Quantitative Microbial Risk Assessment for faecal management – health consequences in the Mekong Delta, Vietnam

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SUMMARY

Vietnam's Mekong Delta (MD) is known as the rice bowl of the country. Rapid development and population growth there have lead to an increasing demand for water use and wastewater treatment. Yet there are no central wastewater treatment plants in the region and water supply systems are generally lacking in rural areas. Only septic tanks (STs) have been introduced to treat human effluent. Small-scale biogas plants, mostly plastic bio-digesters (PBDs) have been promoted to treat animal slurries. However, the operation and maintenance of both systems are unregulated and their microbial treatment efficacy has not been a priority. Poor sanitary practices of local people add to this creating a potentially serious health hazard. This study aims to analyse the microbial risk associated with faecal management in MD as it impacts on public health.

The topic is explored by three vehicles: pilot study, field study and quantitative microbial risk assessment (QMRA). The pilot study replicates tropical conditions to determine microbial reduction and related factors of anaerobic treatment. Reduction rates of phages and bacteria in river water and on terrestrial spinach were determined. The field study was conducted in MD to verify the microbial make-up of faecal substrates, surface water and aquatic spinach. Pathogen treatment efficacy of PBDs was considered. A survey was also conducted to find out human exposure to contaminated sources. All data were used for the QMRA study, which calculates the probability and annual risk of infection via @Risk 5.5 (Palisade Corporation).

The pilot study showed there was a hygienic effect in the anaerobic treatment of excreta but microbe reduction rates were low. The reduction of phages (somatic coliphage, male-specific bacteriophage) and bacteria (*Escherichia coli*, *Salmonella* Senftenberg, *Enterococus faecalis*) in lab-scale PBDs increased with longer hydraulic retention time (HRT). Longer HRT played a vital role in yielding more gas. Besides HRT pathogen reduction also depended on initial concentration, species tested and substrate type. High levels of volatile fatty acids (VFAs) had no effect on microbial reduction at neutral pH. Moreover phage and bacteria reduction also depend on operation conditions – batch-

wise or continuous digestion. Microbial reduction in STs was not significant even at maximum HRT (3 days).

Anaerobic digestion in tropical PBDs had little effect on the inactivation of *Ascaris suum* ova. Yet helminth ova do settle in the sludge at the reactor's base if HRT is long and the relationship between an ova's viability rate and sludge retention time was established by exponential equation. A few *Ascaris suum* ova survived in sludge for up to one year. There was no difference between the viability of *Ascaris suum* ova in biogas or septage sludge.

Faecal substrates sampled during the field study contained high levels of microbial indicators and pathogens. *E. coli* and *Enterococcus* spp. were detected in all pig slurry and septage samples. *Salmonella* spp. were detected in over 60% and coliphages in over 50% of samples. Helminth ova were present in 80% of pig slurry samples, 95% of untreated septage samples, and in all septage sludge samples in high concentrations. Ten ova varieties were found in pig slurries and twelve in septage.

Field study results suggest that the functionality of PBDs and STs is not optimal to inactivate microbial indicators and pathogens. Volume of PBD is not compatible to the amount of pig slurry. PBDs are rarely desludged and STs are emptied only when blockages occur. Thus reduction of bacteria was < 1 $\log_{10}$ and phages < 1.5 $\log_{10}$ while their influent concentrations were high (up to 6.2 $\log_{10}$ CFU ml^{-1}). *Salmonella* spp. were detected more frequently in effluents than in influents. In most PBDs helminth ova did not settle but were released to surface water via effluents, the highest concentration being 175,000 no. l^{-1}. Most PBD effluents and overflows from full STs flow directly and contaminate the surface water, which is used by many people every day.

Surface water and aquatic spinach samples were contaminated. The average *E. coli* level in canal water was over the total coliform limit set by the Vietnamese Surface Water Quality Standard (TCVN 5942-1995). *Salmonella* spp. were routinely detected. Decimal reduction time (T_{90}) of phages and bacteria in Mekong river water was over 2 days. Aquatic spinach was contaminated much like its habitat. *Enterococcus* spp., *E. coli*, somatic coliphage and *Salmonella* spp. were all found in samples, though average *E. coli* concentrations on spinach grown in urban canals was twice that of those grown

in fishponds receiving PBD effluent. On terrestrial spinach microbial reduction was 0.2 – 0.4 $\log_{10}$/day.

By QMRA infection risk was high, ranging in descending order from helminth to rotavirus to Salmonella. The probability of salmonellosis and helminthiasis was higher per exposure to PBD effluent than with pig slurry. MD sewage workers were most at risk due to constant exposure to faecal matter. Incidental ingestion of pig slurries, bathing/swimming in canals, drinking untreated surface water and eating raw spinach constituted chronic exposure scenarios for MD people generally. All mentioned scenarios were found to exceed acceptable risk levels.

Besides health programs and personal hygiene routines, barriers reducing risk of infection include wastewater treatment (e.g. PBDs), due time between last crop irrigation and harvest, treating water before consumption and food preparation. Risks were reduced when PBDs ran at HRTs of 15 and 30 days as effluent was assumed to be free of helminth ova. The high pathogen load of surface water means this is only potable when boiled. Aquatic spinach is not safe to eat unless cooked. Spinach irrigated with improved PBD effluent (HRT $\geq$ 15 days) can be eaten raw, but then only when the time between final irrigation and harvest is long enough. As a rule spinach should be washed prior to consumption.

Current faecal management practices in MD equate to high infection risks for its population. The microbial treatment efficacy of anaerobic digestion there can be improved by relatively simple changes to operations and maintenance. To reduce infection rates a campaign that integrates faecal management, water supply and behavioural change is recommended. While QMRA data collation and modelling requires much effort communicating health risks to the government and public is challenging. Thereby lasting technical, legislative and cultural can be changed so as to improve the public health effectively.

ZUSAMMENFASSUNG (SUMMARY IN GERMAN)

Das Mekong Delta (MD) ist die Reiskammer von Vietnam. Die rasche wirtschaftliche Entwicklung und das hohe Bevölkerungswachstum haben den Wasserbedarf und den Bedarf an Abwasserbehandlungssystemen stark erhöht. Allerdings fehlen im ländlichen Bereich noch immer Wasserversorgungsnetze und in ganzen MD zentrale Abwasserbehandlungsanlagen. Für häusliches Abwasser wurden biologishe Klärgraben (STs) eingeführt und für Abwässer aus der Tierhaltung werden kleine Kunststoff-Biogasanlagen (PBDs) empfohlen. Für beide Systeme steht die Hygienisierung des Abwassers nicht im Vordergrund und sie unterliegen auch keiner geregelten Kontrolle und Wartung. Besonders im ländlichen Bereich, wo Abwässer auch ohne jegliche Behandlung in Gewässer eingeleitet werden, stellt der mikrobielle Eintrag durch Abwasser in die Vorfluter ein hohes Risikopotenzial dar. Diese Arbeit analysiert das mikrobielle Risiko der vorhandenen Reinigungssysteme für die öffentliche Gesundheit im Mekong Delta.

Im ersten Teil der Arbeit wurde im Rahmen einer Laborstudie das mikrobielle Abbauverhalten in den PBDs unter tropischen Bedingungen untersucht. Reduktionsraten von Phagen und Bakterien in Flusswasser und auf Spinat wurden untersucht. Eine Feldstudie im Mekongdelta stellte den zweiten Teil der Arbeit dar, in dem die mikrobielle Belastung von Fäkalsubstraten, Oberflächengewässer, und Wasserspinat analysiert wurden. Der Hygienisierungseffekt der Kunststoff-Biogasanlagen wurde betrachtet. Darin enthalten war auch eine Untersuchung, in welchem Umfang Menschen mit mikrobiell verunreinigten Substraten exponiert sind. Im dritten Teil der Arbeit erfolgte anhand der erhobenen Daten eine quantitative mikrobielle Risikoabschätzung (QMRA) mit Hilfe der Software @Risk 5.5 (Palisade Corporation).

Die Laborstudie zeigte einen Hygienisierungseffekt der Fäkalien im Rahmen der Anaerobbehandlung, allerdings waren die Abbauraten der Mikroben gering: Die Abbauraten der Phagen (somatischen Coliphagen, male-specific Bacteriophagen) und Bakterien (*Escherichia coli*, *Salmonella* Senftenberg, *Enterococus faecalis*) stiegen bei höherer hydraulischer Verweildauer (HRT) – parallel zum Biogasertrag – in den Biogasanlagen. Weitere Faktoren, die die mikrobiellen Abbauraten beeinflussten, waren

die ursprüngliche Höhe der mikrobiellen Verunreinigung, mikrobielle Spezies und der Substrattyp. Hohe Konzentrationen von organischen Säuren bei neutralem pH hatten keinen Einfluss auf den Abbau. Auch die Art der Behandlung – als Batch oder im kontinuierlichen Sytem – beeinflusste die Abbaurate. Selbst bei hohen HRT von 3 Tagen war der mikrobielle Abbau in Abwasserfaulraumen nicht signifikant.

Die Anaerobbehandlung in den untersuchten Biogasanlagen hatte nur einen sehr geringen Effekt auf die Inaktivierung von *Ascaris suum* Eiern. Allerdings sedimentieren Helmintheneier im Fermenter auf den Boden, so dass bei hohen HRT und entsprechender Schlammrückhaltung die Eier im Schlamm über die Zeit inaktiv werden. Ein exponentieller Zusammenhang wurde in der Arbeit beschrieben. Einige Eier von *Ascaris suum* waren noch nach einem Jahr Schlammrückhaltung aktiv. Zwischen Biogas- und Klärgrabenschlamm gab es keine Untersiede hinsichtlich der Überlebensfähigkeit der *Ascaris suum* Eier.

Fäkalsubstrate, die während der Feldstudie untersucht wurden, wiesen hohe Gehalte an mikrobieller Verunreinigung und Pathogenen. *E. coli* und *Enterococus* spp. waren in allen Fäkalschlammproben tierischer (nur Schweine) und menschlicher Herkunft enthalten. *Salmonella* spp. konnte in mehr als 60%, Coliphagen in mehr als 50% der Proben nachgewiesen werden. Helmintheneier waren in 80% der Schweinegülleproben, in 95% der Proben von unbehandeltem häuslichem Abwasser und in allen Proben des Schlammes von ST in hohen Konzentrationen zu finden. Die Eier von 10 verschiedenen Helminthenarten wurden in Schweinegülle und von 12 Arten in häuslichem Abwasser gefunden.

Die Ergebnisse der Feldstudie machten deutlich, dass weder PBDs noch STs geeignet sind, mikrobielle Fäkalindikatoren und Pathogene zu inaktivieren. Das kann daran liegen, dass die PBDs nicht an die anfallende Menge an Schweinegülle angepasst sind. Zudem werden sowohl PBDs als auch STs nur sehr selten entschlammt, z.B. wenn Blockaden vorliegen. Die Bakterien- (<1 $\log_{10}$) und Phagenreduktion (< 1,5 $\log_{10}$) waren entsprechend gering, wobei deren Anfangskonzentrationen hoch waren (bis zu 6,2 $\log_{10}$ CFU ml^{-1}). *Salmonella* spp. waren öfter im Abfluss als im Zufluss in den Anlagen zu finden. Helmintheneier sedimentierten in PBDs nicht, sondern wurden aus den Anlagen ausgespült (Maximalkonzentration: 175.000 l^{-1}). Der Abfluss der meisten

PBDs und STs wird direkt in Oberflächengewässer geleitet, welches von der Bevölkerung täglich genutzt wird.

Sowohl Oberflächenwasser als auch Wasserspinat waren kontaminiert. Die mittlere *E. coli* Konzentration überschritt die nationalen Vorschriften zur Oberflächenwasserqualität (TCVN 5942-1995). Regelmäßig konnte *Salmonella* spp. nachgewiesen werden. Die dezimale Reduktionszeit (T_{90}) betrug im Fluss mehr als zwei Tage. Entsprechend waren alle Wasserspinatproben mit *Enterococcus* spp., *E. coli*, somatischen Coliphagen und *Salmonella* spp. belastet. In städtischen Kanälen war die *E. coli*-Konzentration auf Spinat etwa doppelt so hoch wie auf Spinat, der in Fischteichen wuchs, welche als Vorfluter für PBDs dienten. Auf terrestrischem Spinat betrug die mikrobielle Abbaurate 0,2 – 0,4 $\log_{10}$/Tag.

Die QMRA bewertete das Infektionsrisiko durch Helmitheneier am höchsten, gefolgt von Rotavirus und Salmonella. Die Wahrscheinlichkeit einer Salmonellose und Helminthiasis durch Kontakt mit Biogasabfluss ist höher als die durch Kontakt mit unbehandelter Schweinegülle. MD Kanalarbeiter sind am höchsten durch den stetigen Kontakt mit Fäkalsubstraten gefährdet. Für die gesamte MD Bevölkerung gilt, dass sie durch die Modellszenarien (versehentliches Verschlucken von Schweinegülle, Baden und Schwimmen in Kanälen, Trinken von unbehandeltem Oberflächenwasser, Verzehr von rohem Spinat) chronisch einem Infektionsrisiko ausgesetzt sind. Alle erwähnten Szenarien überschreiten akzeptable Risikowerte.

Neben Gesundheitsprogrammen und persönlichen Hygienemaßnahmen sind Abwasserbehandlung (wie die PBDs), das rechtzeitige Einstellen der Bewässerung vor der Ernte, Wasseraufbereitung vor dessen Verzehr bzw. Nutzung zum Zubereiten von Speisen mögliche Barrieren, die das Infektionsrisiko reduzieren. Das Infektionsrisiko ist bei PBDs durch längere HRTs (15-30 Tage) reduzierbar, da dann der Abfluss als Helmintheneier frei angenommen werden kann. Die hohen Konzentrationen von Pathogenen im Oberflächenwasser verlangen ein Abkochen vor dessen Nutzung. Darüber hinaus ist aquatischer Spinat erst dann risikofrei zu konsumieren, wenn er gekocht wurde. Spinat, welcher mit Abfluss einer PBD mit einer HRT > 15 Tage bewässert wurde, kann roh konsumiert werden, wenn die Zeit zwischen Bewässerung

und Ernte ausreichend lang ist. Generell sollte Spinat vor dem Verzehr gewaschen werden.

Die gegenwärtigen Methoden zum Umgang mit Fäkalsubstraten belasten die Bevölkerung im MD durch hohe Infektionsrisiken. Die mikrobielle Abbaurate von Anaerobsystemen kann durch relativ einfache Änderungen im Betrieb und in der Wartung verbessert werden. Um das Infektionsrisiko zu senken, ist eine Kampagne notwendig, die den Umgang mit Fäkalsubstraten, die Wasserversorgung und die persönliche Hygiene integriert. Die Kommunikation der hier untersuchten Infektionsrisiken bei Entscheidungsträgern und Öffentlichkeit stellt eine besondere Herausforderung dar. Denn nur so können technische Vorschriften, rechtliche Grundlagen und kulturelle Gewohnheiten nachhaltig im Sinne einer besseren öffentlichen Gesundheit angepasst werden.

TÓM TẮT (SUMMARY IN VIETNAMESE)

Vùng đồng bằng sông Cửu Long của Việt Nam (ĐBSCL) nổi tiếng là vựa lúa của cả nước. Sự phát triển kinh tế và gia tăng dân số nhanh ở khu vực này làm gia tăng nhu cầu sử dụng nước và xử lý nước thải. Tuy nhiên, vùng này vẫn chưa có hệ thống xử lý nước thải tập trung và ở nông thôn thì thường thiếu nước cấp sinh hoạt. Chủ yếu có hầm tự hoại xử lý phân người và túi ủ biogas được sử dụng để xử lý phân chuồng. Sự vận hành và bảo trì của hai hệ thống này không tốt và hiệu quả xử lý vi sinh không được ưu tiên. Thêm vào đó, hành vi vệ sinh cá nhân chưa tốt và lối sống truyền thống của dân cư ĐBSCL có thể là mối nguy hiểm cho sức khỏe chính họ. Mục tiêu của nghiên cứu này là phân tích rủi ro vi sinh có liên quan đến việc quản lý chất thải người và gia súc ở ĐBSCL bởi vì nó ảnh hưởng đến sức khỏe cộng đồng.

Đề tài này được thực hiện thông qua 3 nghiên cứu: nghiên cứu thí điểm trong phòng thí nghiệm, nghiên cứu thực tiễn, và đánh giá định lượng rủi ro vi sinh (ĐĐRV). Nghiên cứu trong phòng thí nghiệm được tiến hành theo điều kiện vùng nhiệt đới nhằm xác định hiệu quả xử lý vi sinh và các nhân tố có liên quan của phương pháp xử lý kỵ khí. Mức sụt giảm số lượng của thể thực khuẩn và vi khuẩn trong nước sông và trên rau muống cũng được xác định. Nghiên cứu thực tiễn ở ĐBSCL tập trung vào đặc tính vi sinh của chất thải người và gia súc; của nước sông rạch và rau muống thủy sinh. Hiệu quả xử lý vi sinh của túi ủ biogas ở ngoài thực tế cũng được chú trọng. Ngoài ra, một khảo sát cũng được thực hiện nhằm tìm ra mức độ tiếp xúc của dân cư với các nguồn ô nhiễm phân. Tất cả các kết quả trên được dùng cho ĐĐRV bằng phần mềm @Risk 5.5 (Palisade Corporation) với các tính toán cho ra xác suất nhiễm vi sinh gây bệnh cho từng tiếp xúc và cho cả năm.

Nghiên cứu thí điểm trong phòng thí nghiệm cho thấy phương pháp xử lý kỵ khí nước thải người và gia súc có hiệu quả xử lý vi sinh nhưng ở mức độ thấp. Sự sút giảm nồng độ của thể thực khuẩn (somatic coliphage, male-specific bacteriophage) và vi khuẩn (*Escherichia coli*, *Salmonella* Senftenberg, *Enterococus faecalis*) trong mô hình thí nghiệm túi ủ biogas tăng cùng với sự gia tăng của thời gian lưu thủy lực. Thời gian lưu thủy lực dài cũng đóng vai trò quan trọng trong việc tăng sản lượng biogas. Ngoài thời gian lưu thủy lực, sự sút giảm vi sinh còn phụ thuộc vào nồng độ vi sinh ban đầu, loài vi sinh và loại nước thải. Ở pH trung tính, nồng độ cao của các axit béo bay hơi không gây

ảnh hưởng đến sự sút giảm nồng độ vi sinh. Hơn nữa, sự sút giảm nồng độ của thể thực khuẩn và vi khuẩn còn phụ thuộc vào chế độ hoạt động của hệ thống xử lý kỵ khí: vận hành theo mẻ hay vận hành liên tục. Sự sút giảm vi sinh ở mô hình hầm tự hoại không đáng kể, ngay cả ở mức thời gian lưu thủy lực cao nhất của hầm tự hoại là 3 ngày.

Xử lý kỵ khí trong túi ủ biogas có rất ít ảnh hưởng đến việc bất hoạt trứng *Ascaris suum*. Tuy nhiên, trứng giun sán lắng xuống lớp bùn ở đáy túi ủ nếu thời gian lưu thủy lực đủ dài. Mối quan hệ giữa tỉ lệ tồn tại của trứng *Ascaris suum* và thời gian lưu bùn được thiết lập bằng một phương trình số mũ. Chỉ một số rất ít trứng *Ascaris suum* tồn tại trong bùn đến một năm. Không có sự khác biệt của sự tồn tại của trứng *Ascaris suum* trong bùn biogas và bùn hầm tự hoại.

Mẫu nước thải từ người và gia súc từ nghiên cứu thực tiễn chứa nồng độ cao vi sinh chỉ thị và vi sinh gây bệnh. *E. coli* and *Enterococcus* spp. hiện diện trong tất cả mẫu nước thải từ chuồng heo, từ nhà vệ sinh cũng như trong bùn hầm tự hoại. *Salmonella* spp. hiện diện trong hơn 60% và thể thực khuẩn coliphage trong hơn 50% số mẫu. Trứng gian sán với nồng độ cao được tìm thấy trong 80% mẫu nước thải từ chuồng heo, 95% mẫu nước thải từ nhà vệ sinh (nước thải chưa qua xử lý trong hầm tự hoại) và trong tất cả mẫu bùn lấy từ hầm tự hoại. Mười loại trứng giun sán được xác định trong mẫu nước thải từ chuồng heo và 12 loại được tìm thấy trong mẫu bùn hầm tự hoại.

Nghiên cứu thực tiễn cho thấy rằng hoạt động của túi ủ biogas và hầm tự hoại chưa phải là tối ưu để bất hoạt vi sinh gây bệnh. Thể tích của túi ủ không tương xứng với số lượng chất thải từ chuồng heo. Bùn trong túi ủ hiếm khi được lấy ra và hầm tự hoại chỉ được hút bùn khi bị nghẹt. Chính vì vậy mà sự sút giảm (đầu ra so với đầu vào) vi khuẩn < 1 $\log_{10}$ và thực khuẩn thể < 1.5 $\log_{10,}$ trong khi nồng độ đầu vào rất cao (lên đến 6.2 $\log_{10}$ CFU/lít). *Salmonella* spp. được phát hiện trong mẫu đầu ra thường xuyên hơn trong mẫu đầu vào túi ủ. Trong hầu hết túi ủ biogas, trứng giun sán không lắng xuống mà được giải phóng thẳng ra nước sông, kênh rạch thông qua đầu ra của túi ủ. Nồng độ trứng giun sán ở mẫu đầu ra của túi ủ lên đến 175.000 trứng/ lít. Đa số chất lỏng đầu ra của túi ủ biogas và nước thải chảy tràn từ hầm tự hoại thải thẳng ra môi trường và làm ô nhiễm nguồn nước mặt.

Mẫu nước mặt và rau muống thủy sinh bị nhiễm vi sinh. Nồng độ *E. coli* trung bình trong nước kênh rạch vượt mức Tổng coliform quy định trong Tiêu chuẩn Việt Nam về

Chất lượng nước mặt (TCVN 5942-1995). *Salmonella* spp. thường xuyên được phát hiện trong mẫu. Thời gian giảm thiểu thập phân (T_{90}) của thể thực khuẩn và vi khuẩn trong nước sông là hơn 2 ngày. Rau muống thủy sinh cũng bị nhiễm vi sinh như chính môi trường sống của nó. *Enterococcus* spp., *E. coli*, somatic coliphage và *Salmonella* spp. được tìm thấy trong mẫu. Nồng độ *E. coli* trong rau muống trồng ở các kênh rạch đô thị cao gấp 2 lần so với rau muống trồng ở ao cá nhận chất lỏng đầu ra của túi ủ biogas. Trên rau muống trồng trên cạn, sự sút giảm vi sinh đạt được từ 0.2 – 0.4 $\log_{10}$/ngày.

ĐĐRV cho thấy mức rủi ro bị nhiễm vi sinh gây bệnh của người tiếp xúc nguồn ô nhiễm phân rất cao, mức độ giảm dần từ trứng giun sán, rotavirus đến Salmonella. Xác xuất nhiễm Salmonella và trứng giun sán khi tiếp xúc với chất lỏng đầu ra của túi ủ biogas cao hơn so với chất thải từ chuồng heo. Công nhân vệ sinh hút hầm cầu chịu nhiều rủi ro nhiễm vi sinh nhất do thường xuyên tiếp xúc với chất thải. Tình cờ nuốt phải nước thải từ chuồng heo, tắm/bơi trong kênh rạch, uống trực tiếp nước kênh rạch và ăn rau muống sống là những tình huống tiếp xúc với nguồn ô nhiễm thường xuyên và bất lợi cho sức khỏe cư dân ĐBSCL. Các tình huống này tạo nên mức rủi ro bị nhiễm vi sinh cao hơn so với các tiêu chuẩn giới hạn rủi ro.

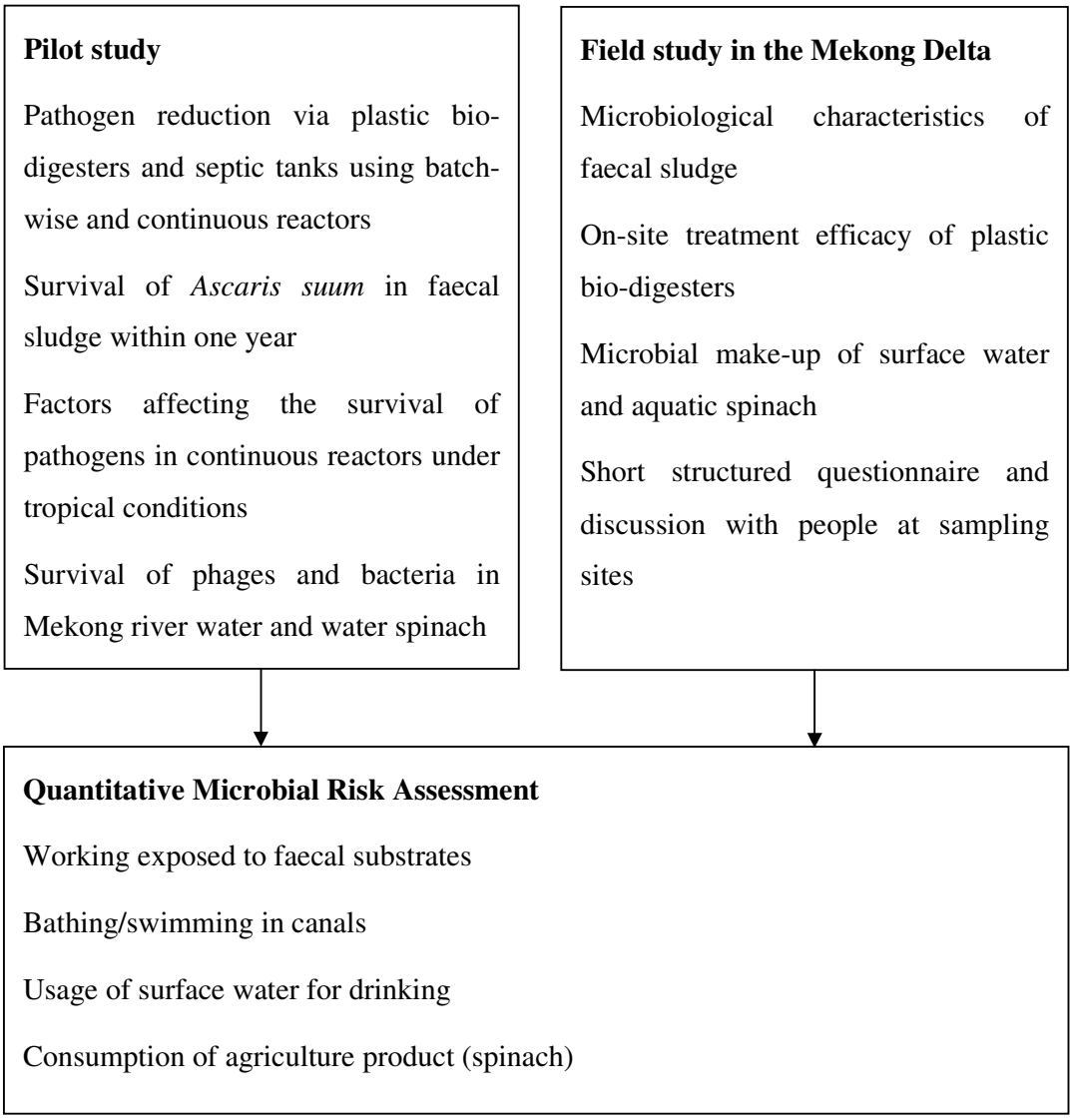
Bên cạnh các chương trình về sức khỏe và hành vi vệ sinh cá nhân, biện pháp giảm rủi ro bị nhiễm vi sinh bao gồm xử lý nước thải (vd như túi ủ biogas), thời gian đủ dài giữa lần tưới nước thải cuối cùng và thu hoạch, xử lý nước mặt trước khi uống và chuẩn bị thức ăn (vd rửa sạch, nấu chín). Mức độ rủi ro giảm khi túi ủ biogas được vận hành ở thời gian lưu thủy lực 15 hoặc 30 ngày vì như thế chất lỏng đầu ra được xem như không còn trứng giun sán. Mức độ ô nhiễm cao của nước mặt cho thấy chỉ uống được an toàn sau khi đun sôi. Rau muống thủy sinh trồng trong môi trường này cũng không an toàn khi ăn sống. Rau muống trồng trên cạn được tưới với chất lỏng đầu ra của túi ủ biogas chỉ có thể ăn sống an toàn khi túi ủ biogas vận hành ở thời gian lưu thủy lực dài hơn 15 ngày, thời gian giữa lần tưới cuối cùng đến khi thu hoạch đủ dài và rau muống phải được rửa sạch đúng cách trước khi ăn.

Tình trạng quản lý nước thải hiện nay ở ĐBSCL mang lại nhiều rủi ro bị nhiễm vi sinh gây bệnh cho cư dân vùng này. Hiệu quả xử lý vi sinh của phương pháp kỵ khí có thể được cải thiện bằng các biện pháp vận hành và bảo dưỡng đơn giản. Nhằm giảm mức độ

nhiễm vi sinh cho dân cư, cần thiết phải có một chiến dịch kết hợp việc quản lý chất thải từ người và gia súc, cải thiện hệ thống nước cấp và thay đổi thói quen của người dân. Trong khi ĐĐVR cần rất nhiều thời gian và nỗ lực, việc tuyên truyền các rủi ro này đến các cấp chính quyền và mọi người dân là một thử thách lớn. Nhưng bằng cách này có thể tác động đến sự thay đổi về kỹ thuật, luật pháp và lối sống truyền thống nhằm cải thiện sức khỏe cộng đồng một cách hiệu quả.

THE STUDY ROAD MAP

The thesis' structure is conventional (introduction, methods, results, discussion and conclusion), and the topic is explored via three studies (a pilot study, field study, and quantitative microbial risk assessment), which this flowchart overviews:



FOREWORD

Cần Thơ gạo trắng nước trong
Ai đi tới đó lòng không muốn về.

Can Tho has white rice and clear water
Once being there, your heart won't leave.

This is a well-known saying about the "capital" of Vietnam's Mekong Delta. Cần Thơ is the biggest city in the Mekong Delta, which possesses enormous potential in terms of its high productive land. Life here has long been dictated to by water. Agriculture and aquaculture, the main productive activities, are dominated by monsoon rains and tidal flows. With its vast network of canals these also influence transportation and infrastructure. In living memory it was easier to get around by boat than by road. There is seasonal variation though generally said the Mekong Delta has abundant water.

Beyond its symbolic and productive uses water is essential for existence. People use it to eat, drink, clean, preserve and dispose – everyday. Water is a measure of public health and more than its abundance is the issue of its management. Nowadays with a dense population and without a centralised wastewater treatment plant in Can Tho City, the first part of the famous saying above seems to be not anymore appropriate. Can Tho has still white rice but not any more clear water! The Mekong Delta is overwhelmingly rural. There are no centralised water supply or treatment plants in the villages and systems are household based. Water is sourced directly from rain, ground and surface water, with wastes often returned directly to these environments. Disease risks from microbial contamination and mineral toxicity are high and chronic illnesses are common. Villagers have low education levels too and surveys show they poorly understand the health risks associated with their water use and wastes disposal practices let alone the vectors of and pathways to illness and wellbeing.

To preserve the environment, especially surface water quality, solutions were introduced to the population. As fishpond toilets are considered unhygienic, septic tanks

have been introduced to households. In rural areas in the Mekong Delta thousands of small-scale biogas plants were built in recent years to utilize the manure for energy. Although these changes are to some extent positive this study quantifies risks associated with the faecal management in the Mekong Delta. The notion of quantitative risk was chosen as it yields values with which to compare sanitation systems and extant accepted risk values.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CFU	Colony forming unit
DALY	Disability adjusted life year
DM	Dry matter
DSM	German Collection of Microorganisms and Cell Cultures
HRT	Hydraulic retention time
NTU	Nephelometric Turbidity Unit, used to describe turbidity
ODM	Organic dry matter
PBD	Plastic biogas digester
PDF	Probability Density Function
PFU	Plaque forming unit
pppy	per person per year
QMRA	Quantitative Microbial Risk Assessment
rpm	rounds per minute
SODIS	Solar Drinking Water Disinfection
ST	Septic tank
T_{90}	Decimal reduction time
TIC	Total Inorganic Carbon
VFA	Volatile fatty acid

1 INTRODUCTION

Rapid development and population growth lead to increasing demands for water as well as discharge of wastewater, which challenge sustainable development. The release of untreated wastewater and faecal sludge to the natural environment has major impacts on the health of communities and results in environmental degradation. In many cities of developed countries the wastewater is treated at the end of the sewer before being discharged into the water bodies. This tradition has been widely established as a standard way of managing wastewater worldwide and known as "end-of-the-pipe" approach or conventional wastewater management approach. In developing countries, however, up to 90% of wastewater is discharged into rivers and streams without any kind of treatment (UNDPI 2003).

In many developing countries, industrial wastewater is less common, though they are severe near large urban centres. Instead, untreated domestic wastewater poses acute water pollution problems leading to low water availability and risks to human health. As a consequence, 80% of illnesses and deaths are attributed to water-borne diseases, taking a child's life every eight seconds (UNEP 2003). Intestinal infections related to water increase malnutrition and it in turn predisposes to severe infections. The poor sanitation not only negatively affects the population's health but also causes financial losses. World Bank (2008) estimated the economic losses of health impact from sanitation in Vietnam to be 262 million US dollar per annum, which is driven mostly by the costs of premature death and treatment of disease. In addition, poor sanitation has also impacts on water resources, environment, tourism and other welfare (**Figure 1.1**). Thus a large portion of misery, sickness and death in the developing world are due to water-related diseases or more accurately should be known as excreta-related disease as pathogens derive from faecal matter.

