine the presence of *Legionella* and amoebae.

SHORT-TERM EFFECTIVENESS OF THE FILTER REFRESHMENT METHOD

The number of *Legionella* in the filter water, measured to be 10^3 CFU/100 ml at the beginning of the experiment, decreased with the increase of residual chlorine concentrations

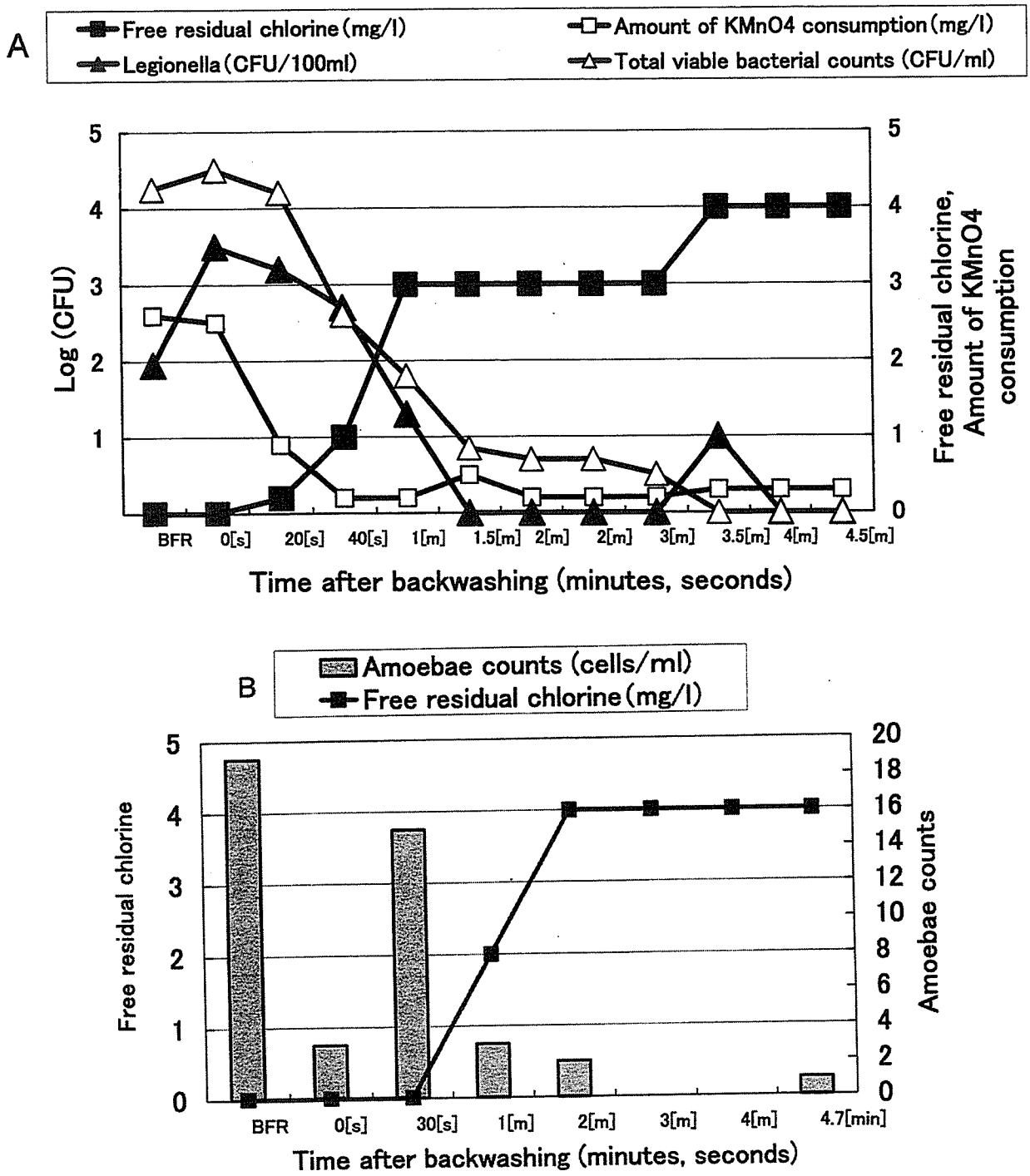

FIGURE 1 Short-term effectiveness of the filter refreshment method. BFR, before filter refreshment; m, minutes; s, seconds.

