Estudi del compostatge de residus sòlids orgànics

Optimització de la mescla inicial i seguiment de paràmetres d'activitat biològica del procés

María Teresa Gea Leiva

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ANTONI SÁNCHEZ FERRER i ADRIANA ARTOLA CASACUBERTA, professor i professora titulars de l'Escola Universitària Politècnica del Medi Ambient, centre adscrit a la Universitat Autònoma de Barcelona

CERTIFIQUEM:

Que l'Enginyera Química María Teresa Gea Leiva ha realitzat sota la nostra direcció, el treball que amb títol **"Estudi del compostatge de residus sòlids orgànics. Optimització de la mescla inicial i seguiment de paràmetres d'activitat biològica del procés"**, presenta en aquesta memòria, la qual constitueix la seva Tesi per optar al Grau de Doctora per la Universitat Autònoma de Barcelona.

I perquè en prengueu coneixement i consti als efectes oportuns, presentem a l'Escola Tècnica Superior d'Enginyeria de la Universitat Autònoma de Barcelona l'esmentada Tesi, signant el present certificat a

Mollet del Vallès, gener 2005

Dr. Antoni Sánchez Ferrer

Dra. Adriana Artola Casacuberta

A mi hermano,

que me recuerda cada día el valor de la nobleza

Un moment...

Cinc anys han passat des del dia en que en Toni em cridà al seu despatx i em va proposar fer el meu treball de recerca en compostatge, encetant així una nova línia de recerca a l'EUPMA. Només teníem un vell compostador de metacrilat, 500.000 de les antigues pessetes i molta il·lusió. No ens podíem imaginar on ens portaria tot allò. En escriure aquesta pàgina, tanco el cicle personal que vaig obrir aquell dia, quan li vaig dir 'D'acord!'. Toni i Adriana, ha estat un plaer treballar i aprendre amb vosaltres, gràcies per oferir-me experiència, coneixements i amistat. Toni, Adriana, Raquel, Estel·la, Fela, Xavi, M Àngels i Luz. Formar part d'aquest grup de recerca ha estat el millor aprenentatge possible, a nivell científic i a nivell personal.

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ÍNDEX

1. INTRODUCCIÓ

La tecnologia de compostatge es presenta com una alternativa sostenible per al reciclatge de la matèria orgànica continguda en gran quantitat de residus, urbans i industrials, que es generen a la societat actual. La legislació vigent, que prioritza el reciclatge i la valorització davant la disposició final de residus, i restringeix l'entrada de matèria orgànica als abocadors, ha fet que els gestors i productors de residus mostrin un interès creixent per aquesta tecnologia.

El nombre creixent de plantes de compostatge per tractar la fracció orgànica de residus municipals (FORM) a Catalunya i la manca de coneixement al nostre país sobre l'aplicació d'aquesta tecnologia a molts residus industrials, fan que l'any 2000 s'impulsi la creació d'una línia de recerca en compostatge a l'Escola Universitària Politècnica del Medi Ambient (EUPMA), centre adscrit a la Universitat Autònoma de Barcelona. La tesi doctoral que es presenta conforma els primers passos del grup de recerca de compostatge de l'EUPMA. Durant els primers 4 anys de treball s'ha construït la infrastructura necessària als laboratoris de l'EUPMA i s'han desenvolupat les tècniques analítiques que han permès la realització de nombrosos treballs experimentals, estenent l'aplicació del compostatge a residus poc estudiats i de naturalesa molt diversa. Molts d'aquests treballs són treballs tècnics d'aplicabilitat immediata, realitzats en contacte directe amb empreses que sol·liciten solucions per als seus residus. La recerca s'ha dut a terme sense oblidar, però, l'enfocament científic, i aplicant els coneixements previs en àrees com enginyeria bioquímica i biotecnologia dels membres del grup. La realització d'aquest treball s'emmarca en dos projectes de recerca subvencionats amb finançament públic per part del Ministeri de Medi Ambient i el Ministeri de Ciència i Tecnologia.