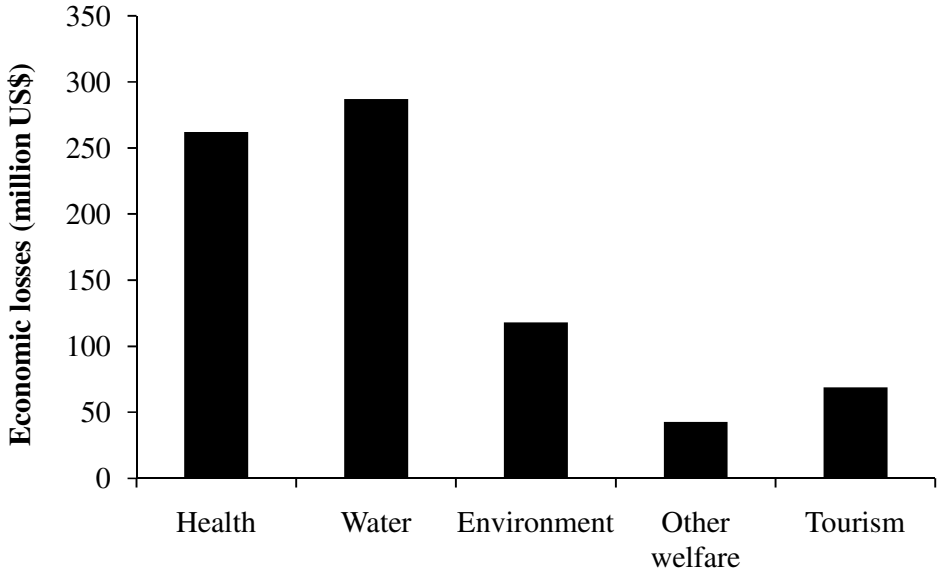


Figure 1.1 | Annual losses by impact category due to poor sanitation in Vietnam. Source: World Bank (2008)

1.1 **Why is faecal treatment needed?**

Faeces are widely acknowledged as a major source of infectious pathogens like enteric bacteria, viruses, protozoa and helminths, which are released from the bodies of infected persons or animals. These agents cause a wide range of diseases (**Table 1.1**). More and more newly recognised pathogens have been detected. According to Sharma *et al.* (2003), this may be due to the development of efficient detection method, an increase of urbanization, the movement of humans from one part of the world to another, multidrug resistance, pathogen gene transfer, and the influence of climate change. Levels of pathogens in faeces or wastewater differ from area to area, depending on its general status of sanitation and hygiene. Thus microbial make-up of different faecal substrates of human and animal origin in the study area was determined.

Table 1.1 | Pathogens in human faeces and in animal manures.

Pathogen	Disease
Virus	
Adenovirus (many types)	Respiratory infections
Astrovirus (many types)	Gastroenteritis
Calicivirus (several types)	Gastroenteritis
Coronavirus	Gastroenteritis
Coxsackie virus A	Herpangina, aseptic meningitis, respiratory illness
Coxsackie virus B	Fever, paralysis, respiratory, heart and kidney disease
Enterovirus (many types)	Gastroenteritis
Hepatitis A virus	Viral hepatitis
Hepatitis E virus	Viral hepatitis
Norovirus	Gastroenteritis
Parvovirus (several types)	Gastroenteritis
Polio virus	Poliomyelitis
Reovirus (several types)	Not clearly established
Rotavirus (several types)	Gastroenteritis
Bacteria	
Campylobacter jejuni	Gastroenteritis, long-term sequelae
Clostridium botulinum	Botulism
Clostridium perfringens	Gastroenteritis, gangrene
Escherichia coli	Gastroenteritis
E. coli O157:H7	Bloody diarrhea, haemolytic uraemic syndrome
Leptospira spp.	Leptospirosis
Listeria monocytogenes	Encephalitis
Mycobacterium tuberculosis	Tuberculosis
Salmonella (1700 serotypes)	Salmonellosis
Shigellae	Shigellosis
Vibrio cholerae	Cholera
Yersinia enterocolica	Yersiniosis, gastroenteritis, diarrhea, long-term sequelae

Table 1.1 (continued)

Pathogen	Disease
Fungi	
Candida sp	Mycoses (skin and systemic)
Tricosporon cutaneum	Skin mycosis
Aspergillus fumigatus	Lung mycosis
Trichophyton sp.	Skin mycosis
Epidermophyton sp.	Skin mycosis
Microsporum sp.	Skin mycosis
Protozoa	
Acanthamoebe (rare)	Meningoencephalitis
Balantidium coli (rare)	Dysentery
Cyclospora cayetanensis	Persistent diarrhea
Cryprosporidium parvum	Cryptosporidiosis, diarrhea, fever
Entamoeba sp.	Amoebic dysentery
Giardia lamblia	Giardiasis
Helminths	
Ancylostoma duodenale and *Necator americanus* (hookworm)	Hookworm infection
Ascaris lumbricoides (roundworm)	Ascariasis
Clonorchis sinensis (liver fluke)	Clonorchiasis
Diphyllobothrium latum (fish tapeworm)	Diphyllobothriasis
Enterobius vermicularis	Pinworm infection
Fasciola hepatica and *Fasciola Gigantic*	Fascioliasis
Hymenolepsis nana	Dwarf tapeworm
Hymenolepsis nana	Dwarf tapeworm
Opisthorchis viverrini (liver fluke)	Opisthorchiasis
Paragonimus westermani (lung fluke)	Paragonimiasis
Schistosoma spp. (blood fluke)	Schistosomiasis, bilharzia
Strongyloides stercoralis	Small roundworm infection
Taenia saginata and *Taenia solium* (tapeworm)	Taeniasis
Trichuris trichiura	Trichuriasis

Source: Filip *et al.* (1988) and WHO (2006a)

Survival of pathogens in the environment is variable. Several of pathogens are very persistent and may even grow outside their hosts. Cysts and oocysts of protozoa and helminth ova remain viable for extended periods in the environment (Cooper and Olivieri 1998). Wang *et al.* (1996) reported that *E. coli* O157:H7 is able to multiply in bovine faeces. Salmonella may survive in slurry for more than 77 days and grow in temperature from 6 to 47^{o}C (Mitscherlich and Marth 1984). It is also documented that several pathogenic and indicator bacteria survive long and multiply in biogas digesters (Sahlström 2003, Gerardi 2003). In order to obtain appropriate survival rates of organisms in the study context, survival of indicator organisms and pathogens in tropical anaerobic digestion as well in water environment, on spinach, in particular conditions were determined.

Pathogens of animals and their potential to infect humans have only recently been acknowledged due to the recognition of zoonotic agents like parasites (Olson and Guselle 2000) and rotavirus (Cook *et al.* 2004). Of the bacteria identified as being a common cause of gastroenteritis, Campylobacter, Salmonella and *E. coli* O157:H7 are zoonoses, able to infect both humans and animals (EPA 2009). In Vietnam, rotavirus G5 was detected in Vietnamese children with diarrhea while this G5 strain was isolated mainly from pigs (Ahmed *et al.* 2007). Thus zoonotic transmission is taken into account in microbial risk analysis in this study.

There are two-sided effects of faeces in faecal management process. On one hand, faeces contain pathogens and on the other they contain nutrients such as nitrogen, phosphorous, potassium (Vinnerås *et al.* 2006). This nutrient source can be recycled to minimise the utilisation of natural resources. However, pathogens should be eliminated before reuse in agriculture or discharge to the environment. In the early human settlements and in places where population is scattered and remote, the release of untreated faecal substrate into the environment can be adequate. However, today, in a crowded world, even in rural areas, lack of wastewater and waste management may lead to disasters in the near future.

1.2 Perspective of anaerobic treatment in tropical regions

In most situations in developing countries, anaerobic digestion is the most appropriate option for wastewater treatment as it is a low-cost, low-maintenance and at the same time high-performance treatment system. Tropical temperatures permit the utilization of efficient anaerobic reactors without heating. This is the main factor that makes the use of anaerobic technology applicable and cost-effective (Foresti 2001).

Anaerobic digestion occurs in three stages: hydrolysis, acid formation (acetogenesis) and methane production (methanogenesis). As the process usually occurs in one reactor (FEC Services 2003) stages run concurrently. In these combined-stage reactors operation conditions (stability, inhibition, toxicity,...) should be maintained so that the degradation rates of all stages are equal. Volatile fatty acid (VFA), total inorganic carbon (TIC) and pH are essential factors in this (Pind *et al.* 2003, FNR 2006). Low pH (<6.5) and a high level of VFA (>1,000 mg l^{-1}) have a significantly toxic effect on methanogenic bacteria in the digester. Hence the production of methane may cease and only carbon dioxide is produced (FEC Services 2003). It indicates that the core of the process (biogas production) should be kept in mind when pathogen reduction trials are conducted in the combined-stage system. However, there are also two-phase anaerobic treatment systems, where hydrolysis and acid forming is encouraged in the first or acid phase while methane production occurs in the second phase in a separate reactor.

Pathogens and indicator bacteria in animal slurries have been reported to reduce under mesophilic anaerobic condition (Kearney *et al.* 1993b, Kumar *et al.* 1999, Juris *et al.* 1996). Yet those studies were conducted at 35-37°C, a higher temperature compared to that of tropical biodigesters (28-30°C). Literature on pathogen reduction efficacy during anaerobic treatment of animal slurries under tropical conditions is scarce. Using Hungate tubes Olsen and Larsen (1987) showed that increasing temperature from 30°C to 35°C significantly shortened average T_{90} values of *Salmonella* Typhimurium, *Enterococcus faecalis* and coliform bacteria. Results observed at 35°C or 37°C may not be applicable to conditions at 28-30°C. Thus one of the objectives of this study is to identify the elimination potential of pathogens in faecal sludge in tropical anaerobic treatment over pilot PBDs and STs.

As well as the temperature, factors such as hydraulic retention time (HRT), VFA, pH, total solids, operation type (batch or continuous digestion), and initial concentrations also affect pathogen survival (Kearney *et al.* 1993a, Kunte *et al.* 1998, Henry *et al.* 1983). VFA has been cited as one of the important factors strongly affecting the survival of pathogens in mesophilic anaerobic digestion of organic waste. Many researchers have shown the influence of VFA on the reduction of pathogens. However those experiments were carried out in different conditions (Fay and Farias 1975, Henry *et al.* 1983, Abdul and Lloyd 1985), only few of them can be applied to the anaerobic digestion of organic waste (Kearney *et al.* 1993b, Kunte *et al.* 1998). To improve the microbial treatment efficacy via anaerobic treatment in the study area, many of factors cited above were considered in the pilot study.

1.3 **Study area**

The study was conducted in the Vietnam's Mekong Delta (MD), so-called Cuu Long Delta (*đồng bằng sông Cửu Long*), the most downstream part of the Mekong river basin. The basin is one of the largest river deltas in Asia. It is a landscape shaped by the waters of the Mekong River that flows from the Tibetan Plateau (China) through Myanmar, Laos, Thailand, Cambodia before entering the South China Sea in the Southwest of Vietnam (**Figure 1.2** and **Figure 1.3**).

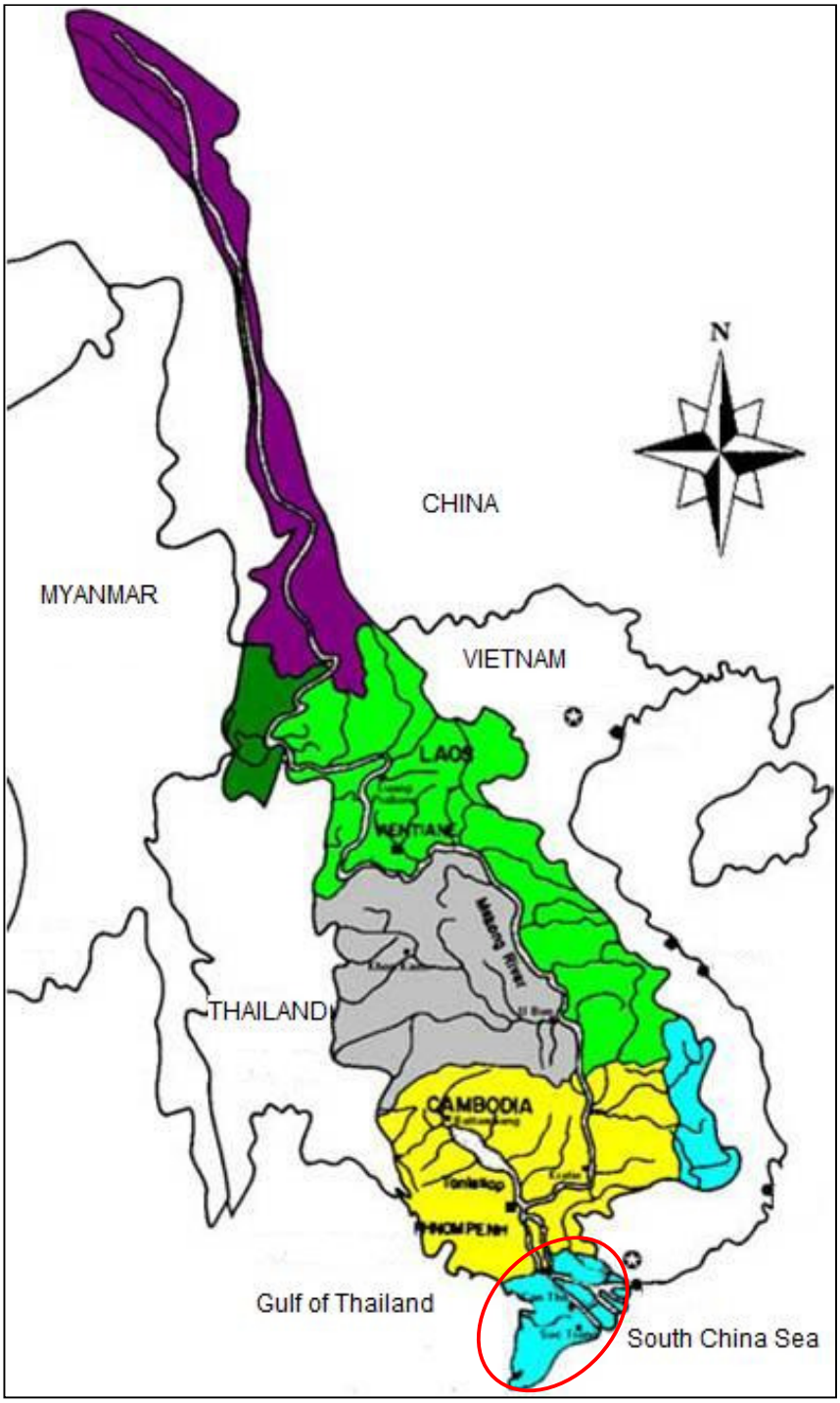


Figure 1.2 | Mekong River basin and location of the Mekong Delta, Vietnam (in circle). Source: Hoanh *et al.* (2003).

Figure 1.3 | Dense network of rivers and canals in the Vietnam's Mekong Delta

Source: Evers and Benedikter (2009).

MD is known as the rice bowl of Vietnam, producing commodities for the domestic market and for export. The region has experienced rapid socio-economic changes in the past few years, with increases in agricultural and aquaculture production. However, important challenges remain. Despite economic growth and development, MD remains among the poorest regions of the country. The challenges with poverty reduction have been linked, among other things, to low educational level of MD population, and resulting lack of human capital (Taylor 2004). Although the MD high population density has in no serious way been a limiting factor for the development (Keskinen 2008), an increasing release of untreated excrement to the environment may pose acute microbial pollution leading to low water availability and risks to human health in the area. In addition, traditional behaviours and routines of MD population (Section 1.3.6) make matters worse.

1.3.1 *A vital agriculture area of the country*

MD extends over 12 provinces and one city (**Figure 1.3**). It covers about 4 million hectares (about 12% of the national area) of which 72.5% (2.9 million ha) is currently used for agriculture and aquaculture, 15% (0.6 million ha) for settlements and infrastructures and 12.5% (0.5 million ha) being mangrove and melaleuca forests (Wassmann *et al.* 2004). Principally the delta's ecosystem is composed of saline, brackish and freshwater habitats. The main freshwater habitats of the delta include the multitude of rivers and canals, floodplain grasslands, melaleuca forests and plantations, as well as wet rice fields and other crops (Duong *et al.* 2001). The saline and brackish habitats remain in the coastal and estuarine zones of the delta offering great resources for shrimp farming. Thus MD possesses enormous economic potential in terms of its high productive land. Comparing to the whole nation, agricultural output of MD accounts for 50%, exported food productions are about 90%, fruit trees and aquaculture products are about 70% (Van 2010).

According to the dynamic development of cultivated areas and the simultaneous emphasis on applying intensive cultivation methods, the agriculture and aquaculture yields keep increasing. Among these, pig breeding was given high priority in MD due to the increasing domestic demand for meat based on the population as well as economic growth. About 26% of the rural households raised pigs (General Statistics Office 2006a) leading to another environmental problem in the rural areas.

The development of delta's agricultural economy has experienced many ruptures. After the unification of the country (1975), the yields decreased as the private trade was banned and farmers faced problems in getting agricultural inputs. Moreover the collectivization decreased farmers' incentives for higher production. The severe decline in agricultural productivity was one of the main reasons for the government to undertake remarkable changes in its policy. In 1986 Vietnam adapted so-called renovation policy (*đổi mới*) that was based on more market-oriented development strategy. The rapid growth in rice production in the late 1980s and 1990s has often been explained by the *đổi mới*. The reallocation of lands and liberalization of production provided more incentives to the farmers. Water-control and irrigation works with the expansion of modern farming techniques have also played a significant role in increasing agricultural production.

1.3.2 *Climate and flooding*

The delta has a tropical monsoonal climate with a dry season from December to April and a rainy season from May to November. Monsoon rains peak in September and October, and combine with floodwaters from the Mekong River causing annual flood and inundation in about 1.2 to 1.4 million ha for two to six months. The average annual rainfall ranges from less than 1,500 mm in the central region and northwest to over 2,350 mm in the south, with some 70-80% of the precipitation concentrated into four months at the height of the rainy season. The mean annual temperature is about 28°C throughout the delta, the difference between the mean monthly minima and maxima being only about 5°C. The relative humidity remains high (80%) throughout the year.

Apart from some hills like Mount Sam (270 m) and Mount Co To (258 m) in An Giang and Kien Giang Province next to the Cambodian border, the region has very low landforms with the range of 0-4 metre above sea level. The very flat area near the Cambodian border such as the provinces An Giang and Dong Thap is thus prone to deep flooding. To protect agricultural and residential areas, dyke systems have been constructed and gradually expanded in MD. There are more than 20,000 km of protection dykes to prevent early floods (MARD 2003). Full-dyke is designed based on the measured and calculated flood peaks to ensure the safety for the people's daily activities and cultivation in the whole flood duration. Semi-dyke is designed to ensure the second crop is harvested before floodwater exceeds the fields.

1.3.3 *Population growth*

The total population of MD is 17.2 million (in 2009) with a population density of 423 persons per square kilometre (more than 1.5 times the country's average). It is the third most densely populated area in Vietnam with 77.2% of the population living in the rural areas. The MD population density is highest in the areas along the Mekong River and the Bassac River (Keskinen 2008).

In general the MD population was increasing rapidly (**Figure 1.4**) despite of some tough periods in its history. When the French arrived in the 1860s to take control over the delta region, the Vietnamese control of the area was still to be established and consolidated. The delta was still a largely virgin area with an estimated population of 1.7 million inhabitants in 1880. French colonialism created a massive ecological as well as economic transformation (Brocheux 1995). Thousands of miles of canals were dug to drain the swamps and vast stretches of mangrove felled. Thus, the Mekong Delta was opened to large-scale human habitation and agricultural cultivation. Consequently, the population has been more than doubled in the last 50 years leading to an explosion of human waste discharged into the environment.

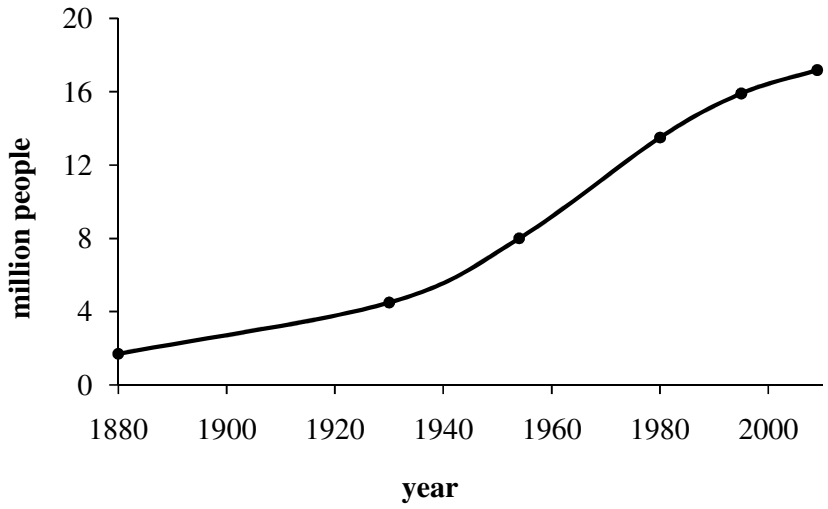


Figure 1.4 | Rapid growth of the population in the Mekong Delta.

1.3.4 *Faecal management*

Vietnam's rapid development and population growth over the last ten years have placed huge demands on its wastewater and waste treatment. However, at present, except for Ho Chi Minh, Can Tho and Da Nang City, which have projects underway to collect domestic wastewater for treatment, none of the other cities or provinces within the country has a centralized wastewater treatment plant.

In MD simple anaerobic treatment has been promoted to treat faecal matter. Only septic tanks (**Figure 1.5**) are introduced for the treatment of human excreta to a larger extent with 23.7% of households having flush toilet connected to STs or sewage pipes (General Statistics Office 2006). The number of households having toilets directly over surface water, so-called fishpond toilets (**Figure 1.6**), is much higher (47%). To treat manure from the increased number of pigs, PBDs were introduced to the population. Thousands of PBDs have been installed and in operation (Lam *et al.* 2006). Their focus is to reduce organic matter and produce cooking gas; hygiene aspects may also be less prioritised. Both treatment systems have been reported to underperform. This situation is typical in the country as a whole. Little information is available about their microbial treatment efficacy as well as how to improve their performance.

Figure 1.5 | Newly built septic tank.

Figure 1.6 | Fishpond toilets.

Plastic bio-digester

PBDs are a cost-effective way to treat animal slurries and produce cooking gas, and have been promoted in many developing countries (An 2002, Yongabi *et al.* 2003,

Brown 2006). PBDs are made from cheap and ubiquitous materials such as polyethylene film (**Figure 1.7**).

Figure 1.7 | Plastic bio-digester.

To increase PBD lifespan they are not exposed to direct sunlight, they are usually fenced off from animals, and their internal temperature is kept at 28–30°C. Bio-digester effluent can be applied to crops (rice, cassava and other perennial crops), vegetables (lettuces, tomatoes, cabbage, and water spinach) and in ponds (fish or water plants) (**Figure 1.8**).

Simple cooker using biogas from PBD.

Use of effluent in fish pond.

Use of effluent to apply on spinach.

Figure 1.8 | Benefit of plastic bio-digester in the Mekong Delta's rural areas.

PBD's design, construction and operation is unregulated, and linked to environmental and health risks. In particular the pathogen reduction efficacy is not well documented, although a few studies in Vietnam's Mekong Delta region have found high concentrations of the indicator bacteria *Escherichia coli* in bio-digester effluents (Kobayashi *et al.* 2003, Rechenburg *et al.* 2007). This study observes the microbial treatment efficacy of PBDs in MD so as to give an overview of their overall performance.

Unlike biogas plants in Europe the output of tropical PBD is low in dry matter. Solids accumulate at the plant's base, often for years. Hence, methane yields cannot be calculated onsite as gas production strongly correlates to the amount of solids present (Nuber and Tien 2008). Conversely, accumulated solids reduce the HRT of PBDs. Of PBDs investigated by Nuber and Tien (2008) 70% showed HRTs of less than six days and the shortest HRT was 1.83 days. Short HRTs may impact negatively on the treatment efficacy of the process as well as the hygienic status of the effluent.

Septic tanks

Most STs in Vietnam receive only black water (Viet-Anh *et al.* 2007, Harada *et al.* 2008) and comprise 2 chambers (Bao 2006). In terms of nutrient recycling the septage sludge might be fit for agriculture. Traditionally human excreta such as sludge from bucket latrines were used as fertilizer in Northern Vietnam. Use of such sludge today is decreasing due to their replacement by STs, in both urban and rural areas (Klingel *et al.* 2001). In some cases septage sludge is used as an alternative fertilizer in agriculture and aquaculture without any prior treatment. Moreover the manual de-sludging of septage may pose a high risk to workers' health. Klingel *et al.* (2001) found that one-third of STs in Nam Dinh (Northern Vietnam) were only ever emptied manually, with workers not taking any health protection measures. Therefore, septage handling practices in Vietnam promote the spread of pathogens, especially helminths.

Maintenance of STs requires septage sludge to be removed on a regular base. In general, STs in Vietnam are emptied only when blockages occur or odour become unbearable, which arises when the tank is full and untreated septage leaks. Harada *et al.* (2008) showed that 89.6% of STs in Ha Noi have never been desludged and when desludged, intervals ranged from 1 to 30 years with an average of 8.1 years. Orders to

empty STs, either by vacuum track or manually, are made by house owners and they have to pay for the service. Klingel *et al.* (2001) found that one-third of STs in Nam Dinh City were only ever emptied manually, with workers not taking any health protection measures. In most cases, the tanks are not constructed for regular maintenance and emptying is combined with reconstruction, e.g. opening the floor for access to the tank. Thus workers are exposed to untreated septage and septage sludge in most cases. However, there is little information about the danger of the substrate they are working with and the chance of contracting an infection.

Disposal of septage sludge and operating and maintaining STs is an increasing problem in Vietnam. Septage is widely acknowledged as a major source of infectious pathogens like enteric bacteria, viruses, protozoa and helminths. It has been recently reported that 75% of Ho Chi Minh City's septage sludge is discharged directly into the environment (Cuong 2008). In Nam Dinh, septage sludge was discharged into fishponds and on fields or wherever the pump truck driver found a place to dump it (Klingel *et al.* 2001).

Surveys of septage and strategies for improving faecal sludge management have been done in some provinces in Vietnam (Klingel *et al.* 2001, Bao 2006). Yet the microbiological make-up of septage in Vietnam is not well documented, especially the variety of helminths whose infection are considered a burden in Vietnam (Trang *et al.* 2007). Survey of 615 people in Nam Dinh showed that 90% were positive for helminth parasites (Dung *et al.* 2007). In Ha Giang 92% of 84 stool samples were positive for at least one parasite (Huong 2006). In 2002 it was estimated that 33.9 million Vietnamese are infected with *Ascaris* (44.4% of population), 17.6 million with *Trichuris* (23.1% of population), and 21.8 million with hookworm (28.6% of population) (Van der Hoek *et al.* 2003). Thus the microbial characteristics of septage need to be quantified so as to give an overview of the nation's health situation and the safe management and possible uses of sludge for agriculture and aquaculture.

As the faecal treatment seems not to be efficient, handling their outputs is another challenge to protect population health. The outputs from the treatment systems as well as untreated faecal matter are often returned directly to the water, which is used by million people every day. There is minimal literature of pathogen survival in the surface water in MD. For most of the MD population, surface water is the basis of living,

serving agricultural production, aquaculture, transportation and daily domestic use, including drinking water in many cases. **Figure 1.9** gives an overview of the faecal management system in MD.

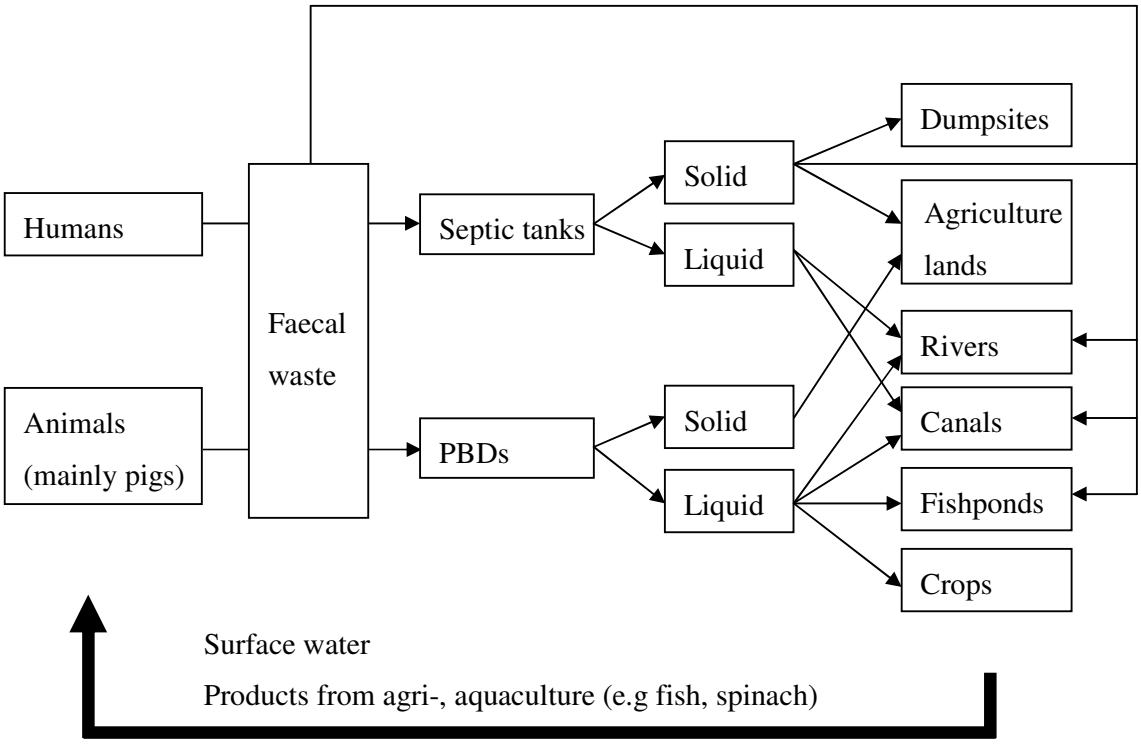


Figure 1.9 | Faecal management in the Mekong Delta, Vietnam.

PBDs = Plastic bio-digesters

1.3.5 *Health issues*

Water-related and water-borne diseases remain a major public health problem in MD. According to the statistics in the recent years from Department of Health and Environment (Centre of Preventive Health of Can Tho City), diarrhea has been the fourth most common diseases after throat infection, respiratory disease, and high blood pressure in Can Tho City. More than 18,000 diarrhea cases were reported in Can Tho

City in 2009 (PHC 2010). Diarrhea is an intestinal infection caused by virus, bacteria or parasites. In addition, typhoid fever – which is caused by *Salmonella typhi* – is endemic in the Mekong Delta (Kelly-Hope *et al.* 2008) and is one of the enteric diseases of significant public health concern in Vietnam (DeRoeck *et al.* 2005). Of 187,318 typhoid fever cases reported in VN between 1991 and 2001, 75.8% were in MD (Kelly-Hope *et al.* 2007). In 1993, a large epidemic of typhoid fever affecting 3,049 people causing two deaths was reported in An Minh district, Kiên Giang Province (Nguyen *et al.* 1993). Among 658 blood cultures in Dong Thap Province, 8.5% were positive for *Salmonella typhi* with an overall incidence of 198 per 100,000 population per year (Lin *et al.* 2000).

Another health burden in MD as well as in the whole country is helminthiasis (Trang *et al.* 2007, Dung *et al.* 2007), which is very common in Vietnam's rural areas. However, helminthiasis develops slowly and remain asymptomatic or mildly symptomatic (Hung *et al.* 2005). As a result, the detection and treatment are often neglected, giving away to a large burden of silent infection, especially in children. Helminthiasis can lead to severe anemia, bowel obstruction, bile duct infection, pancreatic duct infection and pancreatitis.

The epidemic may be ascribed to different causes: lack of safe water supply in rural area; faecal pollution caused by inhabitants of this endemic area such as defecating directly in the waterways; ingestion of contaminated food, especially vegetables sprayed with polluted water; low level of public sanitation and individual hygiene. The diseases are almost exclusively transmitted by food and water contaminated by the faeces and urine of patients and carriers. Polluted water is the most common source of disease transmission. In Dong Thap, drinking river water was associated to 83% of typhoid fever cases (Luxemburger *et al.* 2001) and about 10% population never boil surface water beforehand (Lin *et al.* 2000). There was clear qualitative evidence that MD people, including children, routinely drink untreated river water and sometimes drink water directly from the environment (Few *et al.* 2010).

1.3.6 *Traditional behaviours and routines as health risk factors*

In addition to the microbial contamination of water, traditional behaviours and life routines of the population constitute a great health risk. Basic knowledge of preventive hygiene measures exists among the population in MD (Herbst *et al.* 2009, Few *et al.*

2010). However, hygiene measures are put into practice in an untimely manner or are applied in an incorrect way, most probably due to the misconception of risks and/or a lack of background knowledge of cause–effect relationships (Herbst *et al.* 2009).

People in direct contact with faecal substrates pay little attention to their health consequences. Compared to conventional wastewater treatment systems, small-scale anaerobic digestion plants demand more personal involvement, which leads to more exposure to pathogens. The handling and reuse of different types of waste products with human or animal origins involve hygiene risks. However, the sewage workers using vacuum trucks and farmers handling pig slurry in MD do not use any health protection measures. They do not even wash their hands properly after work. The situation is even worse when the sludge is emptied manually and the workers have to stand for hours in sludge. For people dealing with PBD effluents risk of infection depends on the microbial quality of the effluent, which can hardly be free of pathogens.

Reuse of faecal matter in agriculture/aquaculture is positive in terms of nutrient recycling but it can be a risk for human health. Different from the Northern Vietnam, people in MD do not have a habit of applying fresh human faeces directly on crops. Instead, fishpond toilet is a typical example of reusing human faeces in aquaculture. Application of PBD effluents on crops, e.g. spinach that can be eaten raw, can be a risk for consumers while little information is available on pathogen survival rates on crops in MD. Similarly, use of septage sludge or accumulated sludge from PBDs on arable lands may place farmers, many of whom walk bare foot and have poor hygienic practices, at risk of helminthiasis. One of the indirect uses of excrements in agriculture is to cultivate aquatic spinach in canals contaminated with domestic wastewater.

Usage of surface water contaminated with excrements may have a greater potential for health problems because the water user is unaware of the wastewater presence. MD inhabitants who concentrate mainly along rivers and canals tend to rely on river water for domestic uses, including provision of drinking water. Bathing/swimming in canals and rivers and use of untreated surface water for bathing are commonplace in the area. In rural areas, bathing takes place either in the home using stored water (with or without flocculation) or directly by swimming in canals. Few *et al.* (2010) found that at least

half of the population in neighbourhoods of Long Xuyen City (An Giang province) regularly bath by swimming.