(4 mg/liter at the maximum) and became undetectable 4 min after the backwashing (Fig. 1A). *Legionella* in the filtering medium was also undetectable (6.0×10^2/g to 0/g) after being backwashed for 5 min. The amoebae count could also be reduced by the backwashing (Fig. 1B). The amount of dissolved organic materials, represented by the amount of potassium permanganate consumed, was also reduced over time after backwashing (Fig. 1A). However, when tap water (0.2 mg/liter residual chlorine concentration) was used for backwashing, *Legionella* was not disinfected effectively and there were no marked differences in *Legionella* counts in the filtering medium before and after backwashing. These findings demonstrated that backwashing with chlorinated water at concentrations ranging from 5 to 10 mg/liter was essential for the removal of *Legionella* from the filtering medium.

LONG-TERM EFFECTIVENESS OF THE FILTER REFRESHMENT METHOD

The number of *Legionella* in both bathing water and filter water was maintained at a level lower than 10 to 70 CFU/100 ml by repeated backwashing with chlorinated water alone once a day; the *Legionella* growth was greatly inhibited (Fig.2) compared to that under non-disinfection conditions (see chapter 102). Amoebic growth could be inhibited to a limited level (Fig. 2). On the basis of these results, daily backwashing by the filter refreshment method is considered to be effective for growth inhibition of both *Legionella* and host amoebae in circulating bathing water.

Together with daily use of the filter refreshment method, addition of chlorine into the bathing water to a minimum concentration of 0.2 to 0.4 mg/liter may ensure the supply of circulating bathing water with increased microbial safety.

It was also demonstrated that the filter refreshment method could prevent the deposition of organic substances in the filter medium from the bathers and, thus, reduce the chlorine smell markedly.

The effectiveness of the filter refreshment method has been confirmed in practice by bathhouses serving the public, and the cost has been proven to be inexpensive.

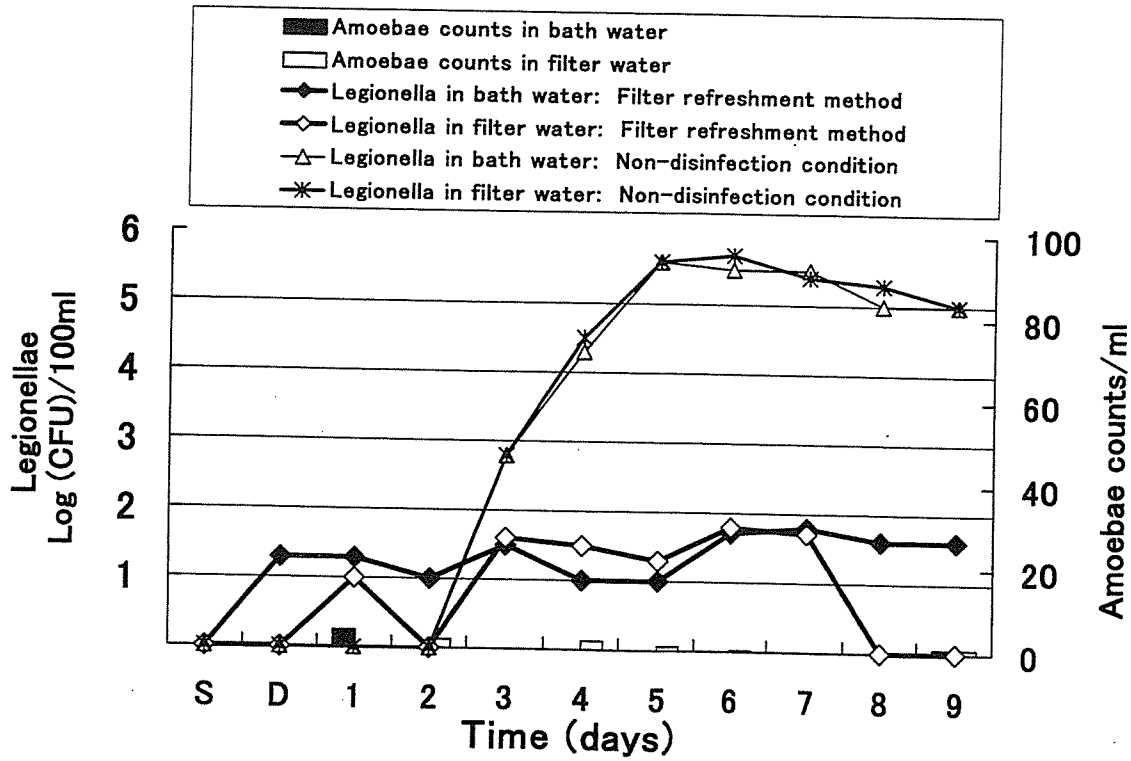

FIGURE 2 Long-term effectiveness of the daily filter refreshment method. S, stop chlorination; D, disappearance of residual chlorine.

The filter refreshment method has been incorporated into the Shizuoka Prefectural Enforcement Ordinances for the Public Bathhouse Law and Hotel Business Law, both of which have been enforced since April 2004.

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