La tesi doctoral, que es presenta com a compendi de publicacions, inclou els següents articles publicats a revistes científiques d'impacte:

article I *Application of experimental design technique to the optimization of bench-scale composting conditions of municipal raw sludge*
Teresa Gea, Adriana Artola i Antoni Sánchez.
Compost Science and Utilization. 2003. Vol. 11(4). p. 321-329.

article II *Composting of wastes produced in the Catalan wine industry*
Teresa Gea, Adriana Artola, Xavier Sort i Antoni Sánchez.
Compost Science and Utilization. 2005. En procés de publicació.

article III *Composting of de-inking sludge from the recycled paper manufacturing industry*
Teresa Gea, Adriana Artola i Antoni Sánchez.
Bioresource Technology. 2005. Vol. 96(10). p. 1161-1167.

article IV *Monitoring the biological activity of the composting process: oxygen uptake rate (OUR), respirometric index (RI), and respiratory quotient (RQ)*
Teresa Gea, Raquel Barrena, Adriana Artola i Antoni Sánchez.
Biotechnology and Bioengineering. 2004. Vol. 88(4). p. 520-527.

Com a documentació addicional es presenten dos articles més, actualment en procés de revisió per a la seva publicació:

article V *Sanitation of sludge from wastewater treatment through composting*
Teresa Gea, Adriana Artola i Antoni Sánchez.
Process Biochemistry. Enviat: juliol 2004.

article VI *Co-composting of sewage sludge:fats mixtures and characteristics of the lipases involved*
Teresa Gea, Pau Ferrer, Gregorio Álvaro, Francisco Valero, Adriana Artola i Antoni Sánchez.
Journal of Biotechnology. Enviat: gener 2005.

L'eix temàtic de la tesi és l'estudi de la compostabilitat de fangs, motivat inicialment per les noves tendències legislatives que obliguen a la higienització dels fangs prèvia aplicació al sòl com esmena orgànica, essent el compostatge una de les tecnologies recomanades per a aquest fi. Així, l'article I presenta un estudi a escala laboratori de la compostabilitat de fangs frescos procedents d'una estació depuradora d'aigües residuals (EDAR) urbanes on s'avalua la influència de les característiques de l'agent estructurant en la compostabilitat potencial d'una mescla de fang amb estructurant. La presència d'agents estructurants en la barreja a compostar en proporció i característiques adequades permeten la bona transferència d'oxigen necessària per al correcte desenvolupament del procés de compostatge. Aquest estudi és ampliat a l'article V, considerant a més fangs digerits anaeròbiament i centrant-se en el potencial d'higienització de les barreges, tal com es requereix als esborranys de les corresponents Directives europees (Comissió Europea, 2001. *2nd draft on Biological treatment of biowaste*) i la normativa americana vigent (*Environmental Protection Agency,* 1995. *A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule*).

A l'article II es presenta un estudi complet sobre la co-compostabilitat dels dos residus orgànics més importants generats en la indústria vinícola, i que habitualment es gestionen de forma externa: el fang generat a l'estació depuradora que tracta les aigües residuals de la indústria, i la rapa (branquillons, fulles i restes de raïm un cop s'han extrets els grans), residu generat durant la verema que presenta característiques que suggereixen la seva utilització com agent estructurant. La possibilitat de co-compostar aquests dos residus a les mateixes instal·lacions on són produïts, utilitzant la rapa com agent estructurant per al fang, i d'aplicar el compost produït en les vinyes de la mateixa empresa, es presenta com una alternativa de gestió sostenible de residus. Aquest estudi analitza la compostabilitat de fangs d'EDAR des d'un punt de vista pràctic, complementant les experiències prèvies a escala laboratori i incloent una experiència a escala industrial.