Surface water is the main source for drinking water of 24.3% households in MD, mainly in rural areas (General-Statistics-Office 2006b). A simple and most common treatment method applied to surface water is flocculation using aluminium sulphate ($Al_2(SO_4)_3.17H_20$). This process causes colloids and other suspended particles in liquids to aggregate, forming a floc. The slow stirring allows the flocs to grow and settle down quickly. Using surface water from MD, Wrigley (2007) showed that alum-flocculation reduced turbidity significantly and about 90% bacteria populations. In Can Tho Province, most people use aluminium sulphate daily, whilst in An Giang many people simply believe that treatment makes no difference (Abrahamsson and Svensson 2000). In MD flood prone areas, many people had no option but to use floodwater for drinking and cooking after hazards struck, often without any treatment (Few and Tran 2010).

Another routine is using surface water to wash vegetables, dishes as well as other utensils. Washing tends to be in flocculated water or directly in untreated surface water. It is found that vegetables became more contaminated after washing in nearby canals after harvesting (Ha *et al.* 2008). As the surface water quality is in most cases unknown, eating raw vegetables is not recommended from Preventive Health Centres in MD though it is not easy to change a traditional eating habit.

MD faces many challenges in water issues. The major constraints of the natural conditions include flooding, salinity intrusion, acid sulphate soils and the spread of acidic water, shortage of fresh water in areas close to the coastline and the impacts of global climate change to sea level rise (Tuan and Wyseure 2007, Truong and Ketelsen 2010). Water pollution from agricultural, industrial chemicals and untreated domestic wastewater just completes the whole picture of water issues in MD. Most studies on water pollution have focused on chemical aspects of the surface water (Minh *et al.* 1997, Long 2001, Toan *et al.* 2010). Little available data is on hand for microbial contamination associated with excreta and its consequence in the area. Thus it is necessary to quantify microbial risks from specific activities which MD population faces.

1.4 Risk assessment and management

1.4.1 *Introduction of Risk Assessment*

Risk assessment is a part of risk analysis that includes risk management and risk communication. After Haas *et al.* (1999) and NAS (1983) term definitions are:

Risk assessment – the qualitative or quantitative characterisation and estimation of potential adverse health effects associated with exposure of individuals to hazards (materials or situations, physical, chemical and/or microbial agents).

Risk management – the process for controlling risks; weighing policy alternatives and selecting the most appropriate action taking into account risk assessment, values, engineering, economics and legal, social and political issues.

Risk communication – the communication of risks to managers, stakeholders, public officials and the public; includes public perception and ability to exchange scientific information.

Quantitative risk assessment was initially developed, largely, to assess human health risks associated with exposure to chemicals (NAS 1983). When QMRA was first undertaken the framework of chemical risk assessment was applied directly for evaluating microbial risk. However some important differences between microbial and chemical agents were identified (Craun *et al.* 1996): 1) pathogens in the environment can grow or decline; 2) microorganisms are not uniformly distributed due to clumping or aggregation; 3) infectious diseases differ from chemical agents as an infected person may proceed to infect additional people; and 4) there is variation in susceptibility to microbial agents because of a complex set of immune responses including short- and long-term immunity that may alter the dose-response relationship and the severity of outcomes. Therefore these authors developed the concept further for assessing risks of human disease associated with pathogenic microorganisms.

1.4.2 *Quantitative Mirobial Risk Assessment*

QMRA is a tool used to predict the consequences of potential or actual exposure to infectious microorganisms and consists of the following steps (Haas *et al*. 1999):

1) Hazard identification: describe range of pathogens as agents of potential significance that are to be considered in the risk investigation.

2) Exposure assessment: determine the size and nature of the population exposed and the route, amount, and duration of exposure.

3) Dose-response assessment: characterize the relationship between various doses administered and the incidence of the health effect.

4) Risk characterization: integrate the information from exposure, dose-response to calculate the likelihood of infection and illness in the exposed population.

Hazard identification for microorganisms is generally straightforward. The major tasks of QMRA are, therefore, focused on exposure assessment, dose–response analysis and risk characterisation.

QMRA was first developed for drinking water (Regli *et al.* 1991) and has lately been applied to practices such as irrigation of crops with reclaimed water (Hamilton *et al.* 2006, Mara and Sleigh 2010a, Mara and Sleigh 2010b), evaluation of health effects of interventions in the urban water system (Labite *et al.* 2010). According to Haas *et al.* (1999) direct measurement of pathogens in combination with QMRA can be used to develop guidelines for food, water and other vehicles. World Health Organization (WHO) guidelines for the reuse of wastewater are based partly on epidemiological investigations and partly on microbial risk assessments. An advantage of QMRA is that it allows prospective studies rather than retrospective ones that typically form the basis of epidemiological studies (Haas *et al.* 1999). The validity of epidemiological studies lies in their focus on actual health effects. Yet waterborne outbreaks and reported cases are often underestimated (Barwick *et al.* 2000) and QMRA may be a way to circumvent this (Haas *et al.* 1999).

1.4.3 *Acceptable risk*

The results of a risk characterisation are used in risk management. The appropriate level for decision-making with respect to micro-organisms is still a matter of controversy. In the case of waterborne protozoa it has been suggested (in the US) that an annual risk of infection of 10^{-4} (i.e. 1 in 10,000) is appropriate for drinking water (Regli *et al.* 1991). WHO guidelines on the safe use of wastewater in agriculture (2006a) and drinking-

water quality (2008) use a default value of $\leq 10^{-6}$ DALY (disability adjusted life year) loss per person per year (pppy) for the tolerable additional burden of disease due to wastewater pathogens. The tolerable infection risks corresponding to tolerable DALY loss value are presented in **Table 1.2**. These values are in accord with the acceptable risk level of 10^{-4} infections per year suggested by Regli *et al.* (1991). Yet Mara and Sleigh (2010b) argue that a tolerable DALY loss of 10^{-4} pppy may be more realistic.

Table 1.2 | Tolerable infection risks (per person per year) of reference pathogens.

Pathogen	DALY loss per case of disease	Tolerable disease risk pppy equivalent to 10^{-6} DALY loss pppy[a]	Disease/infection ratio	Tolerable infection risk[b]
Rotavirus^{LC}	2.6×10^{-2}	3.8×10^{-5}	0.05	7.7×10^{-4}
Rotavirus^{HC}	1.4×10^{-2}	7.1×10^{-5}	0.05	1.4×10^{-3}
Salmonella	1.5×10^{-2}	6.2×10^{-5}	0.1	6.2×10^{-4}
Ascaris	8.3×10^{-3}	1.2×10^{-4}	1	1.2×10^{-4}

[a] Tolerable disease risk = 10^{-6} DALY pppy ÷ DALY loss per case of disease

[b] Tolerable infection risk = tolerable disease risk ÷ disease/infection ratio

^{LC} applicable to low-income countries

^{HC} applicable to high-income countries

Source: DALY loss per case of disease: rotavirus^{HC} (Havelaar and Melse 2003), rotavirus^{LC} (Havelaar and Melse 2003, Mara and Bos 2010), *Salmonella* (Haagsma *et al.* 2008), *Ascaris* (Chan 1997); Disease/infection ratio: rotavirus (Mara and Bos 2010), *Salmonella* (Glynn and Bradley 1992), *Ascaris*: a worst-case scenario was assumed, i.e. all those infected with *Ascaris* develop ascariasis.

1.5 Objectives and scope of the study

The objective of this study is to analyse the microbial risk associated with faecal management in MD as it impacts on public health. Specifically the study aims to:

- determine the microbial characteristics of faecal substrates, surface water and spinach cultivated in surface water in MD
- identify the elimination potential of pathogens in faecal sludge in anaerobic treatment over pilot PBDs and STs
- observe the treatment efficacy of PBDs in MD
- determine the survival of phages and bacteria in Mekong River water and on terrestrial spinach
- quantify microbial health risks associated with faecal management in the Mekong Delta via QMRA.

2 MATERIALS AND METHODS

2.1 Pilot study

An overview of pilot trials is given in **Table 2.1**.

Table 2.1 | Trials conducted within the pilot study

Batch experiment: pathogen reduction in animal slurries
Slurry type: swine and cattle slurries
Low and high initial levels of phages and bacteria
Survival of *Ascaris suum*
Regular feeding experiments
Pathogen reduction in plastic bio-digesters
Different hydraulic retention time
High volatile fatty acid level
Pathogen reduction in septic tank
Survival of *Ascaris suum* in biogas and septage sludge
Survival of phages and bacteria in Mekong river water
Survival of phages and bacteria on terrestrial spinach

2.1.1 *Batch experiment*

Behaviour of target organisms was identified firsthand in batch reactors as a pre-trial, which determined optimal techniques for experiment conditions (pathogens, substrates, inoculums, etc.).

500 ml bottles connected to a gas collection system (**Figure 2.1**) were used as batch digesters. Digesters were fed with 300 ml of slurry and seeded with 10% inoculum from a continuous reactor. Substrates then were spiked with low (10^3–10^4 CFU ml^{-1}) and high (10^6–10^7 CFU ml^{-1}) microbial concentrations.

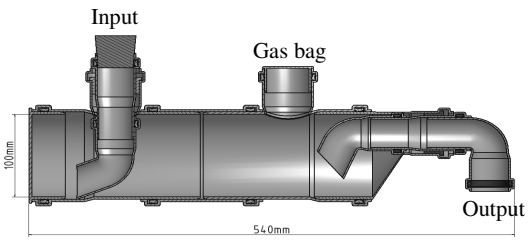
Figure 2.1 | Batch experiment setting

Organisms included somatic coliphages, *Escherichia coli, Salmonella* Senftenberg, *Enterococcus faecalis. Ascaris suum* eggs were placed in bags prior to incubation (10,000 eggs per bag). Substrates without inoculates were used as controls. The temperature of 30°C replicates an average tropical biogas, though the retention time of 45 days is longer than average. The longer retention time was chosen to fully monitor microbial reduction.

Two types of substrates (swine and cattle slurry) and two different concentrations of phage (PFU ml^{-1}) and bacteria (CFU ml^{-1}) at inoculation (low: 10^3 – 10^4; high 10^6 – 10^7) were tested with 4 replications. Digesters were sampled on days 0, 1, 2, 4, 8, 16, 32 and 45 for analyses of phages and bacteria. Bags of *Ascaris suum* were removed every two weeks for analysis.

2.1.2 *Regular feeding experiments*

These trials using constructed reactors replicated tropical condition to determine pathogen reduction in PBDs and STs. Reactors were placed in incubators at 30°C. As the trials were conducted in anaerobic condition, biogas and some chemical parameters were measured to ensure that the setup functioned well.



Polyethylene tubes were used to build reactors of 3 l volume that were filled with 2.5 l of substrate (**Figure 2.2**). They are comparable in dimensions to a domestic bio-digester, which has the length:diameter ratio of 5:1.

Figure 2.2[1] **|** Constructed reactor.

Experiment set-up for pathogen reduction in pilot plastic bio-digesters

- Trial with different hydraulic retention time

The objective of this study was to evaluate the gas production and the hygienic quality of the effluent from PBDs in relation to the HRT. The *in situ* bio-digester conditions at three different HRTs were replicated at bench scale. A HRT of 15 days was chosen, because 13–17 days is considered the best anaerobic treatment time for pig slurry (FEC Services 2003). An HRT of 30 days was chosen because a literature review from Thy *et al.* (2005) showed that biogas production peaks at around day 30, and then declines. Finally, a HRT of 3 days was chosen to see the effect of short HRT on the reduction of pathogen and indicator microorganisms, and this HRT represents the present situation in most PBDs in the Mekong Delta, Vietnam.

The triplicate experiment was carried out with three HRTs (3, 15 and 30 days). Reactors (**Figure 2.2**) were filled with pig slurry (2 g ODM per litre) and seeded with 10% inoculum sourced from a wastewater treatment plant. They were incubated for 8 days without feeding for microorganisms to adapt to the anaerobic conditions. The reactors were then fed once a day with a fixed daily input of 2.5 g ODM of fresh pig manure with different amounts of water for 50 days.

[1] Reprinted from Yen-Phi *et al.* (2009) with permission from IWA Publishing.

Tested microorganisms included somatic coliphages (φX174), male-specific bacteriophage (MSB) and bacteria (*E. coli, Salmonella* Senftenberg*, Enterococcus faecalis*). Bacteria were spiked to the daily feeding material at a final concentration of 10^5–10^6 CFU ml^{-1}. Phages were spiked to the feeding material at 10^5–10^6 PFU ml^{-1}.

Reactor influents and effluents were sampled daily for pH, dry matter (DM) and organic dry matter (ODM). Chemical parameters (NH_4^+-N, VFA and total inorganic carbon (TIC)), gas production and tested organisms were analysed weekly. Samples were stored at 4°C and analysed within 24 hours of sampling. The exception were VFA samples, which were stored in a freezer (–15 to –20°C) and analysed within 3 weeks. Accumulated sludge at the reactor's base was determined at the end of the experiment. The sludge was removed from the reactors and analysed for DM, ODM and the microorganisms concerned.

- Trial with volatile fatty acid

This trial aimed to (1) determine the amount of VFA input that tropical PBDs can handle; and (2) evaluate the effect of VFA on the reduction of micro-organisms at optimal pH values in combined-stage anaerobic reactors. The pig slurry used in the trial contained low VFA. Hence acetic acid (the shortest chain of VFA found in animal slurries) was used to amend the total VFA concentration as acetic acid constituted 65 - 70% of the total VFA (Patni & Jui 1985). Shorter-chain VFA was reported to have a stronger antibacterial effect (Chaveerach *et al.* 2002). Sodium bicarbonate was then used to neutralise the feeding substrate as it is routinely used to adjust pH in anaerobic digesters (Gerardi 2003).

HRT of 15 days was chosen for the trial with VFA. Preparation, operation, sampling and analyses were conducted as described in the trial with HRT. Feeding material was added with acid acetic (neutralised with sodium bicarbonate to pH 7) to a final concentration of 5 g l^{-1}. Pure feeding material was used for control reactors. This experiment was performed in triplicate for 8 weeks. Phages (somatic coliphage and male-specific bacteriophge) and bacteria (*E. coli, Salmonella* Senftenberg*, Enterococcus faecalis*) were then spiked to the daily feeding material at a final concentration of 10^5–10^6 PFU ml^{-1} or CFU ml^{-1}.

Experiment set-up for pathogen reduction in septic tanks

This triplicate trial aimed to determine the reduction of phages and bacteria in STs. The biogas reactor (**Figure 2.2**) was connected to another chamber to form a 2-chamber ST model (**Figure 2.3**) with a volume of 4.5 litres. A HRT of 3 days was chosen to evaluate the maximum treatment efficacy. According to the requirements for new and upgraded STs, which are specified in 20 TCN-51-84 Vietnamese Standards, the HRT should be from 1 to 3 days.

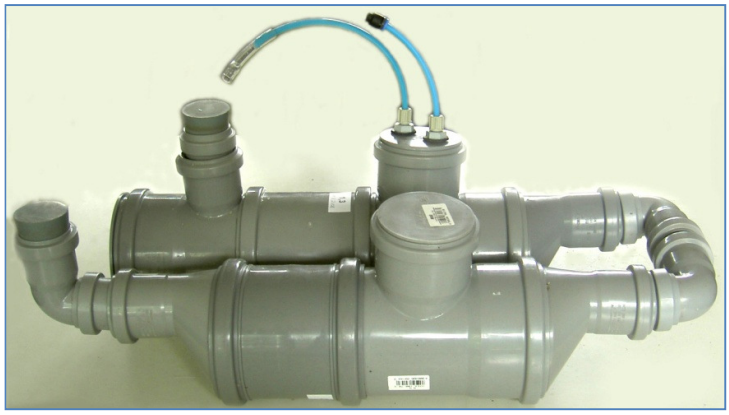


Figure 2.3 | Constructed 2-chamber septic tank model.

Reactors were filled with fresh black water collected from flush toilets and seeded with 10% inoculum sourced from a wastewater treatment plant. The reactors were incubated for 8 days without feeding for microorganisms to adapt to the anaerobic conditions. The reactors were then fed once a day with a fixed daily input of 1.5 litre of fresh black water for one month. Influent and effluent were sampled every two days.

Tested microorganisms included somatic coliphages (φX174), male-specific bacteriophages and bacteria (*E. coli, Salmonella* Senftenberg*, Enterococcus faecalis*). Bacteria and phages were spiked to the daily feeding material at a final concentration of 10^5–10^6 CFU ml^{-1} or PFU ml^{-1}. Reactor influents and effluents were sampled daily for pH and tested organisms. Gas was collected and measured at the trial's end.

Experiment set-up for survival of Ascaris suum in biogas and septage sludge

- Trial with *Ascaris suum* ova survival in biogas sludge

Reactors and sludge accumulated during the HRT trial presented in Section 2.1.2 were used for this experiment. The sludge, collected from 6 reactors with HRT of 15 days and 30 days, was mixed and distributed to 6 new reactors. Reactors were then fed once a day with a daily input of 170 ml of feeding material (15g ODM l^{-1} of fresh pig manure and water) for one week. Two bags of *Ascaris suum* ova were inserted in the sludge in each reactor. Then feeding was maintained once per week for one year with one litre of the feeding material. Samples were taken every two months. At sampling, one reactor was opened and two bags of *Ascaris suum* ova were removed. Biogas was collected and measured at the trial's end.

- Trial with *Ascaris suum* ova survival in septage sludge

Reactors with substrates used in the trial of pathogen reduction in STs presented above were used in this experiment. Four bags of *Ascaris suum* ova were inserted in the sludge in every reactor. The reactors were then fed once a week with 1.5 litre of 2% ODM of human faeces and fresh brown water for one year. Samples were taken every two months. At sampling, one reactor was opened and two bags of *Ascaris suum* ova were removed. Biogas was collected and measured at the trial's end.

2.1.3 ***Survival of phages and bacteria in river water***

Water was sourced from Hau river, Can Tho City and measured for pH and turbidity. Nine-hundred ml of river water was distributed to one-litre beakers. This triplicate trial was conducted at local room temperature (28 to 30^{o}C). Phages (somatic coliphage and male-specific bacteriophage) and bacteria (*E. coli*, *Salmonella* Senftenberg, *Enterococcus faecalis*) were spiked to the water at a final concentration of 10^{5}–10^{6} PFU ml^{-1} or CFU ml^{-1}. Ten ml of fresh river water was added daily to the beakers to compensate for the evaporation and to exchange water environment. Water was then stirred using a glass stick. Samples were taken on day 0, 1, 2, 3, 4, 5, 6, 7, 12 for analyses of concerned phages and bacteria. Analyses were done at the Environmental Biology and Engineering Laboratory, College of Environment and Natural Resources, Can Tho University.

2.1.4 *Survival of phages and bacteria on terrestrial spinach*

Spinach was cultivated from seeds on ready-to-use compost, which was made of organic waste collected from a local market. Phages and bacteria were not detected in the compost. Three pots of water spinach were placed under a roof so that they got sunlight but were not exposed to rain. For the first three weeks, spinaches were irrigated one per week with tap water that contained no organisms. Spinaches were then irrigated once per week for 5 weeks with water spiked with phages (somatic coliphage) and bacteria (*E. coli*, *Salmonella* Senftenberg, *Enterococcus faecalis*) at a final concentration of 10^5–10^6 PFU ml^{-1} or CFU ml^{-1}.

About 5 g of spinach per sample were taken before the irrigation and 45 minutes after it to account for evaporation. Samples were then taken daily until the next irrigation. After the final irrigation samples were taken within two weeks. Samples collected were placed in sterile 500-ml Erlenmeyer-flasks. Fifty ml of distilled water was added and samples were shaken at 120 rpm for one hour before analysis. Samples were measured for dry matter and concerned phage and bacteria. Analyses were done at the Environmental Biology and Engineering Laboratory, College of Environment and Natural Resources, Can Tho University.

2.2 **Field study**

This study was conducted between September and November 2008 in Can Tho City, which is located in the centre of MD. Microbiological make-up of faecal sludge (pig slurry and septage), surface water were determined. Pathogen treatment efficacy of PBDs was also taken into account. In urban area, septage samples were taken from STs in the centre of Can Tho City. Microbial quality of aquatic spinaches cultivated in fishponds and urban canals were examined. Analyses were done at the Environmental Biology and Engineering Laboratory, College of Environment and Natural Resources, Can Tho University.

2.2.1 *Samples from plastic bio-digester*

Samples were taken from 18 PBDs in Phong Dien District (**Figure 2.4**) during the morning's routine cleaning. Influent samples were collected directly at the biogas

digester inflow. Five-hundred ml of the inflow were collected every 20 seconds and transferred into a 40-litre bucket. The material was then stirred well and one-litre sample was taken for analysis. One-litre effluent samples were collected about 5 minutes after effluence.

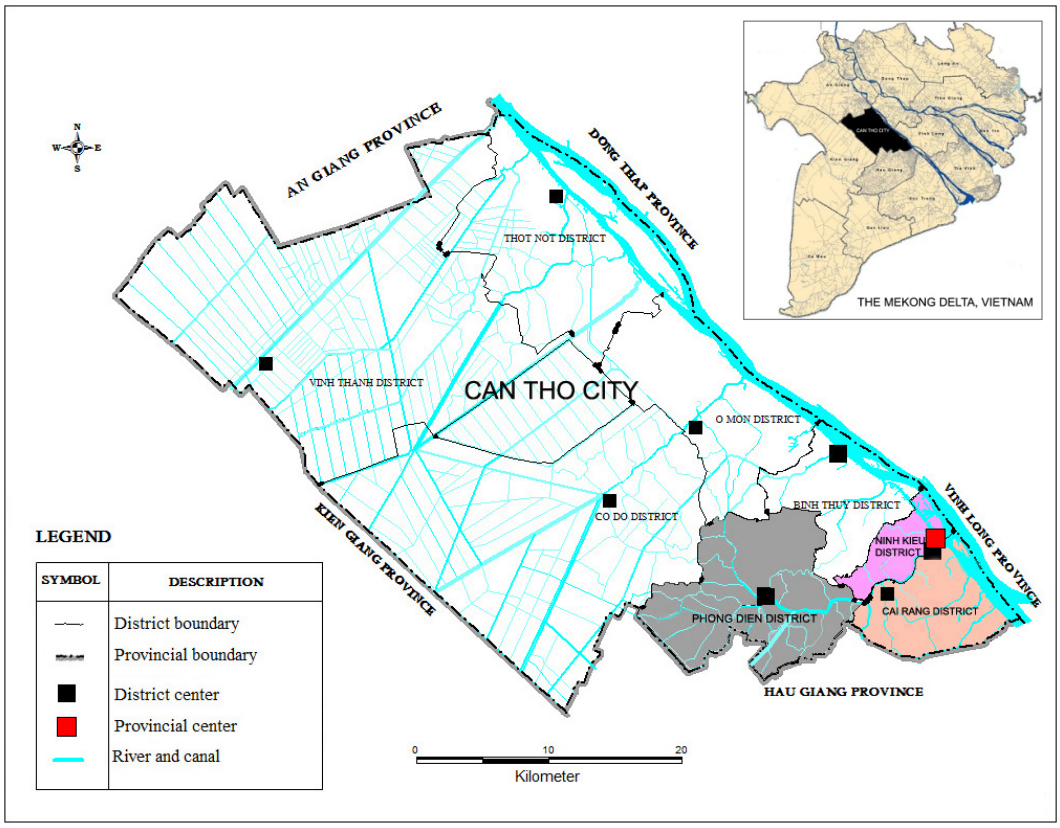


Figure 2.4 | Sampling areas (coloured) within the field study in Can Tho City. Data source: Department of Agriculture and Rural Development of Can Tho City 2009.

A questionnaire survey was conducted during sampling that included the volume and age of PBDs sampled, input sources, de-sludging conditions, and discharge/use of sludge and liquid output. The overall condition of the PBDs was also recorded during sampling.

Samples were analysed for pH, dry matter (DM), somatic coliphages, male-specific bacteriophages, *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., and helminth ova.

2.2.2 *Samples from septic tanks*

This part of the study examined the pathogenic content and indicator organisms in septage in Can Tho City to give an overview of the population's health situation and possible uses of sludge for agriculture and aquaculture. Besides septage sludge, untreated septage was also tested since it is the effluent of full STs. This untreated effluent is often discharged directly to surface water without any treatment.

Samples were taken from STs from 20 single-family dwellings in Ninh Kieu and Cai Rang District (**Figure 2.4**) as they were being emptied by a pump truck. Two samples were taken from each tank: one at a depth of 10 cm (untreated septage), the other from the centre (septage sludge) when the tanker had extracted half the contents. All STs were full at sampling. The emptying intervals ranged from 1 to 20 years. STs with two compartments and a storage volume of 1 – 2 m^3 predominated (16 out of 20 tanks). The number of users of ST per household ranged from two to ten with an average of five. No readymade inspection hatches existed in the surveyed tanks. Workers had to damage house floors as the STs were located in the basements (**Figure 2.5**). Extracted septage was transported to the neighbourhood province (Hau Giang) and discharged to a dump (**Figure 2.6**).

Figure 2.5 | Septage desludge.

Figure 2.6 | Septage is discharged into a dump located in Hau Giang province.

Samples arrived at the laboratory within one hour and were stored at 4^{o} C before analysis. Samples were processed within 24 hours of sampling with the exception of helminth ova. Analyses included pH, dry matter (DM) and somatic coliphages, male-specific bacteriophages, *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., and helminth ova.

2.2.3 *Surface water samples*

Microbial characteristics of surface water were determined in small canals in both rural and in urban areas. Surface water in rural areas receives faecal matter from animals and human beings while in urban areas the main source of faecal contamination is from humans. Moreover the usage of surface water is also different in those two areas. For instance, it is used for drinking in rural but not in urban areas, where tap water exists.

Twenty samples were taken from canals in rural areas where people bath/swim and use the water for drinking and cooking (Phong Dien District, **Figure 2.4**). Another 15 samples were taken from small canals in a downtown area of Can Tho City (Ninh Kieu District, **Figure 2.4**). Tham Tuong and Cai Khe canal were excluded because of its heavy pollution (PCCTC 2003). They are not representative of urban canals in MD. Moreover, there is almost no bathing or swimming here due to the offensive smell. As people usually use surface water at high tide, samples were taken also at high tide. A one-litre sample of surface water was taken at about 20-cm depth, some 1 to 2 meters from the bank. Samples were analysed for somatic coliphage, *E. coli*, *Salmonella* spp. and *Enterococcus* spp and helminth ova.

2.2.4 *Aquatic spinach samples*

In rural areas, aquatic spinach is usually planted in fishponds that receive PBD's effluent. Spinach is commonly used for human as well as porcine consumption. Fifteen aquatic spinach samples (about 20 g per sample) were taken from 15 fishponds receiving PBD's effluent in Phong Dien District (**Figure 2.4**). Samples were placed in sterile 500-ml Erlenmeyer-flasks. Fifty to one-hundred ml of distilled water was added and samples were shaken at 120 rpm for one hour before analysis. In addition, 15 spinach samples were taken from urban canals in Ninh Kieu District (**Figure 2.4**). All

samples were measured for dry matter, somatic coliphage, *E. coli*, *Salmonella* spp., and *Enterococcus* spp.

2.3 **Physiochemical and microbiological analysis**

2.3.1 ***Physicochemical analysis***

COD was analysed by test kit (COD Cuvette test, Merck). The gas quality was analysed by an infrared analyser (VISIT 03). Gas amount in the gasbags was measured with a RITTER gas counter, and then converted to normal conditions (norm litre). VFA were measured by titration method. Other parameters were determined using Standard Methods for the Examination of Water and Wastewater (Eaton *et al.* 2005).

2.3.2 ***Microbial strains used in the experiment***

All phages and most bacteria were obtained from the German Collection of Microorganisms and Cell Cultures (DSM) and the American Type Culture Collection (ATCC) (somatic coliphage DSM 4497, Male-specific bacteriophage ATTC 15597-B1, *Salmonella* Senftenberg DSM 10062, *Enterococcus faecalis* DSM 20478). At the beginning, a batch-wise trial was conducted with similar conditions as in the bio-digesters to compare the reduction of indigenous versus collection strains at low initial concentration (10^2 to 10^3 CFU or PFU ml^{-1}). No significant difference between the reductions of indigenous and collection strains was found, except for *E. coli*. Therefore, *E. coli* was isolated from fresh pig slurry then verified by biochemical tests (Api 20E; Biomerieux) and this indigenous *E. coli* strain was used for further study.

Ascaris suum ova bags (**Figure 2.7**) were sourced from Department of Microbiology, Swedish University of Agricultural Sciences (Uppsala, Sweden). Approximately 10^4 ova were inserted into every permeable nylon bag (mesh 35µm, Ø 6cm) which is held in physiological saline solution (0.9%) at 4°C until use within one week.

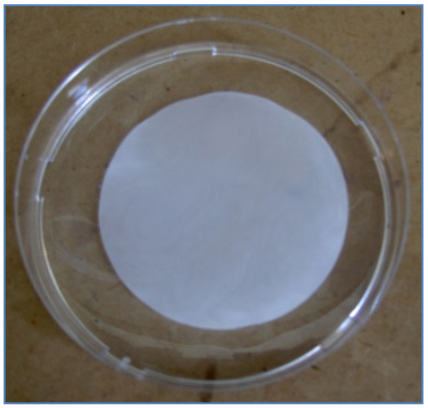


Figure 2.7 | *Ascaris suum* ova bag.

2.3.3 *Inoculum preparation*

The bacteria suspensions for spiking were made in 0.9% NaCl solution from fresh colonies grown on Columbia blood agar (5% sheep blood, Oxoid) using McFarland (BioMerieux) standards. Phages were cultivated according to ISO 10705. Bacteria and phages were then spiked to the substrate of the trials at a final expected concentration.

2.3.4 *Microbiological analysis*

Somatic coliphages and Male-specific bacteriophages were counted by the single-agar-layer technique as described in ISO 10705-2 and ISO 10705-1, respectively. *E. coli* was counted on Chromocult®Coliform Agar (Merck) after 24 hours of incubation at 36 ± 1°C.

Salmonella Senftenberg was enumerated on Rambach agar (Merck) after 24 to 48 hours of incubation at 36 ± 1°C. *Enterococcus faecalis* was counted on Enterococcus Selective Agar according to Slanetz and Bartley (Merck) after 48 hours of incubation at 36 ± 1°C.

Salmonella spp. in wastewater were counted via the most probable number (MPN) method in Rappaport-Vassiliadis broth (48-hour incubation at 36 ± 1°C) and Hektoen agar (24-hour incubation at 36 ± 1°C). Colonies from *Salmonella choleraesuis* (DSM 4224) were used as control strain. To calculate the results MPN tables from WHO Laboratory Manual of Parasitological and Bacteriological Techniques (Ayres and Mara 1996) were used.

Salmonella spp. in surface water and in aquatic spinach were determined by using presence/absence test since they are not often detected in those samples. One-hundred ml of surface water was used directly for analysis. For aquatic spinach, about 80 – 100 g samples collected were placed in sterile 500-ml Erlenmeyer-flasks. Then about 200 ml of distilled water was added and samples were shaken at 120 rpm for one hour before analysis. One-hundred ml of liquid samples were incubated with 100 ml Rappaport-Vassiliadis broth (48-hour incubation at 36 ± 1°C) and transferred to Hektoen agar (24-hour incubation at 36 ± 1°C). Colonies from *Salmonella choleraesuis* (DSM 4224) were used as the control strain.

Results from presence/absence tests were transformed into most likelihood estimate based on an equation from Haas *et al.* (1999):

$$\bar{\mu} = -\frac{1}{V} ln \frac{n-p}{n} \qquad \text{(Equation 1[2])}$$

Where

$\bar{\mu}$: most likelihood estimate (MPN/100 ml)

n: number of samples

p: number of positive (presence) samples

v: volume of tested sample

Helminth ova were determined by using WHO Laboratory Manual of Parasitological and Bacteriological Techniques (Ayres & Mara 1996). Ova with incomplete walls and empty ova were not counted. Only fertile specimens of *Ascaris lumbricoides* ova were counted.

For *Ascaris suum* ova in pilot study, the ova bag were removed at sampling and incubated for 4 weeks at room temperature (20°C - 22°C) in 0.1 N sulphuric acid to allow larvae development of all viable ova. Two bags were incubated from the beginning of the trial for initial viability count. Viability counts were performed under microscope by withdrawing ova from the bag using a syringe. A viability count was

[2] Applicable only to samples with n > p, otherwise other methods are employed.

performed for approximately 500 ova per bag. When the viability rate was too low (<5%), at least 1,000 ova were counted. Ova developing into larval stage were considered viable.

2.4 **Quantitative Microbial Risk Assessment**

2.4.1 ***Exposure scenarios***

Four scenarios were evaluated for QMRA as presented in **Table 2.2**. Since it is not feasible to do the risk assessment for the whole population in every situation, few typical circumstances associated with faecal matter management in the study area were considered. Risks from secondary transmission, i.e. contact with infected people, are not taken into account. Only adults were taken up in the risk model as available dose-response parameters used in risk calculation were obtained generally from feeding studies of healthy adults.

Table 2.2 | Exposure scenarios and exposed population.

Scenarios	Exposed population
1. Working exposure	
1.1 Fresh pig slurry	Farmers
1.2 Liquid effluent of PBDs	Farmers
1.3 Liquid effluent from improved treatment (HRT = 15 days)	Farmers
1.4 Liquid effluent from improved treatment (HRT = 30 days)	Farmers
1.5 Untreated septage	Sewage workers
1.6 Septage sludge	Sewage workers
2. Recreational activities	
2.1 Bathing/swimming in rural canals	Rural inhabitants
2.2 Bathing/swimming in the urban canals	Urban inhabitants
3. Drinking surface water	
3.1 untreated	Rural inhabitants
3.2 after alum flocculation	Rural inhabitants
3.3 after alum flocculation plus boiling	Rural inhabitants
4. Consumption of spinach	
4.1 cultivated in fish ponds received PBD's effluent	Rural inhabitants
4.2 cultivated in urban canals	Urban inhabitants
4.3 cultivated on fields fertilised by PBD's effluent	Rural inhabitants
4.4 cultivated on fields fertilised by PBD's effluent at HRT=15d	Rural inhabitants
4.5 cultivated on fields fertilised by PBD's effluent at HRT=30d	Rural inhabitants

PBD = plastic bio-digesters

HRT = hydraulic retention time

2.4.2 *Hazard identification*

Various groups of pathogenic microorganisms are excreted in faeces and transmitted to human via the faecal-oral route and cause many enteric diseases. These organisms may be zoonotic, i.e. can be transmitted from animal to humans. The organisms were chosen based on the following criteria:

- cause endemic disease in the investigated population
- have severe consequences
- have great persistent
- have low infectious doses

Rotavirus was chosen due to prevalence and rapid spread. As representative for bacteria group, *Salmonella* was chosen because salmonellosis is endemic in the study area. Helminth and *Ascaris* ova were included as they are recognized as persistent, and able to survive in the environment for long periods. Viruses generally have lower infectious dose than bacteria but the lowest being attributable to helminth/Ascaris (parasite) where, in theory, one egg is enough to cause an infection.

2.4.3 *Exposure assessment*

The purpose of the exposure assessment is to determine the amount, or numbers of organisms that correspond to a single exposure (dose) or the total amount or number of organisms that constitute a set of exposures (Haas *et al.* 1999). Amongst three main routes of exposure (ingestion, inhalation, and dermal absorption), only the ingestion route was taken into consideration in this study as faecal-oral transmission is considered the main route of spreading of enteric diseases. Pathogens were modelled by probability density functions (PDFs) for their concentrations detected in substrates.

Density of pathogens in wastewater and surface water as input for risk analysis

Except for *Salmonella* levels in studied substrates, rotavirus and helminh/Ascaris concentrations were further calculated for their risk model inputs. Rotavirus levels in faecal substrates of human origin were calculated based on the average ratios of somatic coliphage: rotavirus in domestic raw sewage and surface water received (un)treated sewage reported by Lodder and Husman (2005). The ratios were 3.7×10^3 in faecal

substrates and 1.5×10^2 in surface water. To calculate the risk of human infection with rotavirus from pig origin based on Iturriza-Gomara *et al.* study (2000), 1% of animal rotaviruses in pig slurry and PBD's effluent in MD were assumed zoonotic.

The amount of viable helminth/*Ascaris* ova in fresh faecal sludge (fresh pig slurry or untreated septage) were calculated according to the viability rate of *Ascaris suum* incubated in 0.1 N sulphuric acid (r_v, cf. Section 3.3). The viability of other helminth ova are assumed similar to that of *Ascaris suum*. Thus Equation 2 is used for both helminth and *Ascaris* ova.

$$N_v = N_o \times r_v \qquad \text{(Equation 2)}$$

where:

N_v: concentration of viable helminth/*Ascaris* ova in 1 l substrate

N_o: concentration of counted helminth/*Ascaris* ova in 1 l substrate

r_v: viability rate of *Ascaris suum*

Helminth ova collected from PBDs' effluents are considered fresh because 1) ova in influents go through PBDs and are discharged in effluents; 2) The velocity of influent flow is so high that some accumulated sludge on top is washed out and contribute to the effluent. Since there is a one-month lag phase of helminth ova in digesters (Section 3.3.1) those ova are considered viable. Thus viable helminth ova counted in PBD's effluents were calculated based on Equation 2. In septage sludge, viable helminth ova were calculated based on the accumulated ova numbers from untreated septage and the exponential equation of *Ascaris suum* survival within one year in sludge (Equation 9, Section 3.3.2).

Input data of microbial concentrations used in the QMRA modelling were presented in **Table 2.3** and **Table 2.4**. Probability density functions (PDFs) presented in the following tables describes the relative likelihood for a continuous random variable to occur at a given point in the data range. The probability of a random variable falling within a given set is given by the integral of its density over the set. The probabilities are modelled using Distribution Fitting function (@Risk 5.5 - Palisade Corporation),

which provides values of the test statistics, and allows users to determine the best fitting distribution of measured/surveyed data. Definition of distribution functions can be found elsewhere (Palisade Corporation 2010). The functions used in this study are briefly described in Appendix 8.3. **Figure 2.8** gives an example of a PDF of *Salmonella* spp. concentrations in pig slurry noted in **Table 2.3**. Loglogistic(-2.2363,12.795,1.8418) returns a log-logistic distribution generated using a location parameter *gamma* value of -2.2363, a scale parameter *beta* value of 12.795, and an shape parameter *alpha* value of 1.8418.

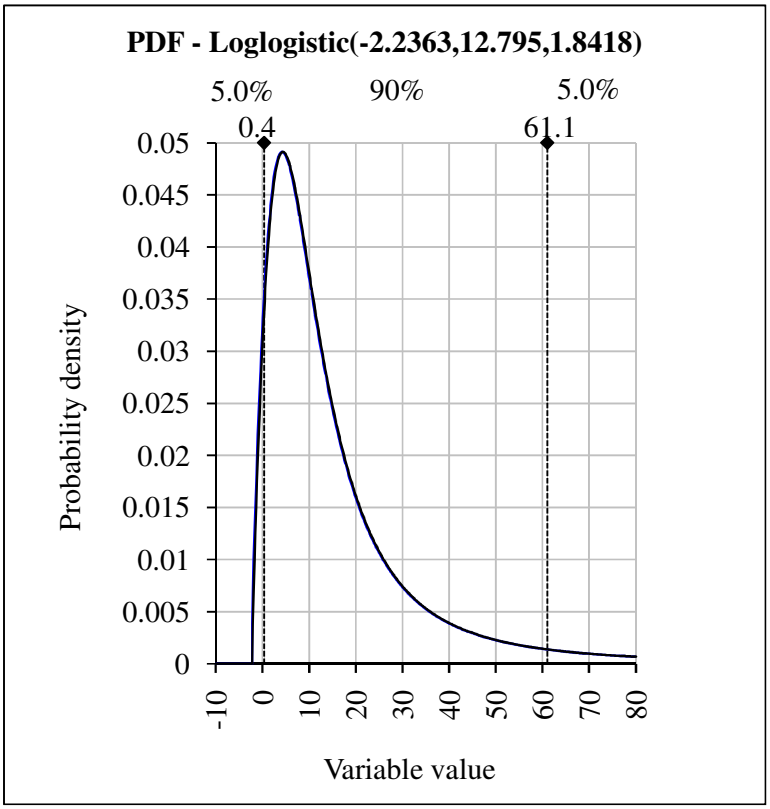


Figure 2.8 | Probability density function of *Salmonella* spp. levels in fresh pig slurries.

Table 2.3 | Input data of source pathogens in faecal substrates for the Quantiative Microbial Risk Assessment modelling.

Organisms	Unit	Probability Density Functions
Pig slurry (Scenarios 1.1, 1.3, 1.4)		
Rotavirus	PFU l^{-1}	BetaGeneral(0.12103,0.42032,0,50270)
Salmonella spp.	MPN/100 ml	Loglogistic(-2.2363,12.795,1.8418)
Helminth ova	no. l^{-1}	Extvalue(433.61,456.3)
Ascaris ova	no. l^{-1}	Expon(164.09)
PBD's effluent (Scenario 1.2)		
Rotavirus	PFU l^{-1}	BetaGeneral(0.16279,0.46397,0,1081.1)
Salmonella spp.	MPN/100 ml	Normal(14,13.306)
Helminth ova	no. l^{-1}	BetaGeneral(0.10048,0.39679,0,120960)
Ascaris ova	no. l^{-1}	Invgauss(16779,127.74)
Untreated septage (Scenario 1.5)		
Rotavirus	PFU l^{-1}	BetaGeneral(0.12734,0.48419,0,32432)
Salmonella spp.	MPN/100 ml	BetaGeneral(0.10498,0.2096,0,7000)
Helminth ova	no. l^{-1}	Expon(449.02)
Ascaris ova	no. l^{-1}	BetaGeneral(0.12424,0.20177,0,416.67)
Septage sludge (Scenario 1.6)		
Rotavirus	PFU l^{-1}	Gamma(0.18971,18191)
Salmonella spp.	MPN/100 ml	BetaGeneral(0.14969,0.23308,0,2800)
Helminth ova	no. l^{-1}	Expon(9147.8)
Ascaris ova	no. l^{-1}	Expon(9147.8)

Table 2.4 | Input data of pathogens in surface water and aquatic spinach for the Quantiative Microbial Risk Assessment modelling.

Organisms	Unit	Probability Density Functions
Surface water in rural areas (Scenarios 2.1, 3.1, 3.2, 3.3)		
Rotavirus	PFU l^{-1}	BetaGeneral(0.11706,0.28735,0,211.11)
Salmonella spp.	MPN/100 ml	Mean = 0.36
Surface water in urban areas (Scenario 2.2)		
Rotavirus	PFU l^{-1}	Extvalue(7.9723,8.1796)
Salmonella spp.	MPN/100 ml	Mean = 0.51
Aquatic spinach in fish ponds received PBD's effluent (Scenario 4.1)		
Rotavirus	PFU l^{-1}	BetaGeneral(0.12741,0.29308,0,3.3444)
Salmonella spp.	MPN/100 ml	Mean = 0.006
Aquatic spinach in urban areas (Scenario 4.2)		
Rotavirus	PFU l^{-1}	Extvalue(0.079723,0.081796)
Salmonella spp.	MPN/100 ml	Mean = 0.005

Pathogen reduction via treatment

Reduction of somatic coliphage, *Salmonella*, and *Ascaris suum* ova over anaerobic digestion and on terrestrial spinach were determined in the pilot study. Coliphage was used as an index organism for rotavirus reduction in water contaminated with faeces (Ottoson and Stenström 2003). Other reduction rates applied in the QMRA modelling were obtained from literature (**Table 2.5**).

Table 2.5 | Input data of reduction rate for the Quantiative Microbial Risk Assessment modelling.

Scenario	Reduction via treatment	Organisms	Reduction rate
1.3	Anaerobic treatment in PBDs at HRT of 15 days	Rotavirus	logRT* ~ Normal(2.5,0.36)
		Salmonella	logRT* ~ Normal(1.74,0.84)
1.4	Anaerobic treatment in PBDs at HRT of 30 days	Rotavirus	logRT* ~ Normal(3.42,0.81)
		Salmonella	logRT* ~ Normal(2.47,0.9)
3.2, 3.3	Flocculation of surface water[a]	Rotavirus	1.9 log unit
		Salmonella	1.7 log unit
3.3	Water boiling[b]	Rotavirus	6.0 log units
		Salmonella	6.0 log units
		Helminth ova	6.0 log units
4.3, 4.4, 4.5	Reduction on terrestrial spinach one week after final irrigation with PBD's effluent	Rotavirus	2.4 log units
		Salmonella	4.1 log units
4	Washing spinach with flocculated/tap water[c]	Pathogens	1 log unit
4	Cooking spinach[d]	Pathogens	6 log units

[a] Westrell 2004, Bennett 2008, Hijnen 2009; [b] WHO 2008; [c,d] WHO 2006a.

* RT = Reduction rate

PBD = plastic bio-digesters

HRT = hydraulic retention time

Volume ingested per exposure and exposure frequency

The volume accidentally ingested was assumed 1 ml in one exposure at emission sources (Scenario 1). This volume has been used in risk assessment for the accidental ingestion of reclaimed wastewater (Asano *et al.* 1992) and source-separated urine used in agriculture (Höglund *et al.* 2002). In scenario 2, the average amount of water swallowed by adults during bathing/swimming in canals was used from Dufour *et al.* (2006). Duration of every bath/swim was estimated from small interviews during sampling combined with author's experience in the Mekong Delta. For the daily water intake applied for drinking surface water (Scenario 3), only published data on tap water were considered. Data on total fluid intake or total water intake including water from beverage or food cannot be representative for the situation of usage surface water for drinking in MD. Thus lognormal distributions to data collected in a survey for tap water intake by adults by Roseberry and Burmaster (1992) were used for the risk model. To quantify the risk associated with spinach consumption the intake amount was arranged by Triang distribution with minimum, most likely and maximum amounts. Frequency of exposure was based on discussions with people at sampling sites. An overview of volumes and frequency ingested is given in **Table 2.6**.

Table 2.6 | Exposure scenarios with assumption on volume ingested and frequency.

Scenario	Volume ingested per exposure	Exposure frequency	Annual frequency
1.1	1 ml	Triang (1, 2, 3) per day	365 days
1.2, 1.3, 1.4	1 ml	Triang (1, 5, 10) per week	52 weeks
1.5, 1.6	1 ml	Triang (1, 2, 3) per week	50 weeks
2.1	16 ml/45 minute[a] × Triang (30,45,60) minutes	daily	365 days
2.2	16 ml/45 minute[a] × Triang (30,45,60) minutes	Triang (1, 2, 3) per week	52 weeks
3	LnIR* ~ Lognormal (7.023, 0.489)[b] (ml)	daily	365 days
4	Triang (50,100,200) (g per meal)	Triang (1, 2, 3) consumption times per week	52 weeks

[a] Dufour *et al.* 2006

[b] Roseberry and Burmaster 1992

* IR: Intake rate

2.4.4 *Dose-response models*

The dose-response models used were the β-Poisson model for rotavirus and Salmonella and the exponential model for helminth ova (Haas *et al.* 1999) and are as follows:

(a) β-Poisson dose-response model

$$P_{inf} = 1 - \left[1 + \frac{d}{ID_{50}}\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha} \quad \text{(Equation 3)}$$

(b) Exponential dose-response model

$$P_{inf} = 1 - e^{-rd} \quad \text{(Equation 4)}$$

or

$$P_{inf} = 1 - e^{-\frac{d}{k}}$$

with

$$k = 1/r \quad \text{(Equation 5)}$$

$$ID_{50} = \ln(0.5)/(-r) \quad \text{(Equation 6)}$$

(c) Annual risk of infection

$$P_{inf(A)} = 1 - \left[1 - P_{inf}\right]^{n} \quad \text{(Equation 7)}$$

where

P_{inf} is the risk of infection of an individual exposed to a single pathogen dose *d*

$P_{inf(A)}$ is the annual risk infection of an individual from *n* exposure per year to the single pathogen dose *d*

ID_{50} is the median infective dose

α is a pathogen "infectivity constant"

r is the probability of one organism initiating an infection

The dose-response relationship for Rotavirus and Salmonella is based on the β-Poisson dose-response model. For *Salmonella* ID_{50} = 23,600 and α = 0.3126; and for Rotavirus ID_{50} = 6.17 and α = 0.2531 (Haas *et al.* 1999). Dose-response relationship for helminth parasites is modelled on the exponential model. For a worse-case evaluation the exact single-hit model (r = 1), which represents the maximum risk curve (Teunis and Havelaar 2000) is used. This was applied for helminth parasites where no dose-response studies on human or animal are available; and in theory, one egg is enough to cause an infection. This model was used in previous studies (Schönning *et al.* 2007, Westrell 2004). Recently, analysing epidemiological data, Navarro *et al*. (2009) developed parameters applied to β-Poisson dose-response model for helminth ova using *Ascaris lumbricoides* ova as indicators:

$$P_{inf} = 1 - \left[1 + \frac{d}{N_{50}}\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha} \quad \text{(Equation 8)}$$

This equation is similar to Equation 3 but N_{50} value is not a measure of the actual median infective dose. Rather it is an empirical value arising from statistical analyses of epidemiological data. The values of N_{50} and α are 859 and 0.104, respectively. This model and its dose-response parameters were used to estimate *Ascaris* infection risks to farmers and children in developing countries (Mara and Sleigh 2010a, Mara and Sleigh 2010b). In the risk calculation conducted in this study, the two models cited were used for *Ascaris* (and helminth ova). Results from both were evaluated and compared with actual disease rates reported in surveillance system in the study area.

2.4.5 *Statistical estimates of risk*

The risk of infection was calculated using @Risk 5.5 (Palisade Corporation), applying 10,000 iterations in the Monto Carlo simulations. Results are presented as probability of infection (P_{inf}) per exposure or annual risk of infection ($P_{inf(A)}$).

3 RESULTS

3.1 Reduction of pathogen and indicator micro-organisms via pilot plastic bio-digesters

3.1.1 *Characteristics of the feeding materials*

In the fresh slurry target phages and bacteria were present in low concentrations before spiking. Seeding sourced from the wastewater treatment plant was free of *E. coli* and *Salmonella* spp. but contained 10^2 CFU ml^{-1} of *Enterococcus* spp. Owing to the different amounts of water added to the pig manure, the chemical and microbial characteristics of the feeding materials varied between HRTs, and an overview is given in **Table 3.1**.

Table 3.1[3] | Average chemical and microbial concentrations of the feeding materials (standard deviations in parentheses).

Parameters	Unit	n	HRT = 3 days	HRT = 15 days	HRT = 30 days
ODM	$g\ l^{-1}$		3	15	30
pH		22	7.54 (0.18)	7.16 (0.22)	7.17 (0.22)
EC	$\mu S\ cm^{-1}$	21	770 (64)	2280 (205)	3160 (543)
TIC	$mg\ HCO_3^-\ l^{-1}$	5	330 (122)	650 (138)	1190 (388)
NH_4^+-N	$g\ l^{-1}$	4	0.07 (0.04)	0.17 (0.08)	0.35 (0.1)
COD	$gO_2\ l^{-1}$	3	2.4 (0.16)	12.2 (0.53)	26.3 (0.4)
VFA	$mg\ l^{-1}$	1	115	645	1290
E. coli	CFU ml^{-1}	2	7×10^3	2.1×10^4	6.3×10^4
Salmonella spp.	MPN/100 ml	2	2×10^o	1.2×10^1	2.0×10^1
Enterococcus spp.	CFU ml^{-1}	2	1.7×10^3	5.1×10^3	1.28×10^4
Somatic coliphage	PFU ml^{-1}	2	3.3×10^2	5.97×10^2	2.97×10^3
Male-specific bacteriophage	PFU ml^{-1}	2	8×10^1	3.2×10^2	6.32×10^2

[3] Reprinted from Yen-Phi *et al.* (2009) with permission from IWA Publishing.

3.1.2 *Performance of reactors*

Physicochemical values differed between the different HRTs. Average pH varied from 6.6 to 7.2. Gas yields increased from 12.8 to 40.3 litre per reactor; the average amount of gas produced was significantly higher at longer HRTs (**Table 3.2**). Yet CH_4 concentration increased with decreasing HRT and pH values.

Table 3.2[4] | Average pH values and sum of biogas produced per reactor for 50 days (standard deviations in parentheses).

Parameters	Unit	n	HRT=3 days	HRT=15 days	HRT=30 days
pH		49	6.6 (0.04)	7.0 (0.01)	7.2 (0.03)
Biogas per reactor	L	21	12.8 (1.2)	31.6 (2.1)	40.3 (1.9)
CH_4	%	21	74 (2.6)	69 (0.4)	65 (2.9)
Biogas efficiency	l per kg ODM fed	21	97 (9)	240 (16)	310 (14)

The influent and effluent ammonium concentrations were below 0.5 g NH_4^+-N l^{-1} over all HRTs. VFA concentrations in the effluents remained low over all HRTs but increased with higher HRT: 108, 235 and 360 mg l^{-1} for HRT of 3, 15 and 30 days, respectively. The COD treatment efficacy (values in the effluents compared with the influents) increased markedly from HRT of 3 days (25%) to 15 days (80%) and 30 days (91%). The accumulated sludge at the base of reactors differed substantially between HRTs: 17%, 35% and 42% of the total ODM fed for HRT of 3, 15 and 30 days, respectively. The TIC – an indicator of process stability – increased gradually for the 15-day HRT to 2,900 mg l^{-1} and 30-day HRT to 4,800 mg l^{-1} for the duration of the trial while the 3-day HRT had a constantly low value of around 700 mg l^{-1}. Therefore, a longer HRT is positive for reactor operation, in terms of reactor stability, effluent stability and thereby also gas production.

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3.1.3 *Reduction of microorganisms tested: comparison of influents and effluents*

All examined phages and bacteria were reduced during the treatment. Generally, bacteria showed higher resistance to treatments than phages. Log_{10} reduction of bacteria ranged from 0.54 to 2.47. Phages reduction ranged from 1.60 to 3.42. The longer the HRT the more efficient the reduction of microorganisms. Reduction during 30-day HRT was about one log_{10} unit higher than that of 15-day HRT, and about two log_{10} units higher than that of 3-day HRT (**Table 3.3**).

Table 3.3[5] **|** Log_{10} reductions of organisms tested comparing inflow and outflow (standard deviations in parentheses; n = 21) in pilot plastic bio-digesters.

Tested organisms	**Unit**	**HRT = 3 days**	**HRT = 15 days**	**HRT = 30 days**
Somatic coliphage	PFU ml^{-1}	1.60 (0.24)	2.50 (0.36)	3.42 (0.81)
Male-specific bacteriophage	PFU ml^{-1}	1.17 (0.39)	2.23 (0.45)	3.00 (0.60)
E. coli	CFU ml^{-1}	0.54 (0.43)	1.79 (0.63)	2.43 (0.71)
Salmonella Senftenberg	CFU ml^{-1}	1.23 (0.83)	1.74 (0.84)	2.47 (0.90)
Enterococcus faecalis	CFU ml^{-1}	1.01 (0.36)	1.77 (0.39)	2.30 (0.50)

E. coli showed less reduction at a HRT of three days compared with *Salmonella* Senftenberg and *Enterococcus faecalis* (**Table 3.3**). In a batch experiment with similar conditions *E. coli* showed a lag phase of 1–2 days before their concentration decreased rapidly. It can be inferred that the *E. coli* population found in effluents from reactors with a HRT of 3 days resulted from this lag phase. With HRTs of 15 and 30 days the reduction of the three bacteria investigated was similar. Log_{10} reduction of somatic coliphage was slightly higher than that of male-specific bacteriophage. The relation of reduction rates between these two phages was comparable for all HRTs (**Table 3.3**).

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3.1.4 *Summary*

Physicochemical values of reactors differed between HRTs. Gas production efficiency was better for longer HRTs. The accumulated sludge at the reactor's base increased with longer HRT. Phages and bacteria examined were reduced, but none was completely eliminated. Log_{10} reduction of bacteria ranged from 0.54 to 2.47. Phages ranged from 1.60 to 3.42. The reduction of organisms at HRT = 30 days was about one log_{10} unit higher than HRT = 15 days and about two log_{10} units higher than HRT = 3 days. The results indicate that the reduction of tested organisms increases with HRT. However the hygienic quality of the liquid effluent does not meet required quality values for surface and irrigation water. Longer HRTs are recommended to increase gas yield and achieve higher pathogen reduction. More barriers should be applied while handling bio-digester outputs to minimise risks to environmental and human health.

3.2 **Reduction of pathogen and indicator microorganisms via pilot septic tanks**

3.2.1 *Characteristics of feeding materials*

Fresh brown water used in the trial had an average organic dry matter of 0.2%. pH varied from 6.7 to 7.0. Target phages and bacteria were present in low concentrations before spiking (*Salmonella* spp. < 10 CFU ml^{-1}; phages < 10^2 PFU ml^{-1}, *E. coli* and *Enterococcus* spp. at 10^3 CFU ml^{-1}). Seeding sourced from the wastewater treatment plant was free of *E. coli* and *Salmonella* spp. but contained 10^2 CFU ml^{-1} of *Enterococcus* spp.

3.2.2 *Performance of reactors*

pH values of the effluents varied from 6.6 to 6.9. Biogas was produced about 120 litre per kg ODM fed with an average CH_4 percentage of 64%. It shows that the reactors functioned well at a 3-day HRT. Biogas production efficiency was higher than that of anaerobic digestion of pig slurry at similar conditions such as temperature, ODM feeding rate, HRT (see Section 3.1.2).

3.2.3 *Reduction of tested micro-organisms: comparison of influents and effluents*

The examined phages and bacteria were slightly reduced during the treatment (**Table 3.4**). At the same HRT (3 days) in anaerobic digesters (cf. Section 3.1.3), phages and *Salmonella* Senftenberg showed greater resistant (log_{10} reduction >1), while *E. coli* and *Enterococcus faecalis* showed similar reduction rates. Generally, reduction of phages and bacteria was not significant in STs as the 3-day HRT is the maximum standard HRT in STs. When the HRT is shorter, lower reduction rates are expected. If the tanks are full of sludge and scum, then the untreated wastewater (black water) flows directly to receiving waters.

Table 3.4 | Log_{10} reductions of organisms tested comparing inflow and outflow (n = 15) in pilot septic tanks at hydraulic retention time of 3 days.

Tested organisms	Unit	Log_{10} reduction	SD
Somatic coliphage	PFU ml^{-1}	0.19	0.09
Male-specific bacteriophage	PFU ml^{-1}	0.67	0.05
E. coli	CFU ml^{-1}	0.72	0.08
Salmonella Senftenberg	CFU ml^{-1}	0.76	0.03
Enterococcus faecalis	CFU ml^{-1}	1.15	0.24

3.2.4 *Summary*

Reduction of phages and bacteria in STs is not significant, although helminth ova are expected to settle into the sludge over time. In Vietnam STs are mostly emptied when full, implying that pathogens, including helminth ova, will contaminate surface waters.

3.3 Survival of helminth in batch digester and in faecal sludge

3.3.1 *Survival of Ascaris suum in batch digester*

The average viability rate of *Acaris suum* incubated in 0.1 N sulphuric acid was 0.82 (82%). The viability of *Ascaris suum* eggs decreased from 82% to 25% after 45 days in both swine and cattle slurries and corresponded to a T_{90} of approximately 90 days (**Figure 3.1**). There was no difference realised in the inactivation rate of *Ascaris suum* in swine and cattle slurry. After 2 and 4 weeks the viability did not reduce significantly. It indicates a lag phase of 4 weeks before the inactivation occurs.

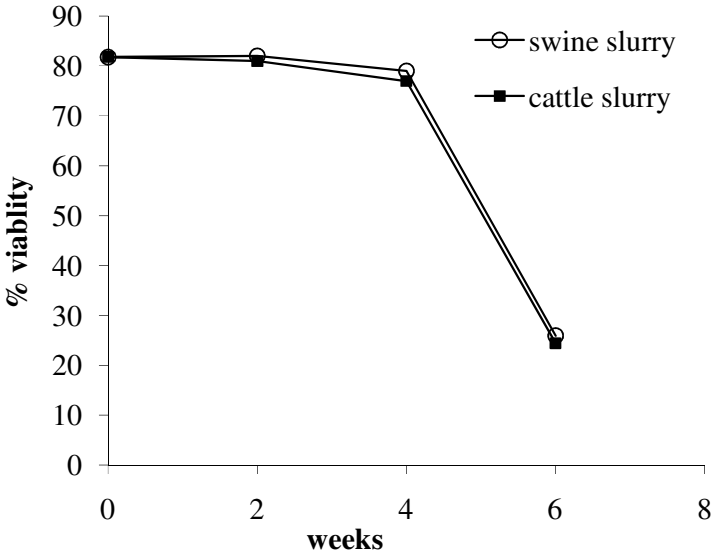

Figure 3.1 | Viability (n = 4) of *Ascaris suum* eggs in batch-wise experiement.

3.3.2 ***Survival of Ascaris suum in biogas and septage sludge***

Performance of the reactors

The pH values of the 2 treatments were optimal for the biogas process (**Table 3.5**) and the biogas production efficiency agreed with the previous trial (cf. Section 3.1.2), thus demonstrating that the reactors ran well during the experiment.

Table 3.5 | pH values and biogas production of the trial reactors.

Parameters	**Unit**	**n**	**Biogas reactor**	**Septic tank**
pH		20	7.05 (0.02)	6.8 (0.01)
Biogas per reactor	l	3	46 (3.3)	370 (12)
CH_4	%	3	65 (2.3)	62 (1.9)
Biogas efficiency	l per kg ODM fed		250 (6.1)	260 (9)

Survival of Ascaris suum ova

The average viability rate of *Acaris suum* ova incubated in 0.1 N sulphuric acid in this trial was 0.836 (83.6%). The viability of *Ascaris suum* ova decreased from 83.6% to 0.3% after 1 year in biogas and septage sludge (**Figure 3.2**) and corresponded to a $\log_{10}$ reduction of 2.5 and a T_{90} of approximately 5 months. No significant difference of the viability was found between the two substrates. The viability decreased rapidly in the first 4 months of the trial (from 83.6 to 5%). It took another 4 months to achieve 0.5% viability and a further 4 months to reach 0.3%, showing that just a few ova were resistant in the long term.

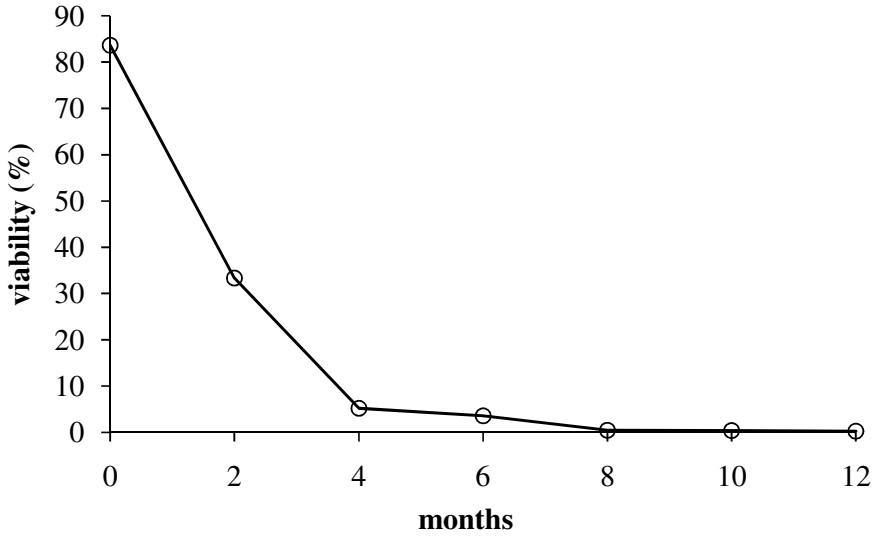

Figure 3.2 | Viability (n = 4) of *Ascaris suum* eggs in biogas and septage sludge.

The reduction of *Ascaris suum* ova as described in **Figure 3.2** is followed an exponential equation (R^2=0.9451):

$$y = 63.883\ e^{-0.502x} \qquad \text{(Equation 9)}$$

where

y:% viability of *Ascaris suum*

x: the retention time in sludge

This equation can be used to estimate the viability of helminth ova accumulated in PBD or septage sludges in tropical conditions.

3.3.3 ***Summary***

The everage viability of *Ascaris suum* ova (r_v) was about 0.8. Anaerobic digestion in tropical PBDs has little effect on the inactivation of *Ascaris suum*. However helminth ova can easily settle at the reactor base if the HRT is long and the input velocity is low. A relationship between helminth ova viability rate and sludge retention time was

established by an exponential equation. A small portion of *Ascaris suum* can survive in sludge for up to 1 year. There is no difference between the viability of *Ascaris suum* in biogas and septage sludges.

3.4 Factors affecting survival of phages and bacteria in anaerobic digestion under tropical conditions

3.4.1 *Hydraulic retention time*

In anaerobic digestion the reduction of pathogens and indicator micro-organisms is limited and a reduction between 1 and 3 orders of magnitude can be expected in a mesophilic process. The reduction of pathogens common to domestic PBDs in tropical regions increases with HRT (cf. Section 3.1.3).

3.4.2 *Initial concentration and substrate type*

Characteristic of animal slurries using in the experiment

The solid content in cattle slurries was higher than in swine slurries. Yet NH_4^+-N values were higher in swine slurries (**Table 3.6**). The pH and COD values were not significantly different between slurries.

Table 3.6 | Physio-chemical characteristic of the raw substrates.

Substrates	pH	Solids (%)		COD	NH_4^+-N
		Total	Volatiles	g l^{-1}	g l^{-1}
Swine slurry	7.74 – 7.85	1.8	1.2	30.05	1.55
Cattle slurry	7.78 – 7.92	2.6	1.7	30.45	0.85

The presence of concerned phage and bacteria in the fresh substrates were at low concentrations (10^3 – 10^4 PFU or CFU ml^{-1}). Seeding used was free of *E. coli* and *Salmonella* spp. but contained 10^3 CFU/100ml of *Enterococcus* spp. The pH status of slurries changed little during the experiment. The NH_4^+-N concentrations differed.

Swine slurries ranged from 1.5 to 1.95 g l^{-1} and cattle slurries ranged from 0.8 to 1.15 g l^{-1} at day 45 of the treatment.

Reduction of tested organisms

Reduction occurred with all phages and bacteria. T_{90} counts varied from 1.44 to >45 days (**Table 3.7**). Initial concentrations and slurry type affected the survival of tested organisms. *E. coli* and *Salmonella* Senftenberg showed a lag phase of 1 – 2 days before their concentrations decreased rapidly regardless of substrate and treatment, indicating a T_{90} of 1 – 2 days. In general the time needed for 90% reduction of *E. coli* and *Salmonella* Senftenberg is from 2 – 4 days. No organism was found after 8 days of treatment. T_{90} values of *E. coli* and *Salmonella* Senftenberg were significantly different ($p \leq 0.001$) across slurries. They survived longer in swine slurry than in cattle slurry.

On the other hand *E. coli* die-off differed significantly when the laboratory strain was used as an inoculate. The undetected level of this *E. coli* population was observed after only one day of anaerobic treatment. In the same experiment *E. coli* tended to die off after 1 – 2 days when the concentration of indigenous *E. coli* is low (10^3 CFU/100ml).

Table 3.7 | T_{90} (decimal reduction time) values of tested phages and bacteria in different substrates and different initial concentrations (n = 4).

Substrate	Initial concentration	Somatic coliphage		*E. coli*		*Salmonella* Senftenberg		*Enterococcus faecalis*	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Swine slurry	Low	> 45		3.13	0.7	1.66	0.1	23.8	3
	high	22.5	3.64	2.86	0	3.5	0.2	15.2	4.4
Cattle slurry	low	20.6	3.47	2.03	0.1	1.85	0	23.2	5.7
	high	13.9	0.91	2.55	0.5	1.44	0.2	15.3	3.5

Enterococcus faecalis survived longer than *E. coli* and *Salmonella* Senftenberg (T_{90} varied from 15.22 to 24.79 days) and it was still viable at 45 days (**Figure 3.3**). Low numbers of *Enterococcus faecalis* were present at day 45 (10^2 to 10^3 CFU/100 ml). *Enterococcus faecalis* showed no significant difference between slurries under identical treatments but the survival rate differed ($p < 0.01$) between low and high initial concentrations, this is probably due to the constant presence of *Enterococcus* spp. in the biogas slurry as it was already found in the initial seed.

Somatic coliphages survived longer in swine than in cattle slurry (**Figure 3.3**) and high initial inoculation showed higher removal rates than with low initial concentration. The results indicate that the viability of tested organisms, with the exception of *E. coli*, relates to initial concentration. Except for *Enterococcus faecalis*, somatic coliphage and bacteria behaved quite differently in swine and cattle slurries.