A l'article III s'estudia la compostabilitat de dos tipus de fangs diferents que es generen en la indústria del reciclatge de paper. El gruix del treball experimental es centra en la compostabilitat dels fangs físico-químics que es generen en l'operació del destintat de paper, fang de característiques oposades a les que presenten habitualment els fangs d'EDAR, com ara un baix contingut en humitat, matèria orgànica i nutrients i una porositat més elevada. Es treballa també amb el fang biològic de l'EDAR de la mateixa indústria, que presenta característiques físiques diferents a la resta de fangs d'EDAR estudiats, especialment pel que fa al contingut en humitat i estructura dels agregats, molt més fibrosos car les aigües residuals arrosseguen fibra de paper.

L'article IV engloba part dels treballs anteriors analitzant els resultats obtinguts des d'un punt de vista biotecnològic, abordant la problemàtica de la monitorització de l'activitat biològica en el procés de compostatge. Inclou, a més de les experiències realitzades amb diferents tipus de fangs, experiments amb FORM. En aquest treball es proposa i discuteix l'ús de diferents índexs basats en l'activitat biològica aeròbia (índexs respiromètrics dinàmics i estàtics i quocient respiratori) com a paràmetre per monitoritzar el procés de compostatge.

Per últim, l'article VI presenta un estudi exhaustiu de la co-compostabilitat de fangs d'EDAR amb greixos animals, materials de característiques físico-químiques complementàries. La indústria alimentària genera anualment gran quantitat de residus amb elevat contingut en greixos, animals o vegetals. La compostabilitat d'aquests residus està poc estudiada i, a l'Estat Espanyol, els pocs treballs publicats corresponen principalment a residus de la indústria de l'oli d'oliva. En aquest treball, a més d'aplicar la metodologia desenvolupada en les experiències anteriors, s'analitza la co-compostabilitat d'ambdós materials des d'un punt de vista biotecnològic, realitzant assaigs enzimàtics com a mesura de l'activitat biològica i emprant tècniques de purificació i identificació d'enzims.

2. OBJECTIUS

L’objectiu principal de la tesi que es presenta és estudiar la viabilitat de les tècniques de compostatge i co-compostatge aplicades com a eines de tractament i estabilització de residus orgànics per a la producció d’un producte final estable. La consecució d’aquest objectiu general passa per la realització dels següents objectius parcials:

Dissenyar, construir i validar un sistema de compostatge a escala laboratori i una planta pilot per poder desenvolupar l’estudi del procés de compostatge aplicat a diferents residus orgànics.

Estudiar de forma sistemàtica, mitjançant la tècnica de disseny d’experiments, el compostatge de diferents tipus de fangs orgànics per determinar la influencia en el procés de determinats paràmetres físics.

Analitzar diferents paràmetres com a eines de seguiment de l’activitat biològica en el procés de compostatge i establir aquells que es puguin emprar de forma rutinària.

Estudiar la viabilitat del procés de compostatge i co-compostatge aplicats a fangs d'EDAR i a residus orgànics produïts a Catalunya la compostabilitat dels quals no està estudiada o aquells de gestió problemàtica.

3. RESUM GLOBAL DE RESULTATS I DISCUSSIÓ

Articles I i V: Compostabilitat de fangs d'EDAR

El primer estudi de la compostabilitat de fangs frescos d'EDAR es va dur a terme a escala laboratori per analitzar la influència de la mida de partícula de l'agent estructurant, la proporció d'agent estructurant afegit al fang, i el volum d'operació en el procés de compostatge. Amb aquest objectiu es va aplicar la tècnica estadística de disseny d'experiments, considerant com a factors les tres variables esmentades i realitzant un total de 32 experiments. Com agent estructurant es van utilitzar encenalls de fusta. La funció objectiu seleccionada va ser la calor relativa generada (CRG), que engloba els paràmetres temperatura assolida i temps i que es considera una mesura del potencial de compostabilitat i d'higienització de la barreja estudiada. El millor ajust per als resultats experimentals, validat estadísticament, es va obtenir amb una funció polinòmica de segon grau (Y) amb interaccions entre les variables estudiades (Equació 1).

$$Y = 49{,}7 - 5{,}96V - 22{,}7M - 24{,}7P - 43{,}6V^2 + 17{,}0M^2 + 8{,}35P^2 +$$

$$+ 8{,}76VM + 5{,}74VP + 11{,}7MP \qquad \text{(Equació 1)}$$

on: V: volum d'operació
M: mida de partícula
P: proporció estructurant:fang

Els coeficients de la funció obtinguda mostraven com les tres variables estudiades influencien el procés amb el mateix ordre de magnitud, i que l'efecte d'aquestes variables està interrelacionat.

L'Equació 1 es va utilitzar per determinar les condicions òptimes per compostar fangs d'EDAR a escala laboratori. La barreja ideal consistia en afegir encenalls de mida 0-5 mm als fangs d'EDAR en proporció volumètrica 1:1. La utilització d'agent estructurant de mida petita afavoreix la integració amb el fang, aconseguint una major reducció en la mida dels agregats de fang, i obtenint com a resultat una millor porositat de la mescla.

L'estudi de compostabilitat de fangs es va ampliar per analitzar i comparar el potencial d'higienització de fangs frescos (FF) i fangs digerits (FD), els dos tipus de fangs produïts de forma habitual a les EDAR's. Els experiments es van realitzar en reactors de 4,5 L i només es van considerar com a factors en el disseny d'experiments la mida de partícula de l'agent estructurant i la relació volumètrica estructurant:fang.

Per avaluar el potencial d'higienització es va seleccionar com a funció objectiu la reducció logarítmica de viabilitat del microorganisme patogen *Salmonella* al material, indicador proposat a la legislació. Aquesta va ser calculada a partir de les corbes de temperatura obtingudes a cada experiment. En general, els valors obtinguts de la funció objectiu per a FF van ser superiors als obtinguts per a FD, d'acord amb el menor contingut en matèria orgànica biodegradable d'aquests darrers.

Ajustant els resultats experimentals obtinguts a una funció Gaussiana (F), es van obtenir les Equacions 2 i 3 per a fang fresc i digerit respectivament.

$$F_{fang\ fresc} = 652{,}4 \cdot \exp\left[-0{,}5\left(\left(\frac{M-(-0{,}779)}{(-0{,}371)}\right)^2 + \left(\frac{P-(-1{,}082)}{(-0{,}272)}\right)^2\right)\right]$$

r= 0,99990 (Equació 2)

$$F_{fang\ digerit} = 2487{,}9 \cdot \exp\left[-0{,}5\left(\left(\frac{M-(-0{,}909)}{(-0{,}329)}\right)^2 + \left(\frac{P-(-1{,}038)}{(-0{,}173)}\right)^2\right)\right]$$

r= 0,99998 (Equació 3)

Els coeficients obtinguts van resultar molt similars per a tots dos tipus de fangs, indicant un comportament anàleg pel que fa a la seva compostabilitat.

La funció objectiu obtinguda es va optimitzar i, en ambdós casos, es van obtenir les mateixes condicions òptimes per a la higienització de fang a escala laboratori: mida de partícula de l'agent estructurant 0-5 mm i proporció volumètrica estructurant:fang 1:1. Aquests valors confirmen els resultats obtinguts a l'estudi anterior.

Els resultats a escala laboratori es van corroborar a escala pilot (100 L), demostrant que els efectes de compactació són negligibles per a la barreja estudiada i que l'òptim trobat proporciona prou porositat i resistència a la compactació en multiplicar el volum per un factor de 20.

La barreja estructurant:fang 1:1 amb encenalls de mida 0-5 mm es va fixar com a estàndard als laboratoris de l'EUPMA per als assaigs de compostabilitat de fangs d'EDAR.

Article II: Co-compostatge de residus de la indústria vinícola

Continuant amb l'estudi de la compostabilitat de fangs d'EDAR, es va realitzar un treball sobre el co-compostatge dels fangs de l'estació depuradora situada a les instal·lacions d'una indústria vitivinícola, i la rapa produïda durant la verema en la mateixa indústria. L'objectiu era utilitzar la rapa com agent estructurant per compostar el fang i obtenir un compost de qualitat aplicable a les vinyes de l'empresa.