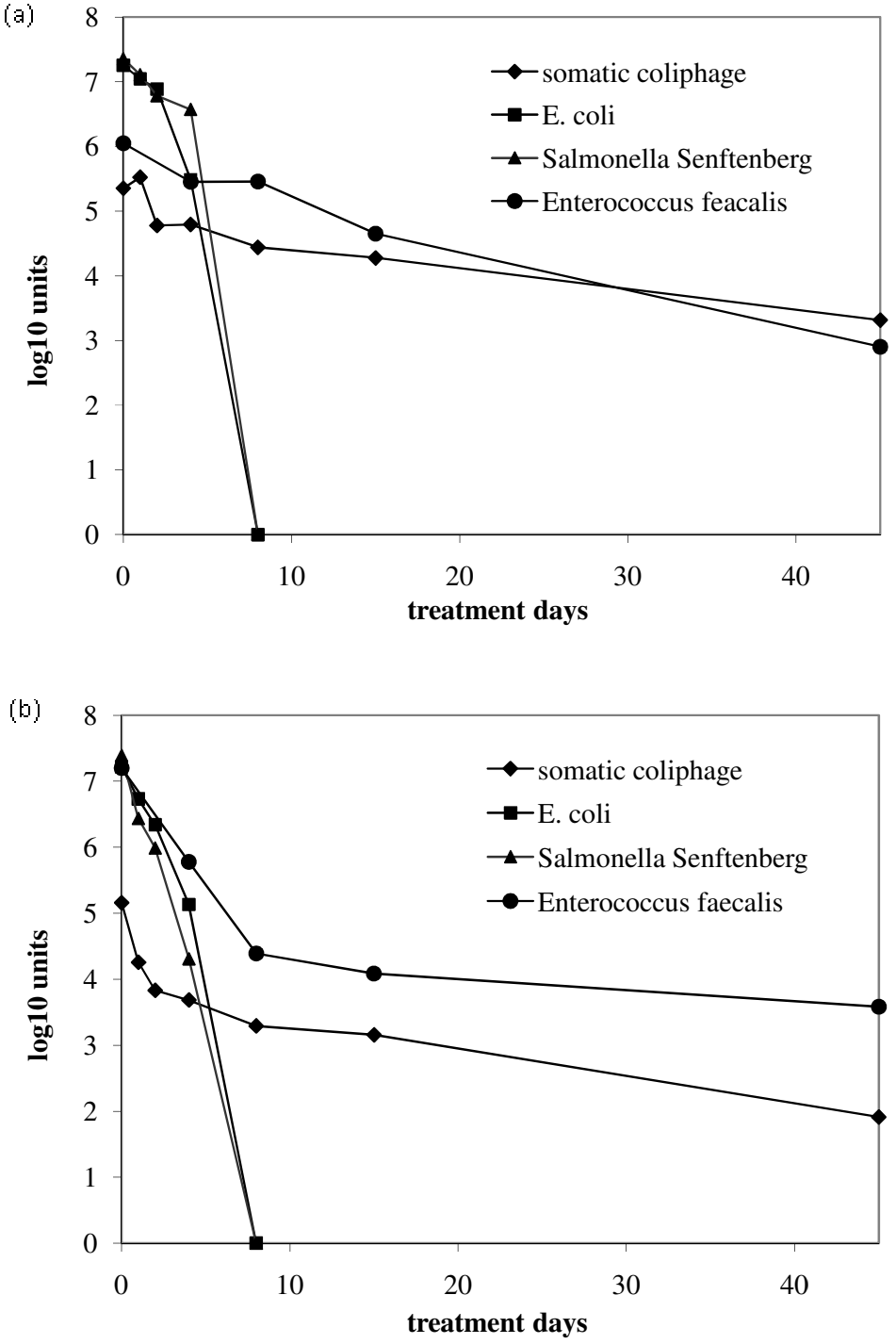

Figure 3.3 | Survival curves of tested phages and bacteria at high initial concentrations in swine slurry (a) and cattle slurry (b)

3.4.3 *Volatile fatty acid*

Characteristics of the feeding materials

In fresh slurry target phages and bacteria were present in low concentrations before spiking (**Table 3.8**). Seeding sourced from the wastewater treatment plant was free of *E. coli* and *Salmonella* spp. but contained 10^2 CFU ml^{-1} of *Enterococcus* spp. The chemical and microbial characteristics of the feeding materials varied between the VFA treatment and the control, and an overview is given in **Table 3.8**.

Table 3.8 | Average chemical and microbial concentrations of the feeding materials (standard deviations in parentheses).

Parameters	**Unit**	**n**	**VFA treatment**	**Control**
ODM	g l^{-1}		15	15
VFA	mg l^{-1}	14	5,000	420
pH		14	7.28 (0.2)	7.01 (0.36)
EC	µS cm^{-1}	14	49,300 (2,600)	2,400 (360)
TIC	mg HCO_3^- l^{-1}	1	10,000	1690
NH_4^+-N	g l^{-1}	8	0.059 (0.01)	0.058 (0.01)
COD	gO_2 l^{-1}	7	12.7 (0.4)	7.9 (1.1)
E. coli	CFU ml^{-1}	2	9.8×10^3	9.8×10^3
Salmonella spp.	MPN/100ml	2	2×10^1	2×10^1
Enterococcus spp.	CFU ml^{-1}	2	1.9×10^3	1.9×10^3
Somatic coliphage	PFU ml^{-1}	2	2.97×10^2	2.97×10^2
Male-specific bacteriophage	PFU ml^{-1}	2	1.2×10^2	1.2×10^2

Performance of reactors

Physicochemical values of daily effluent samples differed between the VFA treatment and the control (**Figure 3.4**). The pH of effluents from VFA treatment was higher than that of the control but both values were optimal for the biogas process. EC values of VFA treatment increased steadily after adding VFA, from 3.4 to 50.4 mS/m while EC remained stable and low in the control. A similar trend for COD was observed: COD increased from 1.1 up to 12.2 g l^{-1} of VFA treatment while COD values of the control varied from 1.2 to 3.4 g l^{-1}. VFA values of effluents from VFA treatment increased while those from the control decreased. At the end of the trial VFA concentration of the accumulated sludge was measured for all relevant parameters and the values were not significantly different from that of the effluent sampled at the same day.

The average amount of biogas produced was significantly higher in control reactors (**Table 3.9**). Yet gas increased noticeably one week after VFA addition and then decreased until the trial's end. VFA in reactors at optimal performance was calculated at a level of 2.5 g l^{-1}, and EC at 2.5 – 3 mS/m. Mass balance based on information of biogas production and influence content shows that the whole VFA treatment process became unbalanced from the second week of VFA amendment.

Table 3.9 | Sum of biogas produced per reactor for 8 weeks (standard deviations in parentheses).

Parameters	Unit	n	VFA treatment	Control
Biogas per reactor	L	3	21.8 (0.4)	31.9 (4.1)
CH_4	%	21	72.9 (6.7)	65.9 (1.5)
Biogas efficiency	l per kg ODM fed	3	156 (2.8)	228 (29)

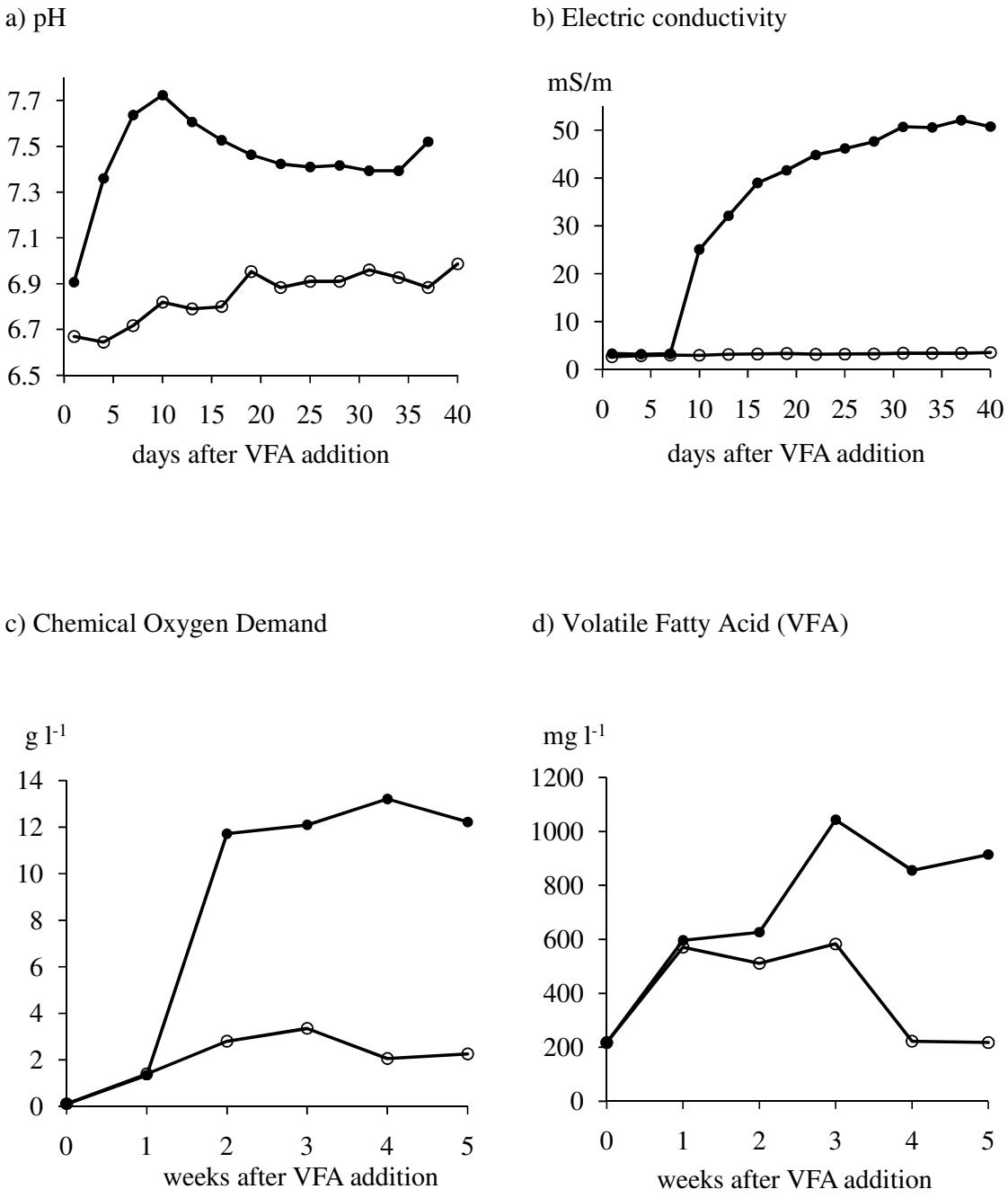

Figure 3.4 | Physicochemical values of effluent samples from volatile fatty acid-amendment treatment (●) and the control/no amendment (○).

Reduction of microorganisms tested: comparison of influents and effluents

All examined phages and bacteria were more resistant in VFA treatment than in the control (**Table 3.10**). Log_{10} reduction of organisms tested concurs with earlier findings at a HRT of 15 days, indicating that high concentrations of VFA did not influence the reduction of tested organisms.

Table 3.10 | Log_{10} reductions of organisms tested comparing inflow and outflow with standard deviations in parentheses (n = 18).

Tested organisms	Unit	VFA treatment	Control
Somatic coliphage	PFU ml^{-1}	1.81 (0.34)	2.49 (0.15)
Male-specific bacteriophage	PFU ml^{-1}	1.44 (1.02)	2.52 (1.26)
E. coli	CFU ml^{-1}	1.65 (0.46)	1.90 (0.33)
Salmonella Senftenberg	CFU ml^{-1}	1.37 (0.47)	1.91 (0.86)
Enterococcus faecalis	CFU ml^{-1}	1.51 (0.31)	1.82 (0.28)

In conclusion, the amount of VFA amendment and the composition of feeding materials depend on the stability of the particular anaerobic process. Tropical PBD running at low loading rates with manure as the only feeding source cannot handle a concentration of VFA > 2.5 g l^{-1}. VFA has no effect on the reduction of indicator micro-organisms and pathogen tested in combined-stage mesophilic anaerobic reactors. Greater reduction rates are expected in two-stage anaerobic digestion systems.

3.4.4 *Batch vs regular feeding trials*

E. coli and *Salmonella* spp. were not found after 8 days of batch-wise treatment (Section 3.4.2), while they were always detected in the effluent as well as in the accumulated sludge of the continuous biogas reactors, even at HRT of 30 days (Section 3.1.3). At the same HRT *Enterococcus faecalis* and Coliphages were more resistant in continuous reactors than in batch-wise ones (**Table 3.11**).

Table 3.11 | Log_{10} reduction of micro-organisms in batch-wise and continuous reactors at hydraulic retention time (HRT) of 15 days.

	batch-wise reactor after 15 days of treatment	continuous reactor HRT = 15 days
Coliphages	1.07	2.5
E. coli	7.25	1.79
Salmonella Senftenberg	7.36	1.74
Enterococcus faecalis	1.40	1.77

3.4.5 ***Summary***

Biogas production is a preferable treatment method to utilize the energy content in the manure, especially in tropical region where most developing countries are located. Tropical temperatures permit the utilization of efficient anaerobic reactors without heating. Thus most of the anaerobic reactors are running at mesophilic condition (28 – 30^{o}C) without pasteurization step. HRT is directly proportional to the reduction rate of indicator organisms and pathogen, and biogas production. Pathogen reduction depends also on the initial concentration, bacteria type and substrate type. In combined-stage reactors such as PBDs, high levels of VFA have no effect on pathogen reduction at neutral pH. VFA levels of over 2.5 g l^{-1} cause inhibition for biogas production in PBDs with low loading rates. The reduction is more pronounced on acidic pH values in reactors. Moreover, the reduction of phages and bacteria is dependent on the operation condition – batch-wise or continuous digestion. Out of many factors affecting the survival of indicators and pathogen in tropical PBDs, HRT is recommended owing to (1) higher HRT can be achieved by using less water to clean the pig sties and regular desludging; (2) higher HRT leads to higher biogas yield.

3.5 **Microbial characteristics of faecal sludge**

3.5.1 ***Pig slurry***

Pig slurry in the Mekong Delta is usually not stored but fed directly into domestic biogas digesters or discharged to surface waters. Thus fresh pig slurry can be found as influent for PBDs. Influence samples were low in dry matter, which ranged from 0.02 to 0.77% with an average of 0.22%. The pH values ranged from 7.3 to 7.5. Occurrence and levels of indicator micro-organisms and pathogens in PBD influents are reported in **Table 3.12**.

Table 3.12 | Occurrence and levels of phages and bacteria in plastic bio-digester's influent samples (n = 18).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU ml^{-1}	ND - 1.9×10^5	17,700	150	5,000	78
Male-specific bacteriophage	PFU ml^{-1}	ND - 3,000	80	10	130	56
E. coli	CFU ml^{-1}	10,000 - 9×10^6	1.6×10^6	32,000	2.5×10^6	100
Salmonella spp.	MPN/100ml	ND - 90	16	10	20	83
Enterococcus spp.	CFU ml^{-1}	900 - 1.9×10^5	31,000	10,500	5,400	100

Helminth ova in fresh pig slurries were detected in 80% samples. When detected, the concentration ranged from 250 to 8,000 no. l^{-1} with an average of 1,300 ova l^{-1}. The ova varieties detected included *Oesophagostomum* spp. (44% of samples), *Ascaris suum* (39%), *Metastrongylus elongates* (33%), *Taenia* sp (28%), *Trichuris suis* (28%), *Fasciolopsis buski* (17%), *Physocephalus sexalatus* (6%), *Schistosoma japonicum* (6%), *Strongyloides ransomi* (6%), *Clonorshis sinensis* (6%).

3.5.2 *Septage sludge*

Characteristics of untreated septage samples

Untreated septage was low in dry matter (average DM = 0.24%). The pH values ranged from 7.3 to 7.5. Occurrence and levels of indicators and pathogens studied in untreated septage are reported in **Table 3.13**. Helminth ova detected were those of *Ascaris lumbricoides*, *Enterobius vermicularis*, *Hymenolepis diminuta*, *Hymenolepis nana*, *Taenia* spp., *Capillaria philippinensis* and hookworm. Their frequency varied from 20 – 40% of samples. The average concentration for each species ranged from 6 – 190 no. l^{-1}.

Table 3.13[6] **|** Concentration of micro-organisms in untreated septage samples (n = 20; average dry matter = 0.24%).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU ml^{-1}	ND - 1.9×10^5	1800	150	25,000	80
Male-specific bacteriophage	PFU ml^{-1}	ND - 1,000	520	600	380	80
E. coli	CFU ml^{-1}	2,000 - 3.5×10^5	68,000	11,000	34,000	100
Salmonella spp.	MPN/100 ml	ND - 7,000	1,300	310	270	70
Enterococcus spp.	CFU ml^{-1}	640 - 24,000	8,500	5,300	4,900	100
Helminth ova	no. l^{-1}	ND - 1,200	450	240	440	95

ND = not detected

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Characteristics of septage sludge samples

Septage sludge had an average dry matter of 5.4%. The pH varied from 6.7 to 7.4. *E. coli*, *Enterococcus* spp., and helminth eggs were detected in all samples tested (**Table 3.14**). There were many varieties of helminth ova in high concentrations (**Figure 3.5**), with the varieties frequency ranging from 10% to 50% and *Ascaris lumbricoides* predominated (**Figure 3.5**).

Table 3.14[7] **|** Concentration of micro-organisms in septage sludge samples (n = 20; average dry matter = 5.4%).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU/g d.w.	ND - 9.7×10^6	1.3×10^6	25,000	3.0×10^6	80
Male-specific bacteriophage	PFU/g d.w.	ND - 6,200	2,100	350	860	80
E. coli	CFU/g d.w.	7,200 - 6.2×10^6	1.1×10^6	2.3×10^5	4.5×10^5	100
Salmonella spp.	MPN/g d.w.	ND - 1,900	570	460	270	60
Enterococcus spp.	CFU/g d.w.	1,500 - 4.0×10^5	78,000	11,000	1.6×10^5	100
Helminth ova	no. l^{-1}	1,000 - 50,000	16,000	13,000	18,000	100

d.w. = dry matter; ND = not detected

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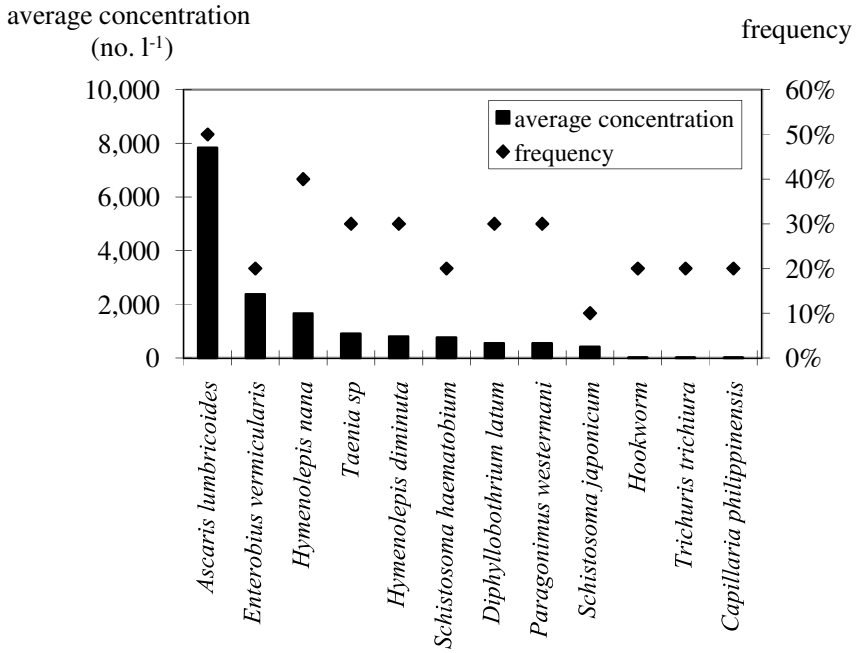

Figure 3.5[8] | Frequency and average concentrations of helminth ova (n = 20) in septage sludge samples.

3.5.3 ***Summary***

E. coli and *Enterococcus* spp. were detected in all pig slurry and septage samples. Coliphages were detected in over 50% of samples. *Salmonella* spp. was detected in more than 60% of samples. Helminth ova were present in 80% of pig slurry samples, 95% of untreated septage samples, and in all septage sludge samples with high concentrations. Ten varieties of helminth ova were found in pig slurries and twelve found in septage. More helminth ova varieties in higher concentrations were found in faecal sludge than those reported from stool samples. The results also show that indicator micro-organisms and pathogens, especially helminth ova, accumulate in sludge.

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3.6 Plastic bio-digesters: effluent microbial characteristics and microbial treatment efficacy

3.6.1 *Plastic bio-digesters*

Description of PBDs surveyed

PBD volume ranged from 7.2 to 15 m^3 with an average of 10.8 m^3. Their age varied from 15 days to 12 years. Influents were sourced from pigsties with the number of pigs varying from 3 to 25 and not proportional to digester volume. Liquid effluents were discharged to fishpond (40%); these combined with gardens (20%); and nearest canals (40%). Only one PBD was desludged, after 1.5 year of operation. Most PBDs (90%) were covered by some kind of roofs and fenced off from animals (see **Figure 1.7**). Ninety per cent of PBDs provided biogas such that it met demands of household cooking.

Characteristics of effluent samples

PDBs' effluents samples had an average DM of 0.65% and ranged from 0.002 to 4.9% while DM content of influent samples varied between 0.02 and 0.77% (**Figure 3.6**). Effluent DM was expected to be less than that of influents. Yet 40% of the former contained more DM than the latter, showing that the loading rate is too low and the sedimentation was not efficient. Effluent pH values varied from 6.7 to 7.4.

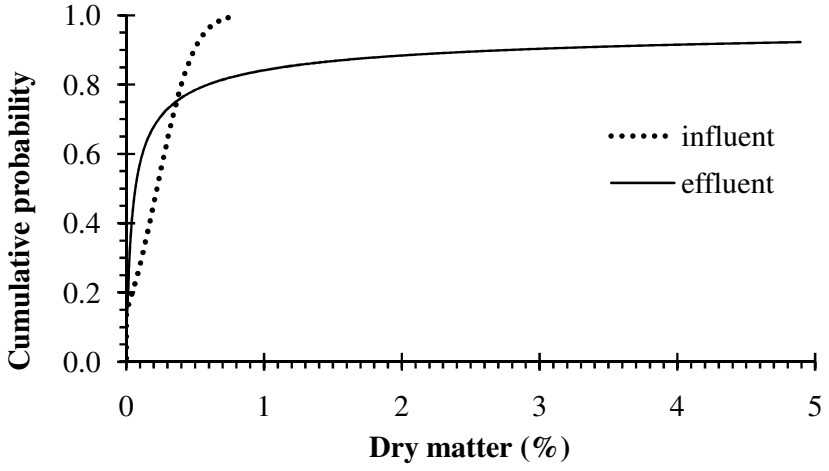


Figure 3.6 | Distribution of dry matter in plastic bio-digester's samples.

E. coli, *Salmonella* spp., and *Enterococcus* spp. were detected in all samples (**Table 3.15**). The detection frequency of *Salmonella* spp. was higher in effluent than influent samples (Section 3.5.1).

Table 3.15 | Occurrence and levels of phages and bacteria in plastic bio-digester's effluent samples.

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU ml^{-1}	ND - 4,000	600	30	1,000	78
Male-specific bacteriophage	PFU ml^{-1}	ND - 100	40	10	5	56
E. coli	CFU ml^{-1}	900 - 1.4×10^6	2.7×10^5	1.2×10^5	3.6×10^5	100
Salmonella spp.	MPN/100 ml	4 - 60	14	9	14	100
Enterococcus spp.	CFU ml^{-1}	90 - 2×10^4	5,500	4,800	5,100	100

Many PBDs (56%) could not hold helminth ova. Ova and DM concentration in effluents were higher than influents. Two effluent samples (11%) were positive for helminth ova while the ova were not found in influents. Effluents with DM over 1% showed significantly high concentration of helminth ova (> 25,000 no. l^{-1}).

3.6.2 *On-site treatment efficacy of pathogens and indictor micro-organisms*

Phages were more resistant to the treatment than bacteria tested (**Table 3.16**). The reduction of phages and bacteria via PBDs in MD was low. It concurs with the previous results (Section 3.1.3) and shows that the $\log_{10}$ reduction of PBDs at a HRT of 3 days was less than 1.6.

Table 3.16 | Log_{10} reduction of phages and bacteria tested via plastic bio-digesters.

	somatic coliphage	male-specific bacteriophage	*E. coli*	*Salmonella* spp.	*Enterococcus* spp.
Influent	4.25	2.79	6.2	1.22	4.49
Effluent	2.78	1.5	5.43	1.15	3.74
log_{10} reduction	**1.47**	**1.29**	**0.77**	**0.07**	**0.75**

An overview of helminth ova detected in PBD influents and effluents is given in **Figure 3.7**. Helminth ova were not detected in either influent or effluent samples of one PBD (5.6%). The owner ran a helminth-control program in pigs and is widely acknowleged as the district's "PBD master". However, the influent DM value of this PBD was low (0.1%) like common situation of PBDs in Vietnam.

Thirty-nine per cent of PBDs with effluent DM values of less than 0.5% showed a reduction of helminth ova. The reduction ranged from 0.2 to 3.0 log_{10}. Significantly 11.2% of effluent samples contained no ova while influent samples had a concentration of between 800 and 1,000 ova l^{-1}. This shows that the operation of these PBDs was optimal for helminth ova settling at the digester base.

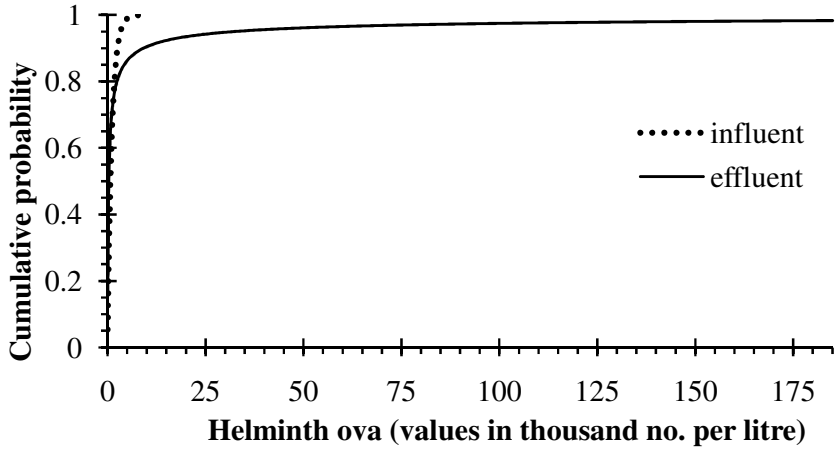


Figure 3.7 | Distribution of helminth ova in plastic bio-digester's samples.

The frequency of helminth ova detected varied between influent and effluent samples (**Figure 3.8**). Except for *Oesophagostomum* spp. other species were detected more frequently in effluents than influents. The reduction rate of helminth ova in sludge was examined in the one-year experiment (cf. Section 3.3.2).

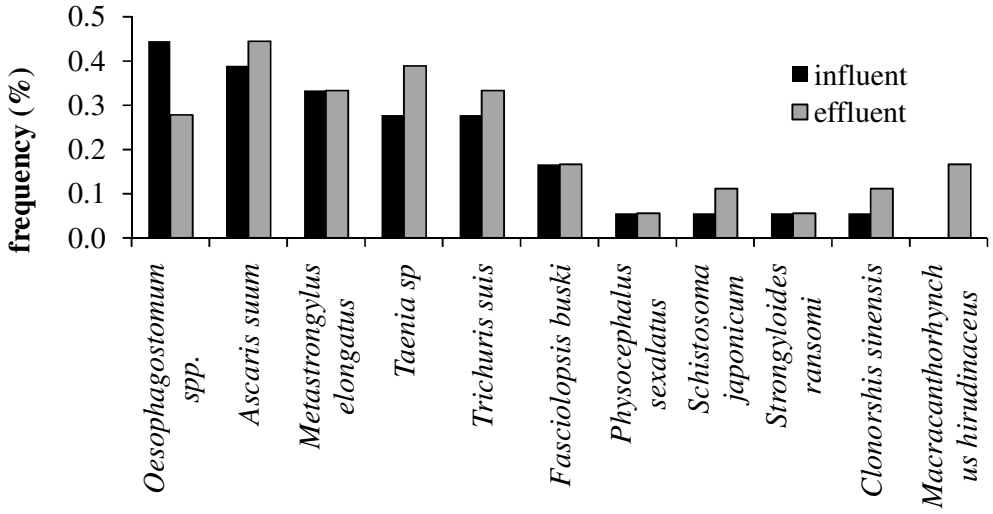


Figure 3.8 | Frequency of helminth ova varieties detected in plastic bio-digester's samples.

3.6.3 ***Summary***

PBDs are common in MD and considered to bring benefits to users. However their design and operation are not optimal. Thus reduction of bacteria was < 1 $\log_{10}$ and of phages < 1.5 $\log_{10}$ while the concentration of phages and bacteria in influents was high. In most PBDs helminth ova did not sediment but were released to surface water via effluents, the highest concentration being 175,000 no. l^{-1}. As 40% of PBD effluents were discharged into nearby canals the situation was not significantly better than the direct discharge of pig slurry to surface water.

3.7 **Microbial make-up of surface water in the Mekong Delta**

In countries where centralised wastewater treatment plants are not yet established, like Vietnam, domestic sanitation systems like PBDs and STs are cost effective. Yet a key challenge is their proper management. In the Mekong Delta untreated septage and

highly contaminated PBD effluents, as well as animal manure and human faeces, are discharged directly to surface water that is used by millions of people for bathing and drinking purposes. Microbial contamination of surface water in rural areas results mainly from animal manure and human faeces. In urban areas the predominant source is human excreta.

3.7.1 *Microbial make-up of water in fishponds receiving plastic bio-digester's effluents*

The reduction of phages and bacteria in MD PBDs is rather low (<1.5 $\log_{10}$). In many cases helminth ova concentrations in effluents are much higher than those in influents. **Table 3.17** shows the occurrence and levels of organisms tested in fishponds receiving PBD effluents. Yet helminth ova were not detected in all samples, and explained by the accumulation of ova in pond sludge.

Table 3.17 | Concentrations of micro-organisms in water from fishponds received plastic bio-digester's effluents (n = 15).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU/100 ml	0 – 2,030	960	170	1,200	60
E. coli	CFU ml^{-1}	5 – 1,600	300	105	575	100
Salmonella spp.	MPN/100 ml		0.69*			50
Enterococcus spp.	CFU ml^{-1}	2 - 200	45	30	48	100
Helminth ova	no. l^{-1}	ND				

* The result based on negative/positive test on 100 ml sample.

3.7.2 *Microbial make-up of water in canals in rural areas*

E. coli were detected at high levels in all samples (**Table 3.18**). The average concentration was higher than that found in fishponds receiving PBD effluents (**Table 3.17**) and higher than the total coliform limit set by the Vietnamese Surface Water Quality Standard (TCVN 5942-1995). That *Salmonella* spp. were detected in 30% of samples shows the high risk of contracting an infection if exposed to such waters.

Table 3.18 | Concentrations of micro-organisms in water from rural canals (n = 20).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU/100 ml	ND - 3,200	920	110	1,200	60
E. coli	CFU ml^{-1}	70 - 4,500	744	309	1,070	100
Salmonella spp.	MPN/100 ml		0.36*			30
Enterococcus spp.	CFU ml^{-1}	3 - 45	26	23	11	100
Helminth ova	no. l^{-1}	ND				

* The result based on negative/positive test on 100 ml sample.

3.7.3 *Microbial make-up of water in canals in urban area*

Phages and bacteria were detected at high levels (**Table 3.19**). The number of positive samples containing somatic coliphage and *Salmonella* spp. was rather high (90% and 40%), though no helminth ova were not detected. It is assumed that ova settle in canal's sludge.

Table 3.19 | Concentrations of micro-organisms in urban canal waters (n = 15).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU/100 ml	0 - 600	190	165	160	90
E. coli	CFU ml^{-1}	4 - 2,400	495	130	1,200	100
Salmonella spp.	MPN/100 ml		0.51*			40
Enterococcus spp.	CFU ml^{-1}	3 - 160	40	27	40	100
Helminth ova	no. l^{-1}	ND				

* The result based on negative/positive test on 100 ml sample.

3.8 **Microbial contamination of cultivated aquatic spinach**

3.8.1 *Aquatic spinach cultivated in fish ponds receiving plastic bio-digester's effluent*

Fishpond spinaches had an average DM of ~10% and were contaminated with pathogens and indicator microorganisms (**Table 3.20**). The ratio of positive samples on

different microbial parameters was lower than that in fishpond water where spinaches were collected **Table 3.17**.

Table 3.20 | Microbial concentration of spinaches cultivated in fish ponds received plastic bio-digester's effluents (n = 15).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU/ g d.w.	0 - 20	15	2.5	12	60
E. coli	CFU/ g d.w.	0 - 430	94	62	105	90
Salmonella spp.	MPN/ g d.w.		0.006*			10
Enterococcus spp.	CFU/ g d.w.	4 - 200	68	46	72	100

d.w. = dry weight

* The result based on negative/positive test on 80 – 100 g fresh sample.

3.8.2 *Aquatic spinach cultivated in urban canals*

Aquatic spinaches collected from urban canals also had an average DM ~10% and contaminated with micro-organisms (**Table 3.21**).

Table 3.21 | Microbial levels of aquatic spinaches cultivated in urban areas (n = 15).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	PFU/ g d.w.	0 - 6	2	1.6	1.6	80
E. coli	CFU/ g d.w.	0 - 1,300	230	150	255	90
Salmonella spp.	MPN/ g d.w.		0.005*			10
Enterococcus spp.	CFU/ g d.w.	5 - 560	110	29	275	100

d.w. = dry weight

* The result based on negative/positive test on 80 – 100 g fresh sample.

In summary, aquatic spinach is contaminated with faecal indicator micro-organisms. *Enterococcus* spp. were detected in all samples, *E. coli* in 90 %, somatic coliphage in

70%, and *Salmonella* spp. in 10% of samples. Average *E. coli* concentration in spinach cultivated in urban canals was more than two times higher than in fishpond receiving PBD effluent.

3.9 **Persistence of phages and bacteria in Mekong river water and on terrestrial spinach in the Mekong Delta**

3.9.1 ***Persistence of phages and bacteria in Mekong river water***

Water was sourced from the Hau River (the lower of 2 branches that constitute the Mekong River in the region) with a pH of 7.35 and turbidity of 144.6 NTU. Somatic coliphage and bacteria were reduced but none was eliminated after 12 days (**Figure 3.9**). T_{90} of somatic coliphage was 3.5 days while that of bacteria ranged from 2.0 to 2.5 days.

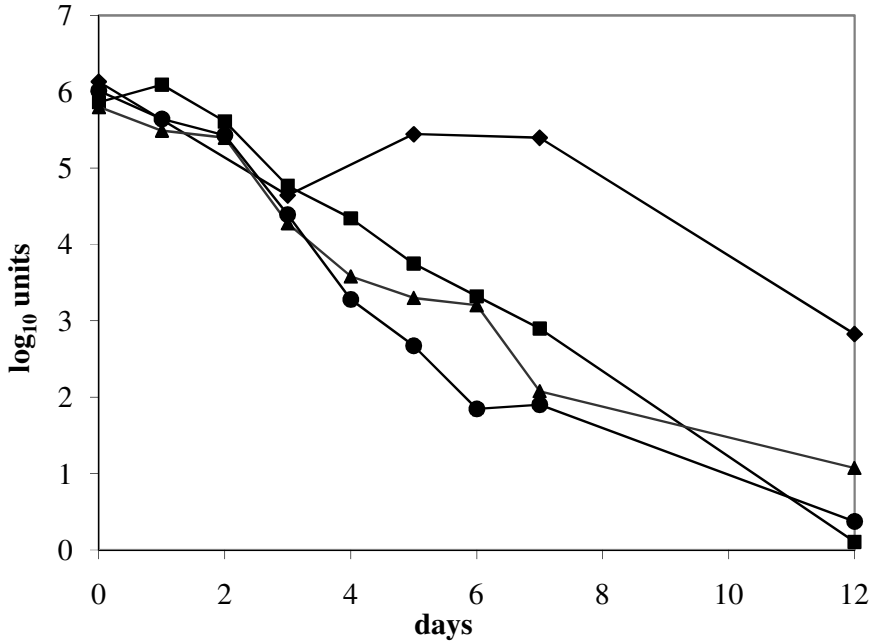


Figure 3.9 | Survival curves of somatic coliphage (♦), *E. coli* (■), *Salmonella* Senftenberg (▲) and *Enterococcus faecalis* (●) in Mekong river water.

3.9.2 *Persistence of phages and bacteria on terrestrial spinach*

Samples taken prior to and 45 minutes after spray irrigation (to allow for evaporation) show that ~2.5 ml of water attach to 100 g of spinach. This was determined by the phages and bacteria on spinach and their known microbial concentrations in irrigated waters. T_{90} of phages and bacteria between final irrigation and harvest ranged from 2.4 to 5.4 days, and is equivalent to 0.2 – 0.4 $\log_{10}$ reduction/day. In contrast to the survival tendency in the river water environment, bacteria survived longer than phages on terrestrial spinaches (**Figure 3.10**).

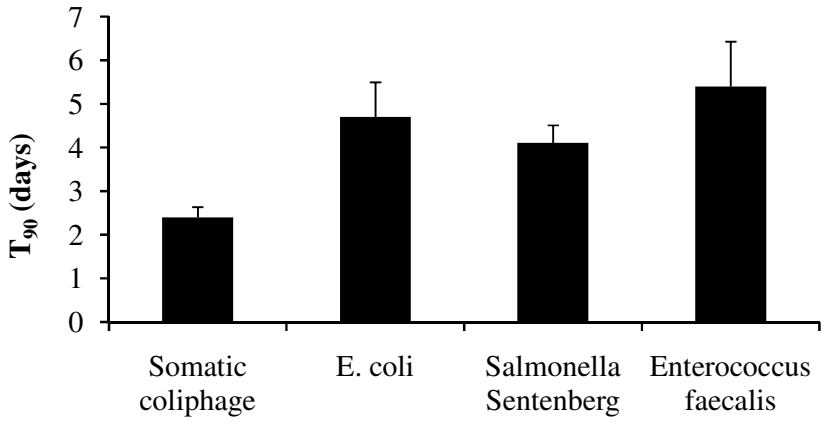


Figure 3.10 | Decimal Reduction Time (T_{90}) of tested organisms on terrestrial spinach. Error bar indicates standard deviation (n = 9).

3.10 **Quantitative Microbial Risk Assessment**

Probability of infection (P_{inf}) with helminth and Ascaris in all scenarios presented were calculated according to risk models introduced by Navarro *et al.* (2009). Results, based on the exponential risk model for helminth and Ascaris are discussed in Section 4.6.3 (**Table 4.1** and **Table 4.2**). Annual risks were compared with accepted levels calculated based on WHO guidelines on safe use of wastewater in agriculture (2006) and drinking water quality (2008): 8×10^{-4} for rotavirus, 6×10^{-4} for Salmonella and 1×10^{-4} for Ascaris (cf. Section 1.4.3).

3.10.1 *Risk of working with faecal substrates in the Mekong Delta*

P_{inf} for sewage workers and farmers exposed to faecal substrates is given in **Figure 3.11**. Results show that MD PBDs effectively reduced the risk of rotavirus infection and P_{inf} was reduced proportional to their higher treatment efficacy. Median probability with salmonellosis from PBD effluent was slightly higher than for pig slurry. This contradicts the mean, median and range of concentrations of Salmonella in pig slurry and PBD effluents (cf. Section 3.5.1 and 3.6.1) as the risk model probability distributions were used as input for exposure model parameters. Thus all PBD effluent samples positive for Salmonella supported a higher probability of infection compared to 83% of pig slurry samples positive for Salmonella with above average concentrations. Interestingly probability of helminthiasis and ascariasis were higher when exposed to PBD effluent than pig slurry, and concurs with helminth ova concentrations in pig slurry (PBD influent) and PBD effluent previously provided (**Figure 3.7**).

P_{inf} with rotavirus was about 100 times higher in untreated septage than septage sludge. In contrast P_{inf} with Salmonella was higher in septage sludge, but in both substrates P_{inf} with Salmonella was $>10^{-4}$. Probability of helminthiasis and ascariasis showed similar trends, with exposure to septage sludge being 0.2 and 0.15 and untreated septage 0.02 and 0.004 respectively. This concurs with helminth and Ascaris concentrations detected in untreated septage and septage sludge (cf. Section 3.5.2).

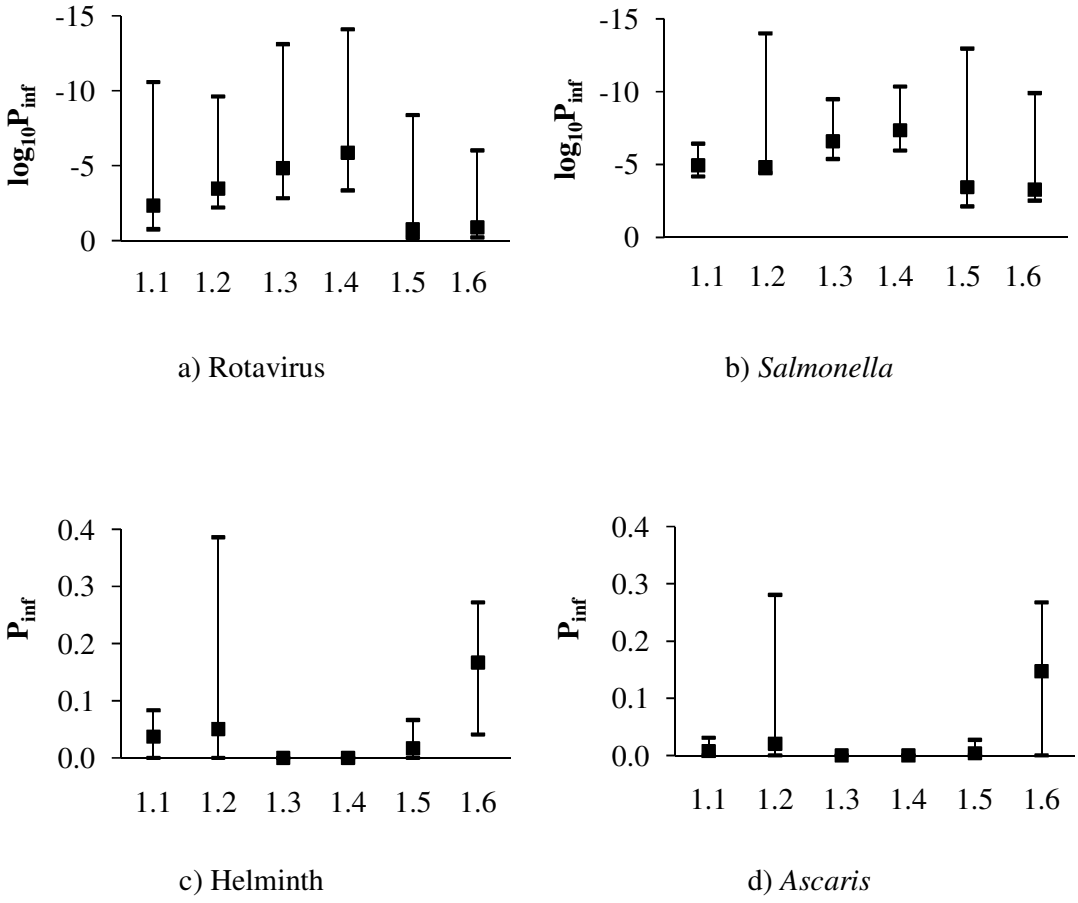


Figure 3.11 | Probability of infections for work with faecal substrates following accidental ingestion of 1 ml of faecal substrates with median values (■) and 95% confidence interval (├──┤).

Fresh pig slurry (1.1); Liquid plastic bio-digester's effluent (1.2); Improved liquid plastic bio-digester's effluent at hydraulic retention time (HRT) of 15 days (1.3); Improved liquid plastic bio-digester's effluent at HRT = 30 days (1.4); Untreated septage (1.5) and Septage sludge (1.6)

Annual infection rates (median values >0.003) for sewage workers and farmers handling slurry and septage in MD today is above WHO guidelines (**Table 3.22**). The chance of helminthiasis or rotavirus infection is nearly 1.0 but the risk of salmonellosis is much lower. Annual risk of salmonellosis and helminthiasis by incidental ingestion of pig slurry was higher than with PBD effluent (**Table 3.22**). While this seems to deny P_{inf} figures in **Figure 3.11** it is explained by the higher exposure frequency of farmers to pig slurry in MD (**Table 2.6**, Section 2.4.3). Results from standardized questionnaire survey showed that just 20% of PBD users applied effluents to crops. For handling PBD effluents with HRTs >15 days the salmonellosis risk was tolerable, but rotavirus infection was inacceptable. Helminthiasis was negligible since lengthy HRTs lowered inflow velocity meaning ova settled in the sludge and infection was only likely during sludge handling.

Table 3.22 | Annual risk of infection for work with faecal substrates (median values and 95% confidence interval in parentheses) following accidental ingestion of 1 ml of the substrates per exposure.

Scenario	Rotavirus	Salmonella	Helminth	Ascaris
1.1	0.96 (2×10^{-8} – 1)	0.008 (3×10^{-4} – 0.05)	1 (0 – 1)	1 (0 – 1)
1.2	0.03 (2×10^{-8} – 0.49)	0.002 (0 – 0.004)	0.99 (7×10^{-10} – 1)	0.88 (0.001 – 1)
1.3	0.001 (8×10^{-12} – 0.14)	3×10^{-5} (3×10^{-8} – 0.0004)	0*	0*
1.4	0.0001 (8×10^{-13} – 4×10^{-2})	5×10^{-6} (4×10^{-9} – 0.0001)	0*	0*
1.5	1 (2×10^{-6} – 1)	0.08 (0 – 0.92)	0.98 (0 – 1)	0.61 (1×10^{-8} – 1)
1.6	1 (0.0002 – 1)	0.12 (3×10^{-8} – 0.65)	1 (1 – 1)	1 (0 – 1)

0 is equivalent to < 10^{-15}

*: No risk calculation was made. Plastic bio-digesters' effluents at hydraulic retention time (HRT) ≥ 15 days are assumed to be free of helminth ova.

1.1: Fresh pig slurry; 1.2: Liquid PBD's effluent; 1.3: Improved liquid PBD's effluent at HRT = 15 days; 1.4: Improved liquid PBD's effluent at HRT = 30 days; 1.5: Untreated septage; 1.6: Septage sludge

3.10.2 *Risk associated with bathing/swimming in canals*

The annual risk of rotavirus infection and salmonellosis when immersed in canals (bathing/swimming) in both rural and urban areas were over 10^{-4} (**Table 3.23**). Helminth ova were not found in surface water so risk of helminthiasis is minimal. Yet it becomes significant if bathers are near STs and PBD effluent. If just 1 ml of effluent is assumed to be ingested the median annual risk of helminthiasis is 1.0.

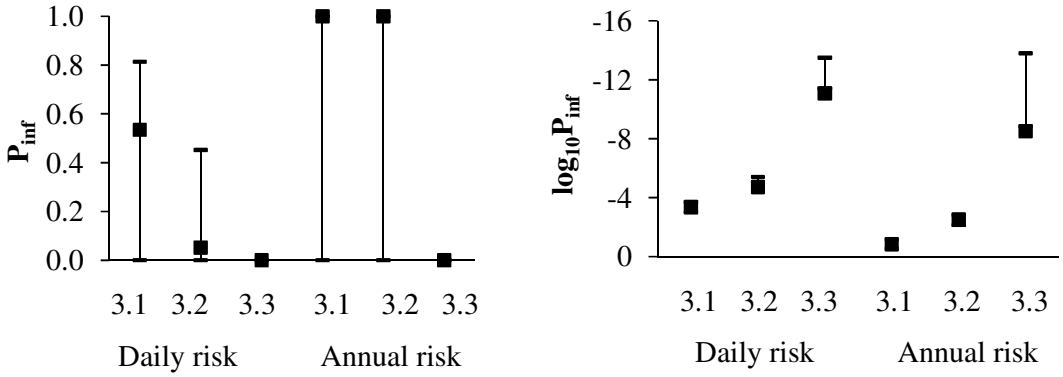
Table 3.23 | Median values of infection probability of bathing/swimming in the canals in rural areas (2.1), in urban areas (2.2). Numbers in brackets represent 95% confidence interval.

Scenario	Rotavirus		*Salmonella*	
	Risk per exposure	Annual risk	Risk per exposure	Annual risk
2.1	0.06 (2×10^{-10} – 0.43)	1 (7×10^{-8} – 1)	6×10^{-6} (5×10^{-6} – 8×10^{-6})	0.002 (0.002 – 0.003)
2.2	0.08 (0 – 0.18)	1 (0 – 1)	9×10^{-6} (7×10^{-6} – 1×10^{-5})	0.0009 (0.0006 – 0.001)

0 is equivalent to $< 10^{-15}$

3.10.3 *Risk of drinking canal water in rural areas*

Drinking canal water mainly occurs in rural areas where there are poor or no water supply systems, so data relate to this zone. Three treatments were assessed: untreated canal water, canal water + alum flocculation, and canal water + alum flocculation + boiling. Flocculation of canal water had a positive health effect (**Figure 3.12**) and has been well documented (Reller *et al.* 2003, Crump *et al.* 2005). Yet corresponding risks of salmonellosis and rotavirus infection are 1 and 0.003 so this treatment is not advised for MD. Boiling after flocculating is cheap and easy, reducing the microbial load of drinking water and so better protects people against gastroenteritis. Risks linked to drinking boiled water were negligible ($<10^{-9}$).



a) Rotavirus

b) *Salmonella*

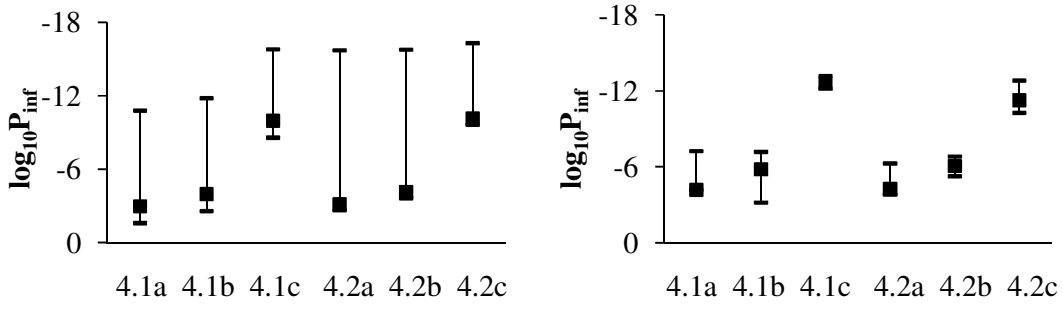
Figure 3.12 | Infection probability of drinking untreated canal water (3.1), after alum flocculation (3.2), and after flocculation plus boiling (3.3); median values (■) and 95% confidence interval (├──┤).

3.10.4 *Risk associated with eating spinach*

Most vegetables need washing or cooking before eating. In MD rural areas untreated or flocculated water is used. In urban areas it is tap water. 'Without proper washing' (cited in tables) means 1) washing with untreated water or 2) no washing at all. Spinach is eaten raw or cooked and both treatments were taken into risk analyses.

Aquatic spinach: spinach cultivated in ponds and canals

Probability of rotavirus infection and salmonellosis was not markedly different if spinach originated in fishponds receiving PBD effluents or urban canals (**Figure 3.13**). Washing spinach with alum-flocculated water or tap water reduced incidence by ~1 $\log_{10}$ of P_{inf} compared to 'without proper washing' scenarios. Eating cooked spinach showed negligible P_{inf} ($<10^{-11}$) per portion.



a) Rotavirus b) *Salmonella*

Figure 3.13 | Probability of infection per portion of aquatic spinach receiving plastic bio-digester effluent (4.1), in urban area canals (4.2) with median values (■) and 95% confidence interval (├──┤).

a) Spinach is consumed raw without proper washing

b) Spinach is consumed raw after washing with alum-flocculated water or tap water

c) Spinach is consumed after cooking

Annual risk analyses show that eating raw aquatic spinach leads a high chance of contracting an infection. For consumption of spinach washed with flocculated or tap water, annual risk of infection with rotavirus was about 1,000 times higher than acceptable risk level while that with *Salmonella* was at acceptable values (**Table 3.24**). Generally aquatic spinaches cultivated in fishponds or in urban canals were not recommended for eating raw. Median annual risks from cooked spinach consumption for both rotavirus and *Salmonella* infections were low ($\leq 10^{-6}$).

Annual risk analyses show that eating raw aquatic spinach promotes infection. Washing spinach in flocculated or tap water reduced annual risks. However, risk of rotavirus infection was about 1,000 times over accepted norms while salmonellosis risk was tolerable (**Table 3.24**). Median annual risk of contracting rotavirus infection and salmonellosis from cooked spinach was low ($\leq 10^{-6}$).

Table 3.24 | Median annual risk of infection from consumption of aquatic spinach receiving plastic bio-digester effluents (4.1), in urban area canals (4.2). Numbers in brackets represent 95% confidence interval.

Scenario	Rotavirus	*Salmonella*
4.1 a	0.1 (2×10^{-9} – 0.93)	0.0007 (0.0004 – 0.0009)
b	0.01 (2×10^{-10} – 0.25)	7×10^{-5} (2×10^{-5} – 9×10^{-5})
c	1×10^{-8} (0 – 3×10^{-7})	7×10^{-11} (4×10^{-11} – 9×10^{-10})
4.2 a	0.07 (0 – 0.22)	0.0006 (0.0003 – 0.0009)
b	0.007 (0 – 0.02)	6×10^{-5} (4×10^{-6} – 0.0001)
c	7×10^{-9} (0 – 3×10^{-8})	6×10^{-11} (8×10^{-11} – 2×10^{-10})

a) Spinach is consumed raw without proper washing

b) Spinach is consumed raw after washing with alum-flocculated water or tap water

c) Spinach is consumed after cooking

Spinach cultivated on fields (terrestrial spinach) fertilised by plastic bio-digester's effluent

P_{inf} from consumption of spinach applied with PBD's effluent at HRT of 30 days was 1 $\log_{10}$ unit lower than that at HRT of 15 days and 2 $\log_{10}$ unit lower than eating spinach applied with PBD effluent in MD (**Table 3.25**). This shows that increased HRTs of PBDs decrease the risk of pathogenic infection. P_{inf} with Salmonella was much lower than other concerned pathogens while P_{inf} with helminth were high if spinach was eaten raw.

Time between final irrigation and harvest also influences crop microbial quality. Results show if spinach was applied with PBD effluent at HRT ≥ 15 days and the time between final irrigation and harvest was ≥3 days and spinach was washed in clean water it could be eaten raw (**Table 3.26**). If elapsed time was 3 days the infection risk was about 1 $\log_{10}$ higher than if 7 days had passed (**Table 3.27**).

Infection was reduced if spinach was washed beforehand. Probability of helminthiasis and ascariasis per portion was reduced by 1 $\log_{10}$ (**Table 3.25**) and annual risk decreased by >0.4 $\log_{10}$ (**Table 3.26**). With high pathogen levels in irrigated water, a one-$\log_{10}$ reduction of pathogens on spinach by washing is not significant. Cooking also reduces infection risk. Risks associated with eating cooked spinach were negligible, even if harvested right after irrigation (data not shown) or the time between final irrigation and harvest was 3 day or 7 days (**Table 3.26**; **Table 3.27**).

Table 3.25 | Median risk of infection per one portion of terrestrial spinach fertilised with plastic bio-digester's effluent (4.3), improved effluent at hydraulic retention time (HRT) of 15 days (4.4), and improved effluent at HRT of 30 days (4.5). Numbers in brackets represents 95% confidence interval. **Time elapsed between final irrigation and harvest is 3 days**. No reduction of helminth ova was assumed in the meantime.

Scenario		Rotavirus	*Salmonella*	Helminth	*Ascaris*
4.3	a	5×10^{-5} ($4 \times 10^{-11} – 0.001$)	8×10^{-6} ($0 – 2 \times 10^{-5}$)	0.10 ($2 \times 10^{-11} – 0.45$)	0.05 ($2 \times 10^{-5} – 0.35$)
	b	5×10^{-6} ($4 \times 10^{-12} – 0.0001$)	8×10^{-7} ($0 – 2 \times 10^{-6}$)	0.02 ($2 \times 10^{-12} – 0.3$)	0.006 ($2 \times 10^{-6} – 0.19$)
	c	5×10^{-12} ($0 – 1 \times 10^{-10}$)	8×10^{-13} ($0 – 2 \times 10^{-12}$)	2×10^{-8} ($0 – 3 \times 10^{-6}$)	7×10^{-9} ($0 – 6 \times 10^{-7}$)
4.4	a	2×10^{-6} ($1 \times 10^{-14} – 0.0002$)	1×10^{-7} ($1 \times 10^{-10} – 2 \times 10^{-6}$)	0*	0*
	b	2×10^{-7} ($0 – 2 \times 10^{-5}$)	1×10^{-8} ($1 \times 10^{-11} – 2 \times 10^{-7}$)	0*	0*
	c	2×10^{-13} ($0 – 2 \times 10^{-11}$)	1×10^{-12} ($0 – 2 \times 10^{-11}$)	0*	0*
4.5	a	2×10^{-7} ($0 – 7 \times 10^{-5}$)	2×10^{-8} ($3 \times 10^{-11} – 6 \times 10^{-7}$)	0*	0*
	b	2×10^{-8} ($0 – 7 \times 10^{-6}$)	2×10^{-9} ($2 \times 10^{-12} – 6 \times 10^{-8}$)	0*	0*
	c	2×10^{-14} ($0 – 7 \times 10^{-12}$)	2×10^{-13} ($0 – 6 \times 10^{-12}$)	0*	0*

0 is equivalent to $< 10^{-15}$

*: No risk calculation was made. PBDs' effluents at HRT ≥ 15 days are assumed to be free of helminth ova.

a) Spinach is consumed raw without proper washing

b) Spinach is consumed raw after washing with alum-flocculated water or tap water

c) Spinach is consumed after cooking

Table 3.26 | Annual risk of infection (median values and 95% confidence interval in parentheses) from consumption of spinach fertilised with plastic bio-digester's effluent (4.3), improved effluent at hydraulic retention time (HRT) of 15 days (4.4), and improved effluent at HRT of 30 days (4.5). **Time elapsed between final irrigation and harvest is 3 days**. No reduction of helminth ova was assumed in the meantime.

Scenario		Rotavirus	*Salmonella*	Helminth	*Ascaris*
4.3	a	0.01 (0 – 0.11)	0.0008 (0 – 0.002)	1 (0.002 – 1)	0.99 (2×10^{-9} – 1)
	b	0.0005 (4×10^{-10} – 0.01)	8×10^{-5} (0 – 0.0003)	0.82 (0.0004 – 1)	0.48 (2×10^{-10} – 1)
	c	5×10^{-10} ($0 - 1 \times 10^{-8}$)	8×10^{-11} ($0 - 3 \times 10^{-10}$)	2×10^{-6} (0 – 0.0003)	7×10^{-7} ($0 - 7 \times 10^{-5}$)
4.4	a	0.0002 (1×10^{-12} – 0.02)	1×10^{-5} (1×10^{-8} – 0.0002)	0*	0*
	b	2×10^{-5} (1×10^{-13} – 0.003)	1×10^{-6} ($1 \times 10^{-9} - 2 \times 10^{-5}$)	0*	0*
	c	2×10^{-11} ($0 - 3 \times 10^{-9}$)	1×10^{-12} ($0 - 2 \times 10^{-9}$)	0*	0*
4.5	a	2×10^{-5} ($1 \times 10^{-13} - 0.007$)	2×10^{-6} ($3 \times 10^{-9} - 6 \times 10^{-5}$)	0*	0*
	b	2×10^{-6} ($1 \times 10^{-14} - 0.0008$)	2×10^{-7} ($2 \times 10^{-10} - 6 \times 10^{-6}$)	0*	0*
	c	2×10^{-12} ($0 - 7 \times 10^{-10}$)	2×10^{-13} ($0 - 6 \times 10^{-10}$)	0*	0*

0 is equivalent to $< 10^{-15}$

*: No risk calculation was made. PBD effluents at HRT ≥ 15 days are assumed to be free of helminth ova.

a) Spinach is consumed raw without proper washing

b) Spinach is consumed raw after washing with flocculated water/tap water

c) Spinach is consumed after cooking

Table 3.27 | Annual risk of infection (median values and 95% confidence interval in parentheses) from consumption of spinach fertilised with plastic bio-digester's effluent (4.3), improved effluent at hydraulic retention time (HRT) of 15 days (4.4), and improved effluent at HRT of 30 days (4.5). **Time elapsed between final irrigation and harvest is 7 days**. No reduction of helminth ova was assumed in the meantime.

Scenario		Rotavirus	Salmonella	Helminth	Ascaris
4.3	a	0.0001 (9×10^{-11} – 0.002)	8×10^{-5} (0 – 0.0003)	1 (2×10^{-3} – 1)	0.99 (2×10^{-9} – 1)
	b	1×10^{-5} (8×10^{-12} – 0.0002)	8×10^{-6} (0 – 3×10^{-5})	0.82 (4×10^{-4} – 1)	0.48 (2×10^{-10} – 1)
	c	1×10^{-11} (0 – 3×10^{-10})	8×10^{-12} (0 – 3×10^{-11})	2×10^{-6} (0 – 3×10^{-4})	7×10^{-7} (0 – 7×10^{-5})
4.4	a	5×10^{-6} (3×10^{-14} – 0.0005)	1×10^{-6} (4×10^{-9} – 3×10^{-5})	0*	0*
	b	5×10^{-7} (0 – 5×10^{-5})	1×10^{-7} (1×10^{-10} – 3×10^{-6})	0*	0*
	c	5×10^{-13} (0 – 5×10^{-11})	1×10^{-13} (0 - 3×10^{-10})	0*	0*
4.5	a	5×10^{-7} (0 – 0.0002)	2×10^{-7} (3×10^{-10} – 6×10^{-6})	0*	0*
	b	4×10^{-8} (0 – 2×10^{-5})	3×10^{-8} (10^{-11} – 6×10^{-7})	0*	0*
	c	5×10^{-14} (0 – 2×10^{-11})	3×10^{-14} (0 – 6×10^{-11})	0*	0*

0 is equivalent to < 10^{-15}

*: No risk calculation was made. PBDs' effluents at HRT ≥ 15 days are assumed to be free of helminth ova.

a) Spinach is consumed raw without proper washing

b) Spinach is consumed raw after washing with flocculated water/tap water

c) Spinach is consumed after cooking

3.10.5 *Comparative risk assessment*

For an overview of infection risk in MD exposure scenarios can be ranked. **Table 3.28** presents annual risk rating of each exposure scenario. The scenarios ranked as being of highest concern were working with faecal substrate and eating raw terrestrial spinach fertilized by PBD's effluent. Thus those who expose directly to faecal substrates such as sewage workers and farmers handling pig slurry are at most risk of infection. The risk rating also shows that heath protection measures (wastewater treatment over PBD, surface water treatment, washing/cooking spinach ...) reduced risks. Compared to the PBD's effluent today in MD, improved effluent at HRT $\geq$ 15 days minimized helminthiasis and lowered significantly rotavirus infection and salmonellosis (**Table 3.28**).

Risk estimates for total MD population differ from exposure scenarios. Risk factors for gastroenteritis in MD were taken into account, with typhoid fever being the exemplar pathogen as it is the best documented. Annual risk of salmonellosis via accidental ingestion was high and ranged from septage sludge (0.12), untreated surface water (0.14) and untreated septage (0.08) scenarios (**Table 3.29**). Yet the potential for salmonellosis in total MD population differed: drinking untreated surface water, to eating raw or poorly washed spinach, and drinking alum-flocculated water due largely to different ratios of people exposed in different the scenarios (**Table 3.29**).

Table 3.28 | Rating of annual median risks following exposure scenarios.

Scenarios	Rotavirus infection	Salmonellosis	Helminthiasis	Ascariasis	**Sum**
1. Working exposure					
1.1 Fresh pig slurry	4	2	4	4	**14**
1.2 Liquid effluent of PBDs	3	2	4	4	**13**
1.3 Liquid effluent from PBD HRT = 15 days	1	0	0[a]	0[a]	**1**
1.4 Liquid effluent from PBD HRT = 30 days	1	0	0[a]	0[a]	**1**
1.5 Untreated septage	4	3	4	4	**15**
1.6 Septage sludge	4	4	4	4	**16**
2. Bathing/swimming					
2.1 in rural canals	4	2	0[b]	0[b]	**6**
2.2 in urban canals	4	1	0[b]	0[b]	**5**
3.Drinking surface water					
3.1 untreated	4	4	0	0	**8**
3.2 after flocculation	4	2	0	0	**6**
3.3 after flocculation + boiling	0	0	0	0	**0**
4. Consumption of spinach					
4.1 cultivated in fish ponds receiving PBD's effluent					
a) raw without proper washing	4	1	0	0	**5**
b) raw after washing with flocculated or tap water	2	0	0	0	**2**
c) cooked	0	0	0	0	**0**

Table 3.28 (continued)

Scenarios	Rotavirus infection	Salmonellosis	Helminthiasis	Ascariasis	**Sum**
4. Consumption of spinach (continued)					
4.2 cultivated in urban canals					
a) raw without proper washing	3	1	0	0	**4**
b) raw after washing with flocculated or tap water	2	0	0	0	**2**
c) cooked	0	0	0	0	**0**
4.3* cultivated on fields fertilised by PBD's effluent					
a) raw without proper washing	3	1	4	4	**12**
b) raw after washing with flocculated or tap water	1	0	4	4	**9**
c) cooked	0	0	0	0	**0**
4.4* cultivated on fields fertilised by PBD's effluent at HRT=15d					
a) raw without proper washing	1	0	0[a]	0[a]	**1**
b) raw after washing with flocculated or tap water	0	0	0[a]	0[a]	**0**
c) cooked	0	0	0[a]	0[a]	**0**

Table 3.28 (continued)

Scenarios	Rotavirus infection	Salmonellosis	Helminthiasis	Ascariasis	**Sum**
4. Consumption of spinach (continued)					
4.5* cultivated on fields fertilised by PBD's effluent at HRT=30d					
a) raw without proper washing	0	0	0[a]	0[a]	**0**
b) raw after washing with flocculated or tap water	0	0	0[a]	0[a]	**0**
c) cooked	0	0	0[a]	0[a]	**0**

*: time elapsed between final irrigation and harvest is 3 days

[a]: No risk calculation was made. Plastic bio-digester's effluent at hydraulic retention time ≥ 15 days are assumed free of helminth ova.

[b]: No helminth ova were found in surface water samples. Yet risk of infection becomes significant if bathers are near septic tanks and plastic bio-digester's effluent.

The numerical value assigned to each category is used to determine the risk rating of reference pathogens in each exposure scenario as follow:

0: acceptable, <0.0001

1: low, 0.0001 to <0.001

2: medium, 0.001 to <0.01

3: high, 0.01 to <0.1

4: very high, 0.1 to 1

Table 3.29 | Estimated annual risk of *Salmonella* infection in the Vietnam's Mekong Delta (MD).

Exposure scenario	Estimated ratios of MD population exposed[a]	Risks for the exposed	Annual risk for MD's population
Accidental ingestion of pig slurry	5%	8×10^{-3}	4×10^{-4}
Accidental ingestion of PBD's effluents	0.01%	2×10^{-3}	2×10^{-7}
Accidental ingestion of untreated septage	0.003%	8×10^{-2}	2×10^{-6}
Accidental ingestion of septage sludge	0.003%	$\mathbf{1.2 \times 10^{-1}}$	$\mathbf{4 \times 10^{-6}}$
Bath/swim in urban canals	0.2%	2×10^{-3}	4×10^{-6}
Bath/swim in rural canals	2%	9×10^{-4}	2×10^{-5}
Drinking untreated surface water	2%	$\mathbf{1.4 \times 10^{-1}}$	$\mathbf{3 \times 10^{-3}}$
Drinking flocculated surface water	10%	3×10^{-3}	3×10^{-4}
Eating raw aquatic spinach without proper washing	40%	7×10^{-4}	3×10^{-4}
Eating raw aquatic spinach with proper washing	20%	7×10^{-5}	1×10^{-5}
Total			$\mathbf{4 \times 10^{-3}}$

[a] Based on Lin *et al.* (2000), Figuié (2003), General Statistics Office (2006b), Danh (2008) and Few *et al.* (2010)

3.10.6 *Summary*

In this study the risks of pathogen infection were recorded in descending order from helminth to rotavirus and Salmonella. In MD it is sewage workers who are most at risk of infection due to their constant exposure to human faecal matter. Yet other exposure scenarios and other people are involved. Incidental ingestion of slurries, eating raw spinach, drinking untreated surface water, and bathing/swimming in canals constitute chronic exposure scenarios for the population in MD. All above cited scenarios were found to be higher than WHO guidelines for the safe use of wastewater in agriculture (2006a) and drinking water quality (2008).