Un dels punts més importants a considerar en aquest cas va ser l'estacionalitat en la producció de la rapa i els requeriments d'emmagatzematge derivats de la mateixa. Durant l'emmagatzematge es produeix la biodegradació dels components més làbils de la rapa, el que provoca canvis en les propietats físiques de la mateixa. Així, l'estudi també analitza l'evolució de la rapa durant l'emmagatzematge al llarg d'un any, i el possible efecte d'aquests canvis sobre el desenvolupament del procés de compostatge.

Durant el temps d'emmagatzematge, s'observà un increment de la porositat i la fracció d'aire lliure (FAL) de la rapa, i una reducció de la densitat aparent i la capacitat de retenció de camp. Aquest fet va ser degut principalment a la degradació de les fulles i les branques més primes que omplen els buits de l'estructura més rígida que proporcionen les branques grans, de degradació més lenta. En conseqüència, es pot preveure un augment en els requeriments d'estructurant en la barreja a compostar al llarg del temps per poder equilibrar el contingut en humitat.

Un dels principals problemes identificats fou l'elevat contingut en humitat dels materials emprats, fang i rapa. Les proves de compostabilitat de diferents barreges rapa:fang a escala laboratori no van assolir el rang termòfil principalment per aquest motiu. Es recomana emmagatzemar la rapa sota coberta per evitar l'entrada d'aigua procedent de la pluja.

A escala industrial es van construir dues piles amb proporcions rapa:fang 1:1 i 2:1. La primera presentava una humitat inicial del 81% i escassa porositat. Després de dues setmanes es va decidir aturar l'experiment ja que la pila no presentava activitat. La pila en proporció 2:1 va assolir temperatures per sobre de 50ºC en la primera setmana. Es van registrar temperatures superiors a 55ºC durant 28 dies, complint amb les recomanacions internacionals per assegurar la higienització de fangs.

Les elevades temperatures assolides i els freqüents voltejos aplicats van contribuir a la regulació de la humitat del material fins a nivells no crítics del procés de compostatge. Aquests resultats constaten les diferències entre els sistemes oberts i dinàmics i els sistemes tancats estàtics i expliquen l'èxit de les experiències a escala industrial en piles voltejades davant els resultats als reactors de laboratori. Si un material a compostar presenta problemes d'excés d'aigua, és recomanable la utilització d'un sistema obert i dinàmic que, a més, contribueix a la millor integració fang:estructurant i a la homogeneïtzació del material. A més, s'ha de tenir en compte que, tal i com es va concloure als estudis previs, la mida de partícula de l'estructurant i el volum d'operació són dues variables relacionades entre si. La mida de la rapa utilitzada podria haver resultat excessiva per als reactors de laboratori de només 4,5 L de volum.

El compost obtingut va presentar uns excel·lents índexs d'estabilitat i maduresa i l'absència d'elements fitotòxics, resultant doncs un material aplicable a les vinyes.

Aquesta estratègia de gestió sostenible dels residus s'està implementant actualment a l'empresa Miguel Torres, S.A.

Article III: Compostatge de fangs de paperera

Es va estudiar la possibilitat de compostar i co-compostar dos dels residus més importants produïts en la indústria del reciclatge de paper: fang biològic (FB, de l'EDAR de la mateixa indústria) i fang físico-químic (FFQ, del procés de destintat del paper). Aquest projecte, dut a terme per a una empresa paperera, va permetre aprofundir en l'estudi dels fangs biològics d'EDAR (FB) i en el seu comportament en ser co-compostats amb un fang físico-químic, el FFQ, de característiques oposades: baix contingut en humitat, matèria orgànica i nutrients.