Barriers can reduce the risk of infection and include wastewater treatment (eg. PBDs), due time between final crop irrigation and harvest, personal hygiene, treatment of water (alum flocculation, boiling) before consumption, and cooking food. Among these PBDs are considered a good for their user benefits (cooking gas, effluents that act as manure for pond and field), as well as for the environment. Yet probability of salmonellosis and helminthiasis is higher per exposure to PBD effluent than pig slurry, and much higher than the acceptable risk levels. Risks are reduced when PBDs run at a HRT of 15 and 30 days as effluent is assumed to be free of helminth ova. Due to the high pathogen load of surface water this is only potable when boiled. Nor is aquatic spinach safe to eat unless cooked. Spinaches irrigated with improved PBD effluent (HRT $\geq$ 15 days) can be eaten raw, but only when the time between final irrigation and harvest is long enough or spinaches are washed properly before consumption.

4 DISCUSSION

4.1 Treatment efficacy of pathogens and indicator microorganisms in plastic bio-digesters and septic tanks

4.1.1 *Situation in the Mekong Delta, Vietnam*

The low reduction rates of phages and bacteria via PBDs in MD concur with previous results showing that the $\log_{10}$ reduction of PBDs at a HRT of 3 days was less than 1.6 (Section 3.1.3). Of PBDs investigated by Nuber and Tien (2008) in MD 70% showed HRTs of less than six days and the shortest HRT was 1.83 days. The high levels of *E. coli* found in effluents of PBDs may be explained by 1) maintenance failure (e.g. short HRT, too much accumulated sludge in reactors); 2) operation conditions (e.g. the whirl flow of influent go directly to the effluent); and 3) growth within the reactor. In addition, the detection frequency of *Salmonella* spp. in PBD effluent samples was higher than that of influents. It may be due to the growth of this bacterium in anaerobic reactors (Ward *et al.* 1999, Gerardi 2003).

Helminth ova as well as DM concentrations found in effluents were higher than those in influents in more than 50% of PBDs. However no correlation between DM and ova concentration was found in all effluent samples. This can be explained by the different operating conditions of PBDs leading to different solid content in effluents. Of course helminth ova in influents depend on the helminth load of the source pigs.

Most helminth ova varieties were detected more frequently in effluents than in influents. This indicates that many ova were washed out due influent velocity and that the operation of only a small proportion of PBDs allowed helminth ova to settle at the base. Thus influent flow should be restricted so ova can settle in sludge and so be inactivated.

4.1.2 *Microbial treatment efficacy from pilot study*

Plastic bio-digesters

That bacteria were more resistant to mesophilic anaerobic treatment of manure than phages is supported by Lund *et al.* (1996). In contrast Gessel *et al.* (2004) showed that somatic coliphages were more persistent than *Salmonella anatum* and faecal coliforms in surface soil treated with liquid pig manure. That may be due to the different

environmental conditions of the trial. It is documented that several pathogenic and indicator bacteria are very persistent and may even multiply in the biogas digester environment (Gerardi 2003).

The reductions in the reactor do not correlate with total reduction efficacy of tested organisms because of the accumulated solids at the digester base. Some pathogens and indicator microorganisms (e.g. helminth eggs) can accumulate in the sludge. However, the concentration of the tested organisms in the sludge at the trial's end was not significantly different from that observed in the liquid output. This concurs with Kearney *et al.* (1993b) who reported that the concentration of *E. coli* and *Salmonella* Typhimurium in separated solid effluents were slightly higher than in liquid effluents.

The lag phase of *Ascaris suum* ova noticed in batch experiment is corroborated by Nordin (2007) and Pecson (2007). According to Fuchs (2006) HRT of the small-scale biogas plants in Vietnam was from 1.2 to 20.4 days, shorter than this lag phase. This shows that the HRT is not the significant factor affecting the survival of helminth ova in tropical PBDs. Hence the operation should be adjusted so that helminth ova can settle in the accumulated sludge at the digester base. In regular feeding experiment, a small portion of helminth ova survived much longer than the rest. This implies that it takes over one year to eliminate 100% of helminth ova in faecal sludge in tropical anaerobic conditions.

Septic tanks

Phages and bacteria were slightly reduced during treatment showing that STs function more like a storage tank than a treatment system. Biogas production efficiency was higher than that of anaerobic digestion of pig slurries under similar conditions. This may be due the make-up of two substrates; compared to human faeces pig slurries contain more cellulose, which is difficult to degrade in a short time.

4.2 **Factors affecting performance and microbial reduction in tropical anaerobic digestion**

4.2.1 ***Effect of operational parameters on plastic bio-digester performance***

The pH of effluents from a HRT of 3 days was lower than that from HRTs of 15 and 30 days, which were optimal for the biogas process (FNR 2006). The TIC was positively affected by a longer HRT. At high TIC values, the fermenter may buffer more organic acids produced in the acetogenic and acetic phase of digestion. High TIC values indicate high process stability inside the reactor and thereby also a potential to add other organic material to the digester: for example, if the pig manure source becomes deficient.

COD treatment efficacy was markedly high at HRTs of 15 and 30 days. A HRT of 15 days is acceptable for COD treatment in tropical PBDs. Yet these values do not correlate with real COD treatments owing to the accumulated solids at the digester base. Besides the PBD design, COD treatment efficacy depends on digester operation and maintenance. With the same HRT, effluent COD values may differ as a result of the velocity of influent flow, suggesting that the speed of input flow should be reduced when it enters the digester.

With a fixed daily input of fresh manure, reactors with longer HRTs produced more biogas. A similar trend was described by Thy *et al.* (2005). The higher gas yields at longer HRT may be due to: (1) prolonged digestion time; and (2) a lower velocity leading to increased sedimentation. The fact that sediment can contribute to biogas production is a point supported by Nuber and Tien (2008). In addition, by the end of the trial the accumulated sludge was more homogeneous in the reactors at longer HRTs.

The higher TIC of the digester's substrate keeps the pH values stable during the anaerobic treatment. This is important for the methanogenesis phase since low pH (<6.5) and high level of VFA have a strongly toxic effect on methanogenic bacteria in the digester (FEC Services 2003). At HRT of 3 days pH and TIC were low while at HRT of 15 and 30 days reactors showed optimal pH for gas production. At low HRTs the methanogenic population is flushed out of the digester because of its long reproduction time, reported to be above 5 days (FNR 2006). As a consequence average

methane production per reactor per day increased significantly from HRT of 3 days (0.2 l) to 15 days (0.4 l) and 30 days (0.5 l).

4.2.2 *Factors affecting microbial reduction in tropical anaerobic digestion*

Initial concentration and substrate type

Initial populations affect reduction rates of most microorganisms tested, and most likely due to competition for nutrients. Thus competition is a factor in reducing the viability of enteric bacteria during mesophilic digestion (Smith *et al.* 2005). *E. coli* and *Salmonella* Senftenberg survived longer in swine slurry than in cattle slurry. This is supported by Olsen and Larsen (1987) who found a sizeable difference in T_{90} values for *Salmonella* Typhimurium and *E. coli* serovar O157 in these slurries, although later research has not cited this (Kumar *et al.* 1999, Côté *et al.* 2006). The reduction rate of *Enterococcus faecalis* was not influenced by slurry type, and supported by Olsen and Larsen (1987).

T_{90} values of *E. coli*, *Enterococcus faecalis* and *Salmonella* Typhimurium in animal slurries with high initial concentrations at 35^{o}C were similar in Olsen and Larsen (1987). That *Enterococcus faecalis* survived noticeably longer than *E.coli* and *Salmonella typhi* is supported by Kumar *et al*. (1999). Literature on the survival of somatic coliphages in slurries at mesophilic anaerobic conditions is scarce.

The reduction of *E. coli* is influenced by the strain used. The *E. coli* population from laboratory strain died off after only one day of anaerobic treatment. A similar trend for coliforms was described at 35^{o}C by Olsen and Larsen (1987); at 6-8^{o}C and 20^{o}C by Larsen and Munch (1986). In contrast Abdul and Lloyd (1985) observed a longer survival of antibiotic-resistant strain of *E. coli* compared to sensitive isolates during anaerobic digestion of pig slurry at 37^{o}C. In this experiment indigenous *E. coli* in swine and cattle slurries showed the same survival rate as the strains isolated from fresh slurries. The results emphasised the importance of using *E. coli* strains, or suitably sensitive organisms, indigenous to experimental substrates to assess the efficacy of treatment conditions on pathogen removal rates. Other bacteria showed no significant difference in reduction rates between laboratory strains and isolated ones.

Hydraulic retention time

A HRT of 3 days showed a very low reduction of organisms tested, especially for *E. coli*. Kobayashi *et al.* (2003) found no significant difference between the concentrations of *E. coli* at the input and output ends of PBDs. Rechenburg *et al.* (2007) also concluded that indicator bacteria are only slightly reduced in PBDs. The high populations of *E. coli* found in PBD effluents in the Mekong Delta are reflected in the results obtained from the reactors with a HRT of ≤ 3 days. When the HRT of the digester is 3 days or less, the $\log_{10}$ reduction of *E. coli* is less than 0.5, while biogas is still produced due to the accumulated sludge at the digester's base. Hence the use of PBDs in such cases does not improve environmental hygiene and poses a health risk if the effluent is not further treated.

Several factors can be related to the higher reduction of pathogen and indicator microorganisms with a longer HRT. One factor is the high level of TIC that, according to Park and Diez-Gonzales (2003) inactivates bacterial pathogens. Another factor is that the longer HRTs result in less easily biodegradable substrates, which affect the survival of facultative anaerobes. Even if the hygienic microbiological quality of PBDs' effluents increases with longer HRTs, a HRT of 30 days was not enough for the effluent to meet WHO (2006a) guideline standards for restricted irrigation, which stipulates a reduction in *E. coli* by 4 log units. If effluent is to be used for food production, other safety barriers will be needed. It is not recommended that effluent is discharged directly to surface water, or applied to vegetables that are consumed raw. Therefore, additional health protection measures, such as allowing substantial time to lapse between final irrigation and harvest, and washing vegetables with clean water prior to consumption, should be applied. Thus the required hygiene levels can be reached, especially for the effluent from reactors with long HRTs.

From this bench-scale study it can be inferred that the reduction of pathogens common to domestic PBDs in tropical regions increases with HRT. Long HRTs, or factors related to longer HRTs such as high TIC, play a vital role in pathogen reduction, while yielding more gas production as well as improving hygiene for PBD users and the general population more broadly. However effluent quality in terms of microbiological hygiene requirements is not good enough to be discharged directly into surface water or

applied to crops that are eaten raw, even with a HRT of 30 days. A HRT of at least 15 days is recommended to increase gas yield and achieve a higher pathogen reduction. In sensitive areas, for example where surface water is used for domestic purposes, a HRT of at least 30 days should be applied.

Volatile fatty acid

The results show that the anaerobic process has its own optimal operation parameters. In this trial reactors could not handle high VFA concentrations (> 2.5 g l^{-1}). Another parameter of interest is EC. The estimated Na^+ concentration (originating from $NaHCO_3$ used for neutralising) in the VFA amendment reactor at the end of the second week was still below the level causing inhibition (Gerardi 2003). Yet EC values in effluents from VFA amendment reactors were much higher than the control from the second week of VFA addition (**Figure 3.4**). It shows that VFA > 2.5 g l^{-1} and EC > 3mS/m inhibits reactor processes. Thus augmenting VFA concentrations to over 2.5 g l^{-1} in a plastic bio-digester in the tropics is not feasible since: (1) high levels of VFA cause a dramatic drop in pH; (2) if pH adjustment is needed then the raised EC values cause inhibition.

High level of VFA may influence the reduction of methanogenic bacteria more than that of the bacteria tested. All bacteria tested are of the Gram-negative group while methane-forming bacteria belong to both Gram-negative and Gram-positive groups. The anti-bacterial effect of VFA on bacteria was more pronounced in Gram-positive bacterium (Raftari *et al.* 2009, Skrivanova *et al.* 2006). The higher sensitivity of Gram-positive bacteria to VFA can be related to the structure of the cell wall in this group (Raftari *et al.* 2009). Gram-negative cell walls have a more complicated structure than do those of gram-positive organisms. The outer membrane serves as a permeability barrier to very large or hydrophobic molecules (Paustian and Roberts 2006).

The effect of VFA on pathogen reduction depends on the stage-based design of anaerobic treatment system. In a well functioning combined-stage reactor, VFA concentrations can reach a high level while pH stays in the optimal range for biogas production (Boe and Angelidaki 2009, Hansen *et al.* 1998, Kearney *et al.* 1993b). In this trial the reduction of indicators and pathogen tested was not influenced by VFA. The reduction rate was higher in the slightly acidic pH in the control reactors. This finding is supported by Hill (2003) who found that the effect of VFA on pathogen inactivation

was more pronounced at acidic pH values. Kearney *et al.* (1993b) found no correlation between VFA concentration and pathogen reduction. However this finding is based on a multi-stage anaerobic digestion process. In the two-phase system, hydrolysis and acid forming is encouraged in the first or acid phase while methane production occurs in the second phase and in a separate reactor. Thus in the first phase VFAs are produced and accumulated causing a decrease in pH. High concentrations of VFA and low levels of pH cause a greater reduction of pathogens (Kunte *et al.* 1998). However acceptable enzymatic activity of acid-forming bacteria occurs above pH 5.0 (Gerardi 2003). In contrast pH value in the second phase (methanogenesis) should be neutral to favour the activity of methanogenic bacteria. Thus pathogen reduction is expected to be more efficient in two-stage reactors.

Batch versus regular feeding trials

The greater decline of *E. coli* and *Salmonella* spp. in batch versus continuous anaerobic digestion is supported by Kearney *et al*. (1993a). This may be due to the growth of these bacteria in the reactors. Growth of faecal coliforms and *Salmonella* spp. in mesophilic anaerobic digester sludge after pasteurization has been reported (Ward *et al.* 1999). The operational conditions of continuous reactors may favour survival of enterobacteriaceae as these are facultative anaerobes. The bacteria were provided with fresh nutrients and a small amount of oxygen. The greater inactivation rate of Coliphages in batch reactors can be explained by a lack of their bacteria host in the substrate.

4.3 **Microbiological characteristics of faecal substrates in the Mekong Delta**

4.3.1 ***Pig slurry***

Many helminth ova species were found in pig slurries. Pigs in MD were shown to be infected with helminth species at a high rate and six helminth ova species were previously reported (Hung *et al.* 2000, Yoshihara *et al.* 1999). Yet this may reflect the fact that PBD slurries are a combination of many animal's manure. *Ascaris suum* and *Taenia* spp., both responsible for zoonotic diseases, were frequently detected in samples and present a health risk to people (Olson and Guselle 2000). *Fasciolopsis buski* was also found and its presence may be due to spinaches that often supplement a pig's diet. Yoshihara *et al.* (1999) found that spinach is a habitat for the intermediate host of *F.*

buski. Since aquatic spinach is often eaten raw it is possible that people will contract this fluke infection.

MD farmers do not handle pig slurries, except cleaning pigsties. Slurries go to PBDs or surface water. Yet this latter is used to bath, wash, cook and drink. Thus untreated pig slurries should not be discharged to surface water. That their use is encouraged in PBDs to produce cooking gas is a positive solution for a region with typical water-based life. However, PBD's functionabiliy should be taken in account to achieve an optimal pathogen reduction and gas production.

4.3.2 *Untreated septage*

When the ST is full, untreated septage often flows directly to surface water bodies, and contaminates them. The average concentration of *E. coli* in the untreated septage does not meet Vietnamese Domestic Wastewater Discharge Standards (TCVN 6772:2000), as it only allows levels of total coliform in the range of 1,000 to 10,000 MPN/100ml. In our study we only measured *E. coli* and found levels above the limit in all samples.

As the studied tanks were filled with sludge, the HRT of waste water was short. Helminth ova in the overflow do not sediment fast enough to be captured in the sludge accumulated at the tank's base, and the ova risk ending up in the surface water used by many people every day. Thus increasing the frequency of emptying means the risk for transmitting helminths to the surface water is reduced. While residents must obtain a construction permit for the design of a ST before houses are built, there are neither regulations nor legislation for their emptying. Thus STs should be built in such a way as to be easily accessed for maintenance, eg. removable access covers should be installed for easy inspection of sludge level and emptying. Beside raising awareness in the general population there must be changes to legal and policy frameworks if STs are to perform optimally and decrease the risk of contamination to surface water.

4.3.3 *Septage sludge*

Phages and bacteria found in septage sludge were in accord with the concentration found in other regions, e.g Australia (Kellogg Brown and Root Pty Ltd 2006) and Europe (Lepeuple *et al.* 2004). Yet the percentage of phage-positive samples differed

spatially depending on the health situation of different human populations. Lucena *et al.* (2003) for instance reported that MSB were detected in all septage sludge samples in Buenos Aires (Argentina), while Calci *et al.* (1998) found only 58% of samples collected in Southern Rhode Island (USA) were MSB positive.

That helminth ova were detected in 100% of septage sludges tested is supported by a study of Bao (2006) in northern Vietnam, although our concentration range is wider. There was no correlation found between helminth ova concentration and retention time of tank sludge (data not shown). It is more likely that the population of helminth ova correlated to the number of users and their helminth loads. From approximately 300 m^3 of septage sludge discharged daily into the environment in Ho Chi Minh city (Cuong 2008) about 5 billion helminth ova were calculated to be released daily into the environment. No data is on hand for septage sludge discharges in Ha Noi, but a concentration of up to 5,730 helminth ova per litre found in the Kim Nguu River (Trang *et al*. 2007) may be due to discharge from the city's sewerage system and direct discharge of untreated septage and septage sludge into the river.

The average concentrations of helminth ova detected in septage sludge is corroborated by other studies. Concentrations were much higher than those found in Bangkok, Manila and Accra (Strauss *et al.* 2000). The incidence of helminth infections in Vietnam is generally higher than those of many other developing countries (Trang *et al.* 2007), but *Ascaris* concentrations were lower than the concentrations found in Bangladeshi slums, with over 100,000 ova l^{-1} (Lloyd and Frederick 2000).

Many helminth ova varieties were found in septage sludge, and concurs with Le Hung *et al.* (2005) who reported multiple kinds of helminth ova found in stool samples in an ethnic minority community in the mountains of southern Vietnam. Most studies on helminth infections among people in Vietnam have focused on soil-transmitted helminths (STH) like *Ascaris lumbricoides*, *Trichuris* sp. and hookworm (Van der Hoek *et al.* 2003, Trang *et al.* 2007). Our results showed that *Ascaris lumbricoides* ova predominated in high concentrations and frequency. Yet ova concentrations of most other varieties found in septage sludge were much higher than those of *Trichuris* sp. and hookworm, which were present at an average of 8 ova per litre. Erdogrul and Sener (2005) also detected a high percentage of *Enterobius vermicularis* in various fruit and

vegetables irrigated by wastewater in Kahramanmaras, Turkey. *Taenia* spp. were detected in 30% of septage sludge samples although Taeniasis is the target of control programs in several countries (Gonzalez *et al.* 2006). This shows that beside STH other parasitic helminths should be also considered in managing a population's health.

4.4 **Microbial contamination of surface water and aquatic spinach in the Mekong Delta**

4.4.1 ***Surface water***

That *E. coli* and *Enterococcus* spp. were detected in all water samples from fishponds receiving PBDs' effluents is in accordance with the occurrence of these bacteria in the effluents. The *E. coli* concentration is consistent with Kobayashi *et al.* (2003) who showed the *E. coli* concentration ranged from 10^2 to 10^5 CFU ml^{-1}.

The *E. coli* levels found in rural canals concur with Kobayashi *et al*. (2003) who reported a range of 10^2 to 10^4 CFU ml^{-1}. *Enterococcus* concentrations are supported by Abrahamsson and Svensson (2000), who worked with drinking water quality in An Giang, Can Tho[9] and Soc Trang (MD provinces). Yet *E. coli* levels in Abrahamsson and Svensson (2000) were low, with an average of 51 CFU per 100 ml. This may be due to the large uncertainty in test results as stated by the authors. The mean concentrations of *Enterococcus* spp. and somatic coliphage are corroborated by studies in other countries on surface water contamination by human and non-human faecal waste (Hot *et al*. 2003, Lucena *et al*. 2003). *Salmonella* spp. were detected in 30% of samples and this shows a risk of contracting salmonellosis while exposed to canal water.

The high percentage of positive samples taken from urban canals may reflect discharge of human faeces, untreated septage, domestic ST effluent and other wastes. *E. coli* concentration was in accord with concentrations found in Ho Chi Minh City canals (Ha *et al.* 2008).

[9] Can Tho (Cần Thơ) Province was split into two new administrative units: Cần Thơ City and Hậu Giang Province in the beginning of 2004.

Microbial concentrations in specific watercourses can vary by season. In the dry season canal and river water levels are low and microbial concentrations can be above average. In contrast wet season water levels are high and microbial loads can be below average, yet storm run-off can increase loads (Kistemann *et al.* 2002). Thus spatial variations may be more significant than seasonal variations. Seto (2002) measured total coliform levels at 2-month intervals from 37 sampling sites in 6 MD provinces from April 2000 to January 2002, and with peaks and lows in various months in different sites found no seasonal pattern. A similar pattern (**Figure 4.1**) was observed in Rechenburg *et al.* (2005) while *Enterococcus* levels did not vary significantly over a year (1×10^3 - 8×10^3 CFU per 100 ml). Thus microbial loads in MD surface waters may depend on watercourse size and local conditions, e.g pollution sources.

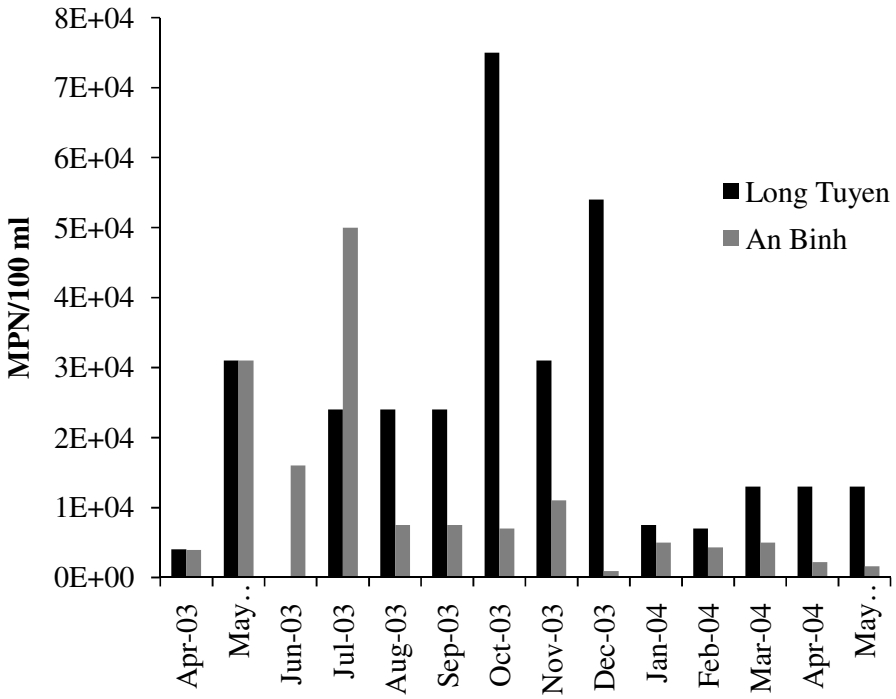


Figure 4.1 | Total coliform concentrations in rural canals in Can Tho.
Data source: Rechenburg *et al.* (2005).

4.4.2 *Cultivated aquatic spinach*

Compared to the microbial load on field vegetables (Ha *et al.* 2008) those on aquatic spinaches were much lower. The latter's habitat only received PBD liquid effluents. No direct faecal sources were seen to discharge into ponds. In Vietnam field vegetables

may be contaminated from polluted irrigation waters or organic fertilizers like animal dung (Ha *et al*. 2008).

Due to the different sources of microbes the average and range of *E. coli* concentrations found in aquatic spinach in urban areas were higher and wider than those in fishponds. Yet *E. coli* levels here were still much lower than on vegetables in Ho Chi Minh City markets, which were reported to be up to 10^4 CFU g^{-1} (Ha *et al*. 2008). No data is on hand for microbial contamination of vegetables in MD markets but it is expected to be less than those in Ho Chi Minh City.

4.5 **Persistence of phages and bacteria in Mekong river water and on terrestrial spinach in the Mekong Delta**

Somatic coliphage and bacteria were reduced slowly in Mekong River samples, revealing the chance of pathogen transmission is great. That phages were more resistant than bacteria in water agrees with Allwood *et al.* (2003). Reduction rates were higher than in other studies conducted at lower temperatures (Wang and Doyle 1998, Santo Domingo *et al.* 2000). These microbes survive longer at lower temperatures (Guan and Holley 2003).

Reduction rates of phages and bacteria on spinaches were lower than WHO guidelines (WHO 2006a), with levels of 0.5–2 log units/day. The volume of water attaching to spinaches after irrigation is much lower than the 10.8 ml of wastewater on 100 g of lettuce noted by Shuval *et al.* (1997). Yet the target vegetables and methods used in this study were different, although it replicates MD conditions used here for risk analysis.

4.6 **Quantitative Microbial Risk Assessment**

4.6.1 ***Risk associated with faecal management in the Mekong Delta***

Annual infection risks of working with faecal sludge were higher than acceptable risks. The results are consistent with Bao (2006) and Bo *et al.* (1993) regarding the impact of septage and sewage sludge on workers' health. Risks of salmonellosis were significantly lower than other concerned pathogens. This trend is supported by Schönning *et al.* (2007) who assessed risks associated with handling and use of human

faeces. Can Tho City sewerage workers operating the pump trucks and farmers cleaning pigsties did not take any health protection measure during this study's sampling. Sewage workers merely washed their hands with a small amount of tap water after work. The situation is even worse when desludging is done manually. In many cases workers have to stand inside the storage tank for hours while removing septage sludge. Thus infection risks for people handling with faecal sludge in MD are not reduced by health protection measures and remain high.

Annual risk of rotavirus infection when immersed in canals in both rural and urban areas is supported by Few *et al.* (2010) who showed a strong association between swimming frequency in surface water and diarrhea diseases in MD. Risk of bathing directly by swimming in canals was above acceptable levels though bathing in canals is a commonplace habit of MD rural habitants. By using flocculated water for bathing in the home, it is estimated that annual risks can be reduced at least 10 times.

Risk estimates show that MD canal water is unsuited to bathing/swimming. *E. coli* and *Enterococcus* spp. concentrations (Section 3.7) are higher than EPA standards (2003) for bathing freshwater with full body contact. According to WHO guidelines for microbial quality of recreational waters (2003), the 95th percentile values of *Enterococcus* spp. suggest an average probability of one case of gastroenteritis per 20 exposures. This ratio (5×10^{-2}) is similar to the calculated infection risk for rotavirus (**Table 3.23**).

Drinking untreated or alum-flocculated surface water leads to high risks of infection. This is backed by Luxemburger *et al.* (2001) who showed that drinking surface water resulted in over 80% of typhoid fever cases in Dong Thap. This occurs mainly in rural areas where substandard water delivery and sanitation systems abound, promoting incidental ingestion of faecal matter. There are several alternative methods for ensuring water is safe to drink. These include boiling the water, chemically disinfecting it, filtering it, using various combinations of the previously stated methods, or buying bottled water. In addition, solar drinking water disinfection, so-called SODIS, is a low-cost, point-of-use water purification method that has been disseminated globally (Mäusezahl *et al.* 2009).

Drinking safe water is possible in rural MD. About 7,500 households in Dong Thap were introduced to SODIS method within the first phase of Vietnam SODIS project (2005 – 2009). Preliminary observation from this project showed that the number of reported diarrhea cases was reduced (Helvetas 2010). SODIS requires sunlight, a clear water source and unscratched, transparent PolyEthylene Terephthalate (PET) bottles. Thus SODIS method may be difficult to apply in rainy season when sunlight is limited and the turbidity of surface water is high. Traditionally boiling water is the best method for making water safe to drink. Boiling water properly kills bacteria, parasites, and viruses causing gastroenteritis. However, Few *et al.* (2010) showed that boiled water had significant contamination. That may be due to the prior contamination of containers or recontamination from a long storage time. It implies that hygiene practices at home play a significant role in reducing infection risks (Herbst *et al.* 2008).

MD locals appear to assume that there is no undue pathogen load when washing spinach in untreated canal water. Even when spinach is washed in flocculated or tap water annual risks reduced but spinach is still not safe for eating. No helminth ova were found in samples so aquatic spinach is assumed to be ova free, yet as spinach is a known snail habitat and snails are an ova's intermediate host, helminthiasis may be contracted (Yoshihara *et al.* 1999, Shuval *et al.* 1986). Under current PBD functionality regimes, spinach applied with PBD effluent is not recommended for eating raw because of the helminth contamination. Thus in the MD it is advisable to cook spinach before eating.

4.6.2 *Fluctuation of risk estimates*

Risk of rotavirus infection linked to immersion in canals and drinking this water may overestimate the reality. Rotavirus populations used in models derive from animal and human faeces. This study did not establish faecal origin so rotavirus levels were taken to be human, and as animal-human transmission is low (Cook *et al.* 2004) infection can be considered a worst-case scenario.

Infection risks may be seasonal. Where there is little seasonal difference like MD rotavirus infections vary little year-round (Nguyen *et al.* (2001). Few *et al.* (2010) also found no strong evidence of an association between seasonality and diarrheal diseases in MD. Yet typhoid fever peaks before the wet season when river levels are lowest (Lin *et al*. 2000, Luxemburger *et al.* 2001). Literature on seasonality of helminthiasis in MD

is scarce, though conditions are suitable for soil transmission of intestinal helminths throughout the year (Hung *et al.* 2005). It indicates that marginal seasonal variations in water quality are unlikely to generate a strong difference in health outcome at the population level. The variation in water source used for daily life and health protection measures applied may mostly influence the health situation of the population.

Infection risks in flood prone areas will be higher than estimated risks due to increased exposure to contaminated water. Except for areas of prolonged flooding like Dong Thap and An Giang, floodwaters breach many houses in MD at high tides in September and October. Water quality will depend on proximity to latrines, animal manure, waste dumps etc. Most of Dong Thap and An Giang's populations are deprived. They work in the fields and drink and cook with untreated surface water so are more exposed to waterborne hazards like diarrhoea in the wet season (Few and Tran 2010).

Infection risk is influenced by pathogen, with risk ranking of exposure to human faeces in descending order being helminthisasis, rotavirus infection and salmonellosis. Helminthiasis is the peak risk as it is a health burden in the general population (Trang *et al*. 2007, Dung *et al.* 2007). However there is no data of helminth infection reported from Can Tho City in 2009. The risk ranking order of rotavirus and Salmonella is confirmed by epidemiological data in Can Tho City, where 18,178 diarrhoea cases and 13 typhoid fever cases were reported in 2009 (PHC 2010). As rotavirus accounts for ~50% of diarrhoea cases in Vietnam (Nishio *et al.* 2000, Nguyen *et al*. 2001, Nguyen *et al.* 2004) there were an estimated number of 9,000 rotavirus-related diarrhoea cases in the city.

4.6.3 *Use of appropriate data and risk models*

Rotavirus levels used in risk models were calculated on the average ratios of somatic coliphage: rotavirus in domestic raw sewage and surface water received (un)treated sewage (Lodder and Husman 2005). Compared to the rotavirus - *E. coli* ratio of $1{:}10^5$ (Mara *et al*. 2007, Howard *et al*. 2007) use of rotavirus concentrations based on somatic coliphage varied from 5 – 29%. Yet variation between the two calculations for surface water was greater (3 to 108 times), showing that a rotavirus-*E. coli* ratio of $1{:}10^5$ may not be applicable for urban water systems as in a study of Labite *et al*. (2010). Thus the rotavirus - *E. coli* ratio mentioned may only be apt for domestic wastewater and raw

sewage systems. *E. coli* and rotavirus in surface water should have a different ratio as they may originate from different sources such as animal manure, garbage etc.

Choice of model can also sway risk outputs. Probability of helminthiasis or ascariasis using an exponential model was generally 1 $\log_{10}$ higher than if the β-Poisson model designed by Navarro *et al.* (2009) was used (**Table 4.1**). The exponential model also gave higher annual risk values in all scenarios, and the difference between the models was greater at lower input doses (**Table 4.2**). Scenario 4.3c showed values below 10^{-4} for ascariasis in both models but that the median result from the exponential model was about 2 $\log_{10}$ higher than the β-Poisson model. This shows that the dose-response relationship developed by Navarro *et al.* (2009) may be suited to calculate helminthiasis risks in developing countries due to endemic immunity there. In addition, current infection rate is likely to be lower than theory (one egg can cause infection (r = 1) and applied in the exponential model).

Table 4.1 | Probability of infection of helminth and Ascaris (median values and 95% confidence interval in parentheses) following two different risk models.

Scenario	Exponential model, r = 1		β-Poisson model, N_{50} = 859 and α = 0.104 (Navarro *et al.* 2009)	
	Helminth	*Ascaris*	Helminth	*Ascaris*
1.1	0.38 (0 – 0.76)	8×10^{-2} (0 – 0.32)	4×10^{-2} (0 – 8×10^{-2})	8×10^{-3} (0 – 3×10^{-2})
1.2	0.51 (1×10^{-4} – 1)	0.22 (0 – 1)	5×10^{-2} (7×10^{-12} – 0.4)	2×10^{-2} (1×10^{-5} – 0.3)
1.5	0.18 (0 – 0.64)	4×10^{-2} (4×10^{-10} – 0.28)	2×10^{-2} (0 – 7×10^{-2})	4×10^{-3} (4×10^{-11} – 3×10^{-2})
1.6	0.99 (0.42 – 1)	0.98 (0 – 1)	0.20 (4×10^{-2} – 0.3)	0.10 (0 – 0.3)
4.3 a	0.50 (4×10^{-4} – 1)	0.86 (2×10^{-10} – 1)	0.10 (2×10^{-11} – 0.45)	5×10^{-2} (2×10^{-5} – 0.35)
4.3 b	7×10^{-2} (4×10^{-5} – 1)	0.18 (2×10^{-11} – 1)	2×10^{-2} (2×10^{-12} – 0.30)	6×10^{-3} (2×10^{-6} – 0.19)
4.3 c	7×10^{-8} (3×10^{-11} – 7×10^{-6})	2×10^{-7} (0 – 4×10^{-5})	2×10^{-8} (0 – 3×10^{-6})	7×10^{-9} (0 – 6×10^{-7})

Accidental ingestion of 1 ml of Fresh pig slurry (1.1); Liquid plastic bio-digester's effluent (1.2); Untreated septage (1.5) and Septage sludge (1.6)

Consumption of spinach fertilised with plastic bio-digester's effluent in the Mekong Delta (4.3); time elapsed between final irrigation and harvest is 3 days. No reduction of helminth ova was assumed in the meantime.

a) raw without proper washing

b) raw after washing with flocculated water/tap water

c) cooked

Table 4.2 | Annual risk of infection of helminth and Ascaris (median values and 95% confidence interval in parentheses) following two different risk models.

Scenario	P_{inf} from Exponential model, r = 1		P_{inf} from β-Poisson model, N_{50} = 859 and α = 0.104 (Navarro *et al.* 2009)	
	Helminth	*Ascaris*	Helminth	*Ascaris*
1.1	1 (0 – 1)	1 (0 – 1)	1 (0 – 1)	1 (0 – 1)
1.2	1 (1 – 1)	1 (1×10^{-2} – 1)	0.99 (7×10^{-10} – 1)	0.88 (1×10^{-3} – 1)
1.5	1 (0 – 1)	1 (9×10^{-8} – 1)	0.98 (0 – 1)	0.61 (1×10^{-8} – 1)
1.6	1 (1 – 1)	1 (0 – 1)	1 (1 – 1)	1 (0 – 1)
4.3 a	1 (4×10^{-2} – 1)	1 (2×10^{-8} – 1)	1 (2×10^{-3} – 1)	0.99 (2×10^{-9} – 1)
4.3 b	1 (4×10^{-3} – 1)	1 (2×10^{-9} – 1)	0.82 (4×10^{-4} – 1)	0.48 (2×10^{-10} – 1)
4.3 c	7×10^{-6} (4×10^{-9} – 8×10^{-4})	2×10^{-5} (0 – 4×10^{-3})	2×10^{-6} (0 – 3×10^{-4})	7×10^{-7} (0 – 7×10^{-5})

Accidental ingestion of 1 ml of Fresh pig slurry (1.1); Liquid plastic bio-digester's effluent (1.2); Untreated septage (1.5) and Septage sludge (1.6)

Consumption of spinach fertilised with plastic bio-digester's effluent in the Mekong Delta (4.3); time elapsed between final irrigation and harvest is 3 days. No reduction of helminth ova was assumed in the meantime.

a) raw without proper washing

b) raw after washing with flocculated water/tap water

c) cooked

4.6.4 *Risk estimates versus epidemiological data in the study area*

Rotavirus is the most common cause of severe diarrhoea in children worldwide but it is just one infectious pathogen involved. Agents include other viruses, bacteria, protozoa and parasites. Thus it is difficult to meaningfully assess the risks of rotavirus infection for diarrhoea. In any case little data on rotavirus and helminth burdens exist for MD so typhoid fever – which is caused by *Salmonella typhi* – is the exemplar disease and is here compared with salmonellosis, an infection of Salmonella bacteria, estimated from the QMRA study.

Annual rates of typhoid fever in MD in the period from 1991 to 2001 ranged from 1×10^{-5} to 2×10^{-3} (Kelly-Hope *et al.* 2007). Based on infection ÷ disease rate of 0.1 (Glynn and Bradley 1992), calculated *Salmonella typhi* infection rates varied from 1×10^{-4} to 2×10^{-2} in MD. The median annual risk of salmonellosis from studied scenarios in MD was estimated at 4×10^{-3} (**Table 3.29**), and is in-range despite not all risk factors being account for and risk of salmonellosis is higher than *Salmonella typhi* infection. While this finding may not capture the situation in Kelly-Hope *et al.* (2007) it does show that risk estimates fit rather well in with general epidemiological trends.

4.6.5 *Sensitive sub-populations*

Certain sections of the population are not well represented in the study. Dose-response parameters used in risk assessments were largely gained from feeding studies of healthy adults (Soller 2006). Children, the elderly, pregnant women and immuno-compromised individuals are excluded. These cohorts are disease-sensitive and more likely to contract infections that become chronic (Gerba *et al.* 1996). Hence cohort-specific research and policies aimed at reducing risk of waterborne infection among a population's vulnerable must be realised (Leclerc *et al.* 2002).

Among the sensitive sub-populations, children should be considered a special case because their neurological, immunological, and digestive systems are still in developing stages (Nwachuku and Gerba 2004). Moreover, children are more exposed to pathogens in the environment because of poor or lack of sanitary habits. U.S Environmental Protection Agency (2006) suggested age-dependent adjustment factors applied for assessing health risks of environmental exposures to children: 10-fold for exposure

occurring before 2 years of age; 3-fold for exposure occurring between the ages of 2 and 16; and no adjustment after 16 years of age. Then risks for children increase proportional to the adjustment factors. In our study, children are assumed not to involve in working with faecal substrates but they can be exposed to septage while playing close to dumpsites. Children's ingestion dose during one bath/swim in canals and frequency of events should be higher compared to that of adults.

4.7 **General discussion**

4.7.1 ***Possibilities to reduce pathogens discharged into the watercourse***

Decimal reduction time of phage and bacteria in Mekong River water was longer than 2 days. Yet contaminates are emitted into the river daily, causing pathogen spread through the delta's network of waterways. There are no plans for central wastewater treatment plants servicing some 80% of the rural population, so pathogen loads should be reduced via small-scale systems before discharge. Direct discharge of human faeces, especially from infected people, should be forbidden. Low-cost anaerobic digestion system like PBDs is a viable alternative for tropical regions. Health control programs must be seen as the best solution – reduce pathogens at source.

Anaerobic treatment of animal slurries has an effect on the reduction of pathogens and indicator micro-organisms. Most organisms tested were reduced but not eliminated. Besides the known benefits of PBDs, gas yield and hygiene status of sludge and liquid outputs should be taken into account. The microbial contamination of PBD sludge is assumed to be similar to that in septage sludge. STs function more like storage tanks than anaerobic treatment systems, as do PBDs. Pathogens and microbial indicators were slightly reduced compared to PBDs. Yet a ST can act as a trap to keep helminth ova in the sludge. When the tank is full untreated septage overflows and contaminates nearby surface water. Hence the design, construction and operation of these plants should be improved for optimal performance.

Besides dealing with faecal management, health control programmes should be introduced. For instance, mass treatment is very important for a rapid elimination of helminth infections while health education and sanitation play a role in preventing re-infection (Hung *et al.* 2005). Yet no control program was cited in the Ministry of

Health's *Orientation on Strategies for Preventive Health Activities to 2010* (Thuong 2000), and helminthiasis is considered a relevant health burden in Vietnam (Trang *et al.* 2007, Dung *et al.* 2007), which this study confirms. Other infectious diseases like shigellosis (bacillary dysentery), typhoid fever, cholera, hepatitis, etc have been incorporated in preventive health activities in Vietnam.

4.7.2 *Safe use of nutrients from faecal sludge*

Phages and bacteria were found in sludge even after many years in STs. This shows that pathogens and indicator organisms, and especially helminth ova, accumulate in the sludge, and concurs with Yen-Phi *et al.* (2009) and Kearney *et al.* (1993b) on the mesophilic anaerobic treatment of animal waste. After application to land sludge-borne bacteria and helminth ova can persist for months or even years (Jimenez 2007, Gerba and Smith 2004), placing Vietnamese farmers, many of whom go about barefoot and have poor personal sanitation and hygienic practices, at risk of helminthiasis. Thus, and in accord with WHO guidelines (2006b), septage sludge and PBD digestate in Vietnam should not be applied directly to farmland regardless of tank or digester retention time.

Use of faecal sludge for aquaculture is another possibility for the recycling of nutrients. However, care should be taken to avoid microbial contamination. Yajima and Kurokura (2008) found that applying animal manures directly to fishponds in Vietnam resulted in faecal contamination of water and the skin of fishes, with the estimated risk of enteric infection being 100 – 1,000 times higher than the US Environmental Protection Agency standard. Use of septage sludge for aquaculture is not significantly different from using manure in fishpond. Moreover helminth ova that accumulate in the pond sludge may survive until the pond is desludged, usually every year or two, and this sludge is then applied directly to arable land. According to Sanguinetti *et al.* (2005) *Ascaris suum* ova remain viable for up to 20 months of in-pond septage storage. In China, up to 10,000 parasite eggs per 100 ml of pond sludge were found where fish ponds had been fertilized with septage sludge collected from public toilets (Bo *et al.* 1993). Thus the direct application of septage sludge to fish ponds is not recommended in Vietnam.

By treating and sanitising the faecal sludge it should be possible to reuse this resource in agriculture. Where centralised wastewater treatment plants are yet to be established, like in Vietnam, small sanitation systems should be considered. Proposed treatments

effective against helminth ova include ammonia (Ottoson *et al.* 2008, Nordin *et al.* 2009) and lime (Cappizzi-Banas *et al.* 2004). An alternative treatment is anaerobic digestion with a high HRT (Yen-Phi *et al.* 2009). Treatments combining faecal sludge dewatering and subsequent co-composting with organic solid waste were also shown to produce hygienic biosolids safe for agricultural reuse (Koné *et al.* 2007). For aquaculture it has been found that the liquid effluent produced via the combined anaerobic and facultative processes to rear fish in bio-stabilization ponds can also be effective (Bo *et al.* 1993).

Waste stabilization ponds are likely to be the most appropriate option for wastewater treatment in most developing countries as they are low cost, low maintenance with high performance systems. In warm climates a series of ponds comprising an anaerobic pond, a secondary facultative pond and 1-2 maturation ponds can achieve a 3-4-log unit pathogen reduction and produce an effluent with 1 helminth egg per litre (Mara 2008). Additionally the anaerobic ponds can be covered to collect the biogas generated within the ponds. Ayres *et al*. (1992) recommended a design equation based on HRT of treatment ponds to meet the WHO guideline of ≤ 1 helminth egg per litre for restricted irrigation.

4.7.3 *Use of Quantitative Microbial Risk Assessment*

The QMRA models presented in this study provide a starting point for managing risks associated with faecal management situation in MD. It can be built upon, refined and adjusted for different scenarios at different sites. For example if alum-flocculated water is further treated by a sand filter system the microbial reduction due to this filter could be added to the model or the total reduction rate can be adjusted. Risk of drinking treated water from the SODIS method can also be assessed by replacing input microbial data of Scenario 3 (see above). Thus there is an opportunity for future reassessment of the risks in this study, including for regional populations and sensitive sub-populations.

Risk estimates give an overview of a population's health when epidemiological data is hard to obtain. The number of gastroenteritis cases, especially diarrhoea, reported to health centres is bound to be lower than actual cases, especially in rural areas. The percentage of outpatient treatment in state health facilities in MD in 2006 was just 52% (General Statistics Office 2006b). The rest visited private clinics, traditional

practitioners or they self-treated. Thus epidemiological data is never complete. In addition QMRA can provide data on which cohorts are at most risk, and the ideal basis from which to create preventive health initiatives in MD.

QMRA is a tool to support the prioritization of preventive health initiatives. The United States Department of Agriculture was the first government agency required by law to include risk and economic analyses in all major regulations dealing with human health, safety and the environment (McElvaine 2001). Ranking of infection risk potential by exposure scenarios enables the identification of control points for better management. **Figure 4.2** gives an overview of factors leading to salmonellosis in MD. It shows that drinking untreated and alum-flocculated surface water leads to an 80% annual risk of salmonellosis. Accidental ingestion of pig slurry, eating raw aquatic spinach constitute to about 10% and 8%, respectively. That Can Tho Preventive Health Centre recommended inhabitants to drink boiled water and eat well-cooked food to prevent salmonellosis (PHC 2010) is in line with the study's results but it seems to be not adequate. Sewage workers face most risk of infection but this cohort is small.

QMRA based on exposure assessments aids in selecting appropriate interventions for better health outcomes. To protect human health it is not enough to just check the total coliforms in water, as is set out in the Vietnamese Standard TCVN 5942-1995. This is a bacterial indicator and overlooks enteric viruses and protozoa (Rose and Gerba 1991). Moreover people are infected through multiple pathways, that include but go beyond water (Briscoe 1984).

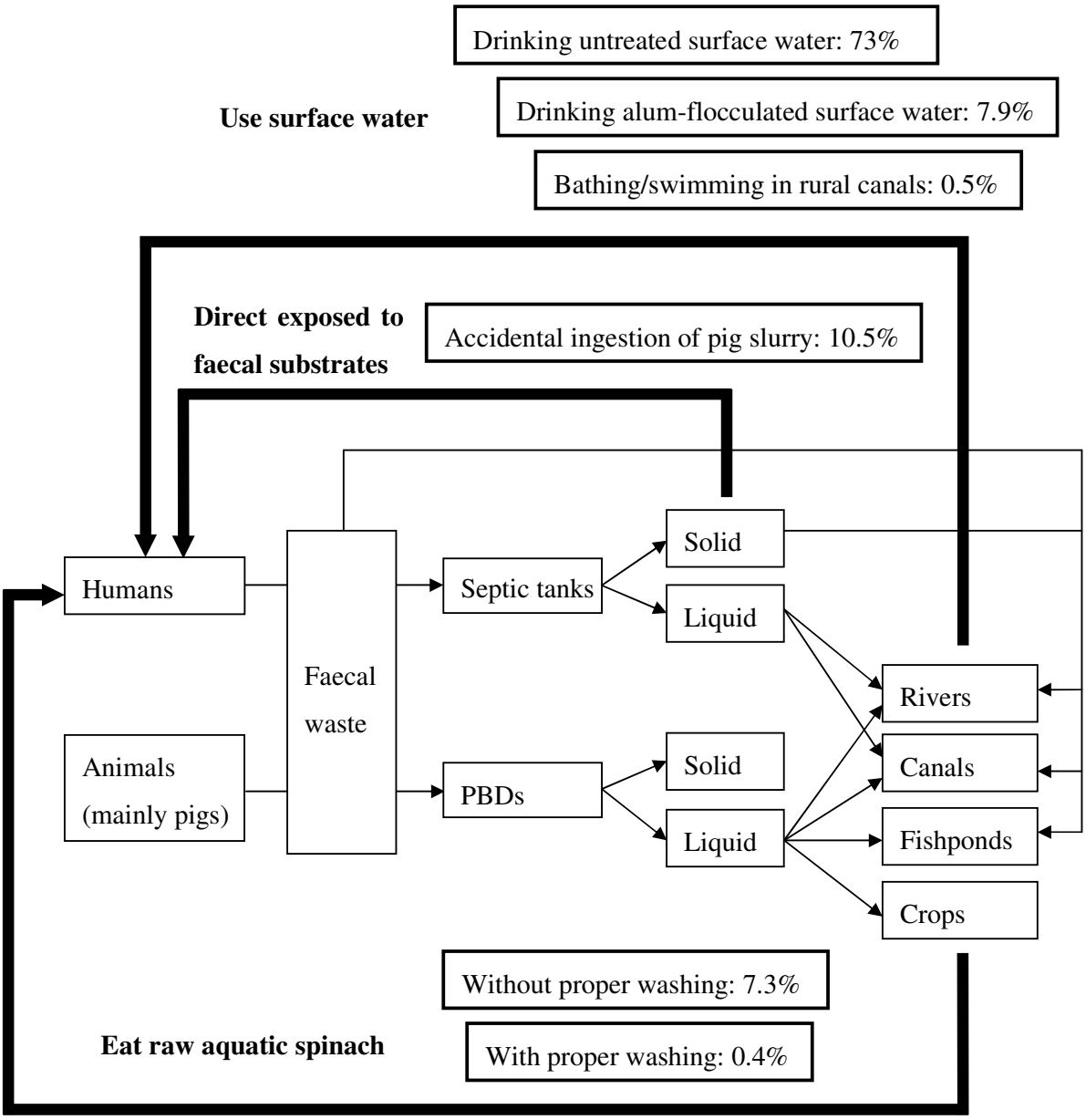


Figure 4.2 | Main factors constituting annual salmonellosis for the Mekong Deta's population. The percentages presented were based on calculations in **Table 3.29**. All other exposure scenarios contribute to 0.2% of the annual salmonellosis.

PBDs = plastic bio-digesters

Risk communication is a tool to exchange information between government officials, technicians, health researchers, private practitioners and the public. While QMRA data collation and modelling requires much effort communicating health risks to the public is challenging. The question is how experts and the public should communicate when each use a different language and has different levels of comprehension. The conflict between experts and public risk perception is at the basis of the social dilemmas of risk management (Slovic 1999). This author suggested that the public participation into both risk assessment and risk decision making is necessary to increase the legitimacy and public acceptance.

Society seems to accept risks to the extent that risks are associated with benefits, and the individual participates on a "voluntary" basis (Starr 1969). For instance, many people go swimming to improve their physical and mental health though there is a risk of drowning. Results show that most of MD canal water is unsuited to swimming and risk of swimming in canals constituted 0.5% of annual salmonellosis for MD population. As water quality differs from area to area, the population can decide whether they take risks or not. But they should perceive risk including evaluations of the probability as well as the consequences of a negative outcome. And so it is likely that this study's findings, in seeking to improve the health, wellbeing and productivity of the Mekong Delta via hygienic and sustainable faecal management solutions will best be directed at preventive heath initiates.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the pilot study it can be inferred that there is a hygiene effect of domestic PBDs and STs in tropical regions although the maximum reduction rate of phages (somatic coliphage) and bacteria (*E. coli*, *Salmonella* spp., *Enterococcus* spp.) is about 3 $\log_{10}$ unit. Long HRTs, or factors related to longer HRTs such as high TIC, play a vital role in microbial reduction, while yielding more gas production in PBDs. The reduction rates over STs are generally lower than PBDs and not significant. *Ascaris suum* ova need about 5 months to reduce 90% viability in both biogas and septage sludge. The ova viability in sludge can be estimated based on sludge retention time from the exponential equation established within the study.

Field study results suggest that the functionality of PBDs and STs is not optimal to inactivate pathogens. Volume of PBD is not compatible to the amount of pig slurry. PBDs are rarely desludged and STs are emptied only when blockages occur. There is a slight reduction of phages, *E. coli* and *Enterococcus* spp. over PBDs. *Salmonella* spp. are detected more frequently in effluents than in influents. In addition, helminth ova do not settle efficiently in the sludge. More ova varieties with higher average concentration are found in effluents than in influents. Most PBD effluents and overflows from full STs flow directly and contaminate the surface water, which is used by many people every day.

While microbial treatment of the main treatment systems in MD is not efficient, the pathogen loads in faecal substrates are noticeably high, especially helminth ova. The ova are detected in 95% untreated septage and in all septage sludge samples. More ova varieties in higher concentrations in septage are found than those reported from stool samples. Thus, the burden of helminth parasites still exists in the MD population. Since human disease can be caused by zoonotic transmission, high pathogen levels found in pig slurry can be taken account to gastroenteritis cases in the population.

The management of (un)treated faecal substrates in MD leads to microbial contamination of the watercourse and some aqua-, agriculture products. Surface water with high concentrations of phage and bacteria leads to microbial contamination of

spinach in this habitat. Helminth ova are not found in surface water as well as on aquatic spinach. However, spinach cultivated on fields fertilised by PBD's effluent is also contaminated with helminth ova. The low inactivation rate of phage and bacteria in MD surface water and on terrestrial spinach plays little role in preventing infection for the exposed population.

The QMRA study shows that the risks of pathogen infection were recorded high and in descending order from helminth to rotavirus and Salmonella. In MD it is sewage workers who are most at risk of infection due to their constant exposure to human faecal matter which contains high levels of pathogens regardless of its retention time in STs. Yet other exposure scenarios and other people are involved. Incidental ingestion of slurries, bathing/swimming in canals, drinking untreated surface water, and eating raw spinach constitute chronic exposure scenarios for the population in MD.

Faecal management in MD leads to high risks of infection for the population. Both existing treatment systems (PBDs and STs) are not efficient in terms of pathogen removal. Technological barriers reduce the risk of infection and include wastewater treatment (e.g. PBDs), due time between final crop irrigation and harvest, treatment of water (alum flocculation, boiling) before consumption, and food preparation. Due to the high pathogen load of surface water this is only potable when boiled. Aquatic spinach is not safe to eat unless cooked.

The current microbial treatment efficacy of anaerobic digestion in MD can be improved provisionally by simple practices of operation and maintenance. Health care program and personal hygiene play an important role in reducing risks of infection. To reduce infections in the population, an integrated plan for faecal management, water supply and behaviour change campaigns including education program for children is needed. MD might serve as good example for many densely populated areas in tropical regions with "abundant water" but low clean water availability.

5.2 Recommendations

5.2.1 *Faecal matter management*

Where there are no plans for wastewater treatment plants for nearly 80% of the population living in the rural areas and not yet centralised plants in cities, faecal management remains a big issue in the whole country. Legislative reforms, public awareness campaigns, and strong law enforcement are needed to conserve the environment and to improve the population's health. Direct discharge of fresh faecal matter (e.g. open defecation in canals, rivers) as well as faecal sludge collection (e.g. septage sludge) into the environment should be strictly forbidden. Instead, on-site anaerobic treatment such as STs and PBDs should be promoted. PBD users should be encouraged to build simple toilets and connect them to PBDs. In addition, regulated use of treatment systems should be taken into account.

While these are largely out of sight and out of mind, STs will remain the main sanitation system for developing country households into the foreseeable future. Their sustainable use relies on knowledge of septage pathogens in specific areas and appropriate protection measures for people who handle septage. Sludge expelled from the STs, despite of retention time, must be sanitized properly prior to reuse in agriculture and aquaculture. STs must be emptied regularly to avoid untreated septage leaking into and polluting the environment. In addition an integrated management plan for septage sludge is needed to minimize infections in the population.

Domestic PBDs are a low-cost and relatively effective local technology. For their risk-free and sustainable use their pathogen reduction efficacy should be taken into consideration. Out of many factors affecting the survival of indicators and pathogen in tropical PBDs, HRT is recommended to implement owing to (1) higher HRT can be achieved in existing PBDs by using less water to clean the pigsties; and regular desludging; (2) higher HRT leads to higher biogas yield. A HRT of at least 15 days is recommended to increase gas yield and achieve a higher pathogen reduction. In sensitive areas, e.g. where surface water is used for domestic purposes, a HRT of at least 30 days should be followed. For a newly built PBD, its volume should be compatible with its manure input. A PBD model with two connected plastic tubes as shown in **Figure 2.3** can be a solution for a better microbial treatment efficacy. In

adition, whirl influent flows should be avoided so that helminth ova can settle efficiently at the digester's base.

PBD effluent quality in MD today regarding microbiological hygiene requirements is not good enough to be discharged directly into surface water or applied to crops that are eaten raw. However the effluent can be applied to non-food crop (timber), food crops that are processed or cooked before consumption (e.g coconut, rice) and food crops that the irrigation cannot reach the eaten parts (e.g banana, orange, grapefruit, etc.). More barriers (further treatment, proper practice) should be applied while handling bio-digester effluent to minimise risks to human health and the environment.

5.2.2 *Human health improvement*

People who deal with faecal substrates must be aware of the health risks and consequences. They should minimize their contact to faecal substrates such as using protective clothing. Hand disinfection should be done right after working. More knowledge of the quality of faecal substrates as well as health protection measures should be distributed among exposed and the general population. Manual desludging should be phased out and pump trucks that are able to traverse narrow lanes should be phased in. A simple solution such as using pumps instead of manual extraction of sludge is proposed as an urgent measure to improve sanitary worker's health. QMRA study suggests that farmers and sewage workers in MD should be dewormed regularly.

Household interventions may be as effective at preventing gastroenteritis as other environmental approaches, such as improved sanitation, hygiene (e.g. hand washing with soap, bathing in home with flocculated water), and drinking boiled water. The population should be informed about the infection risk of bathing by swimming in canals, rivers. Household interventions should be strongly encouraged, particularly because of evidence that they are cost-effective and that the target population may in fact be willing to pay for all or a portion of their cost.

5.2.3 *Further research*

A number of issues can be identified as subjects that need to be investigated further.

- More pathogens should be taken into account for QMRA: Campylobacter, Giardia and Crytosporodium.
- QMRA of direct usage of septage and PBD sludge in agriculture and aquaculture.
- Possibilities to eliminate helminth ova at high concentration in septage.
- Contamination of fish raised in fishponds and in rivers.
- Development of guidelines for the safe use of faecal sludge as fertilizing agents.

6 GLOSSARY

Ascariasis	An infection of the small intestine caused by *Ascaris lumbricoides*, a large roundworm.
DALYs (Disability-Adjusted Life Years)	DALYs are a measure of the health of a population or burden of disease due to a specific disease or risk factor. DALYs attempt to measure the time lost because of disability or death from a disease compared with a long life free of disability in the absence of the disease.
Decimal reduction time	The time required to kill 90% of the organisms being studied
Disinfection	The process of killing infectious agents, microorganisms that can cause infectious diseases, involving disinfecting agents or physical processes.
Disease	Symptoms of illness in a host, e.g. diarrhea, fever, ect.
Effluent	Liquid (e.g. treated wastewater) that flows out of a process or confined space.
Enteric diseases	Diseases classified as enteric enter through the mouth and intestinal tract and are usually spread by contaminated food, water, or contact with contaminated vomit or faeces.
Excreta	Faeces and urine.
Exposure	Contact of a chemical, physical or biological agent with the outer boundary of organisms (through inhalation, ingestion or dermal absorption).
Hazard	A biological, chemical, physical or radiological agent that has the potential to cause harm.
Helminthiasis	Infection with one or more intestinal parasitic worms (e.g. roundworms *Ascaris lumbricoides*, whipworms *Trichuris trichiura*, hookworms (*Necator americanus* and *Ancylostoma duodenale*), etc).

Hydraulic retention time	Time the wastewater takes to pass through the system.
Immuno-compromised	having an impaired immune system and therefore incapable of an effective immune response, usually as a result of disease, such as AIDS, that damages the immune system.
Log_{10} reduction	Organism removal efficiencies: 1 log_{10} unit = 90%; 2 log_{10} unit = 99%; 3 log_{10} unit = 99.9% and so on.
Median	The middle value of a sample series (50% of the values in the sample are lower and 50% are greater than the median).
Methanogenesis	The formation of methane by microbes known as methanogens or methane producing bacteria.
Pathogen	A disease-causing organism (e.g. viruses, bacteria, protozoa, helminths).
Plastic bio-digester	A low-cost, small-scale biogas plant made of polyethylene tube.
Risk	The likelihood of a hazard causing harm in exposed populations in a specific time frame, including the magnitude of that harm.
Salmonellosis	An infection with Salmonella bacteria
Septage	Combined untreated human waste, liquid (supernatant) and solid or semi-solid materials, removed from septic tanks or any other container which holds untreated human waste.
Sludge	An organic solid or semi-solid residual material accumulated in the septic tank's base or digester's base.
Septic tank	An underground tank for receiving wastewater consisting of one or more compartments, in which the sanitary flow is detained to permit concurrent sedimentation and sludge digestion.

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8 APPENDICES

8.1 Structured questionnaire used during plastic bio-digester sampling

Date of sampling: ……

Code of samples:

……

I. General Information of Household

1. Name of household owner: .....

2. Address, telephone number

.....

.....……

…….....

II. PBD condition

1. Volume: ..... m^3

Length (m): .....

Diameter (m): .....

2. PBD age: .....year(s) .....month(s)

3. Times of emptying since PBD to be in use?

Not yet 1 time

2 times 3 times

> 3 times

4. Mode of emptying?

Mechanical Manual

If manual, how many people involved, for how long?

.....

…….....

.....…

5. Source of input

- Pig manure, from ..... sows, ... piglets, ... pigs for meat

- Other sources: .....

6. Discharge/use of effluent

fish ponds gardens

nearest canals Other uses: .....

Other observations:

.....

.....

.....

.....

.....

.....

8.2 Structured questionnaire used during septage sampling

Date of sampling/septic tank emptying:

……

Code of samples:

……

I. General Information of Household

1. Name of household owner: .....

2.Address:.....

.....

….....

II. Water Supply

1. What is your water source?

Private house-connection

Public tap

III. Situation of Environment Sanitation and Septic Tank Emptying

1. Toilet connected to the municipal sewerage system?

Yes No

2. Duration of septic tank since built ?

< 1 year 1 – 3 years > 3 years

3. Volume of septic tank?.....m^3

Number of chambers/compartments? .....

4. Times of emptying since septic tank to be in use?

Not yet 1 time

2 times 3 times

> 3 times

5. Duration between emptying times?

……

6. Service Providers?.....

Private Public

7. Mode of septic tank's emptying before?

Mechanical Manual

If manual, how many people involved, for how long?

.....

…….....

.....…

Other observations:

.....

.....

.....

.....

.....

.....

8.3 Distribution functions used in the risk models

Table 8.1 | Description of the distribution functions used in the risk models.

Distribution function	Description
BetaGeneral	**BetaGeneral**(*alpha1,alpha2,minimum,maximum*) specifies a beta distribution with the defined *minimum* and *maximum* using the shape parameters *alpha1* and *alpha2*. The BetaGeneral is directly derived from the Beta distribution by scaling the [0,1] range of the Beta distribution with the use of a minimum and maximum value to define the range. The PERT distribution can be derived as a special case of the BetaGeneral distribution.
Expon	**Expon***(beta)* specifies an exponential distribution with the entered beta value. The mean of the distribution equals beta. This distribution is the continuous time equivalent to the Geometric distribution. It represents the waiting time for the first occurrence of a process which is continuous in time and of constant intensity. It could be used in similar applications to the Geometric distribution (e.g. queuing, maintenance and breakdown modelling), although suffers in some practical applications from the assumption of constant intensity.
ExtValue	**ExtValue**(*alpha, beta*) specifies an extreme value distribution with location parameter *alpha* and shape parameter *beta*.
Gamma	**Gamma**(*alpha,beta*) specifies a gamma distribution using the shape parameter *alpha* and the scale parameter *beta*. The Gamma distribution is the continuous time equivalent of the Negative Binomial i.e. it represents the distribution of inter-arrival times for several events from a Poisson process. It can also be used to represent the distribution of possible values for the intensity of a Poisson process, when observations of the process have been made.

Table 8.1 (continued)

Distribution function	Description
Invgauss	**Invgauss**(*mu,lambda*) specifies an inverse Gaussian distribution with mean *mu* and shape parameter *lambda*.
Loglogistic	**Loglogistic**(*gamma,beta,alpha*) specifies a log-logistic distribution with location parameter *gamma* and shape parameter *alpha* and scale parameter *beta*.
Lognorm	**Lognorm**(*mean,standard deviation*) specifies a lognormal distribution with the entered *mean* and *standard deviation*.
Normal	**Normal**(*mean,standard deviation*) specifies a normal distribution with the entered *mean* and *standard deviation*. This is the traditional “bell shaped” curve applicable to distributions of outcomes in many data sets.
Triang	**Triang**(*minimum,most likely,maximum*) specifies a triangular distribution with three points — a *minimum*, *most likely,* and *maximum*. The direction of the "skew" of the triangular distribution is set by the size of the *most likely* value relative to the *minimum* and the *maximum*.

8.4 Vietnamese Standard of Surface Water Quality (TCVN 5942-1995)

Table 8.2 | Parameter limits and maximum allowable concentrations of pollutants in surface water in Vietnam (TCVN 5942-1995).

No.	Parameter and Substance	Unit	Limitation Value	
			A*	B**
1	pH value		6 – 8.5	5.5 - 9
2	BOD5 (20ºC)	mg l^{-1}	4	25
3	COD	mg l^{-1}	10	35
4	Dissolved oxygen	mg l^{-1}	≥6	≥2
5	Suspended solids	mg l^{-1}	20	80
6	Arsenic	mg l^{-1}	0.05	0.1
7	Barium	mg l^{-1}	1	4
8	Cadmium	mg l^{-1}	0.01	0.02
9	Lead	mg l^{-1}	0.05	0.1
10	Chromium, Hexavalent	mg l^{-1}	0.05	0.05
11	Chromium, Trivalent	mg l^{-1}	0.1	1
12	Copper	mg l^{-1}	0.1	1
13	Zinc	mg l^{-1}	1	2
14	Manganese	mg l^{-1}	0.1	0.8
15	Nickel	mg l^{-1}	0.1	1
16	Iron	mg l^{-1}	1	2
17	Mercury	mg l^{-1}	0.001	0.002
18	Tin	mg l^{-1}	1	2
19	Ammonia (as N)	mg l^{-1}	0.05	1

Table 8.2 (continued)

No.	Parameter and Substance	Unit	Limitation Value	
			A*	B**
20	Fluoride	mg l^{-1}	1	1.5
21	Nitrate (as N)	mg l^{-1}	10	15
22	Nitrite (as N)	mg l^{-1}	0.01	0.05
23	Cyanide	mg l^{-1}	0.01	0.05
24	Phenol compounds	mg l^{-1}	0.001	0.02
25	Oil and grease	mg l^{-1}	ND	0.3
26	Detergent	mg l^{-1}	0.5	0.5
27	Coliform	MPN/100ml	5,000	10,000
28	Total pesticides (except DDT)	mg l^{-1}	0.15	0.15
29	DDT	mg l^{-1}	0.01	0.01
30	Gross alpha activity	Bq l^{-1}	0.1	0.1
31	Gross beta activity	Bq l^{-1}	1.0	1.0

* applied to the surface water using for source of domestic water supply with appropriate treatments.

** applied to the surface water using for the purposes other than domestic water supply. Quality criteria of water for aquatic life are specified in a separate standard.

ND = not detectable

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