El treball es va iniciar amb una bateria d'experiments a escala laboratori per determinar la compostabilitat dels materials, realitzant diferents proves amb FFQ i barreges de FFQ i FB, amb i sense addició d'agent estructurant. En les proves en que es va addicionar agent estructurant, les temperatures màximes assolides i el temps que es mantenien van ser inferiors que amb les barreges corresponents sense estructurant. L'estructura fibrosa d'ambdós fangs proporciona suficient porositat i evita la necessitat d'estructurant a escala laboratori. És necessari però, analitzar els possibles efectes de compactació en aquests materials treballant a escala industrial. Les barreges de FFQ i FB van assolir el rang termòfil i temperatures d'higienització, si bé els FFQ sense barrejar van oferir la millor compostabilitat tenint en compte aquest paràmetre.

Posteriorment, es va dur a terme una prova a escala pilot per estudiar en detall el procés de compostatge dels FFQ. El procés es va desenvolupar de forma correcta, assolint temperatures dins el rang termòfil durant dues setmanes i assegurant així la higienització del material. No es van observar efectes de compactació, donada l'estructura dels agregats. Es va obtenir una reducció de pes del 22% i de matèria orgànica del 33,5%. També es va seguir l'evolució del contingut de cel·lulosa, doncs s'esperava que fos un dels principals components de la matèria orgànica dels FFQ. El perfil de cel·lulosa va resultar molt similar a l'obtingut per a la matèria orgànica i la reducció total en cel·lulosa va ser del 68%. El material obtingut en aquest assaig presentava un elevat grau de maduresa i estabilitat en finalitzar el procés.

Article IV: Monitorització de l'activitat biològica

A partir dels experiments de compostatge duts a terme amb diferents residus orgànics, es van calcular 3 índexs biològics diferents: l'índex respiromètric dinàmic (IRD), l'índex respiromètric estàtic (IRE) i el quocient respiratori (QR). L'objectiu era determinar si aquests índexs són vàlids com a paràmetres de monitorització de l'activitat biològica en el procés de compostatge, donada la manca de paràmetres que descriuen aquest aspecte del procés. Així mateix, es pretenia comparar els índexs respiromètrics dinàmic i estàtic i estudiar els factors que afecten el QR. Els residus compostats van ser: fracció orgànica de residus municipals (FORM), fangs frescos (FF), fangs digerits (FD) i fangs paperera (FP).

L'IRE es va determinar a dues temperatures diferents: l'IRE_{37} a 37ºC (temperatura recomanada per la bibliografia quan s'utilitza l'IRE com a mesura d'estabilitat) i l'IRE_{T} a la temperatura de procés (temperatura del material en el moment de mostreig). Tots dos índexs van seguir una evolució similar a la temperatura, presentant valors màxims a l'etapa inicial de descomposició termòfila i una ràpida reducció durant les etapes de refredament i maduració. Així doncs, l'IRE es podria utilitzar com indicador de l'activitat biològica. Durant la primera etapa de descomposició (etapa termòfila) els valors de l'IRE_T resultaren significativament superiors als IRE_{37}. Probablement en realitzar el test a 37ºC, la població termòfila present en el residu en el moment de mostreig presenta una activitat limitada. En canvi, els valors d'IRE_{37} i IRE_T són molt similars durant la fase de maduració, en la qual la temperatura del material al reactor es troba al rang mesòfil. Comparant els valors obtinguts per als diferents materials, es va observar com l'IRE era superior per a la FORM i els FF que per als FD i els FP, d'acord amb la biodegradabilitat esperada d'aquests substrats.

El perfil obtingut per a l'IRD també va resultar similar al perfil de temperatura, de manera que també es podria considerar un paràmetre indicador de l'activitat biològica. Ja que la determinació del IRD és més costosa i complicada a nivell industrial que la del IRE, es van comparar els dos paràmetres, IRD i IRE_T. Tot i que tots dos índexs presentaren un perfil similar, els valors d'IRD van resultar superiors als de l'IRE_T durant l'etapa termòfila, probablement degut a limitacions difusionals en la respirometria estàtica. A la fase mesòfila de maduració, per contra, tots dos paràmetres presentaren valors similars, probablement degut a que el factor limitant en el consum d'oxigen passa a ser l'activitat biològica en lloc de la difusivitat de l'oxigen a la matriu sòlida.

El QR es va mantenir aproximadament constant durant el procés de compostatge, de manera que queda descartat com paràmetre de monitorització de l'activitat biològica. Tanmateix, el fet de que es mantingui constant pot ser d'utilitat en la modelització del procés, donat que és un paràmetre íntimament lligat a l'activitat metabòlica dels microorganismes.

Article VI. Co-compostatge de fangs d'EDAR amb greixos

Els residus rics en greixos que es produeixen en alguns sectors industrials del nostre país, especialment la indústria alimentària, es caracteritzen per tenir un elevat contingut energètic, baixa biodegradabilitat i elevada hidrofobicitat. La seva gestió mitjançant la tecnologia de compostatge només es pot plantejar com el co-compostatge amb un residu que aporti la humitat i els nutrients que manquen als greixos. Així, es va estudiar el procés de co-compostatge de greixos amb fangs d'EDAR, residus de característiques complementàries. Donada l'escassa porositat d'ambdós residus, a la barreja fang:greix se li va afegir estructurant en la proporció i de la mida prèviament determinada com a òptima per a barreges a escala laboratori (1:1, 0-5 mm). Aquest estudi va permetre la utilització d'una nova mesura d'activitat biològica més específica d'aquest residu, l'activitat enzimàtica lipolítica responsable de la degradació dels greixos, i l'aplicació de tècniques de purificació i identificació d'enzims.

El primer objectiu fou estudiar el comportament del procés per a diferents proporcions de greix afegit al fang i determinar els límits de compostabilitat i les proporcions òptimes. Es van realitzar experiments amb barreges que contenien del 0 fins al 80% de greix. En aquests, s'observà que les barreges fins a un 50% de greix compostaven correctament però per a concentracions més elevades de greix, les mancances en contingut d'humitat i difusió d'oxigen resultaven limitants per al procés.

També es va observar que, a major quantitat de greix afegit al fang, major era la durada de la fase termòfila. Aquest fet és d'especial interès doncs apunta a la utilització de greixos com a co-substrat per a altres residus orgànics de baix potencial energètic amb els que resulti difícil assolir el rang termòfil de temperatures o sostenir-lo per garantir la higienització. Tot i que les barreges fins un 50% de greix poden ser compostades, es recomana treballar a menors concentracions per evitar temps de procés massa elevats, especialment si s'ha de realitzar aquest procés de compostatge a escala industrial. A partir dels resultats obtinguts, es pot considerar com límit màxim un 30% de greix en termes de reducció en matèria seca (40%) i contingut en greix (85%) i de durada de la fase termòfila. Aquesta mescla també va presentar els valors més elevats d'activitat lipolítica de les barreges estudiades a escala laboratori.

El treball es va completar amb un estudi detallat a escala pilot de la barreja amb el 30% de greix, màxim recomanat, per monitoritzar paràmetres biològics rellevants. El procés es va desenvolupar correctament, oferint un perfil de temperatura típic d'un procés de compostatge i es van assolir els requeriments d'higienització. Degut a la naturalesa altament hidrofòbica dels greixos, es van produir grans quantitats de lixiviats que van provocar la reducció del contingut en humitat fins a valors limitants per a l'activitat biològica. En conseqüència, el contingut en humitat esdevé un factor de control crític per al correcte desenvolupament del procés de compostatge quan hi ha greixos presents.

La reducció en matèria seca i en contingut total en greix, van ser similars a les obtingudes a escala laboratori. Tot i així, no es va detectar activitat enzimàtica lipolítica durant els primers dies de procés, tot i que l'acumulació d'àcids grassos de cadena llarga evidenciava que aquesta activitat existia. Aquest fet podria estar relacionat amb l'afinitat de les lipases per adsorbir-se a la superfície de la fase sòlida, el que podia provocar que el mètode d'extracció de l'enzim utilitzat hagués estat insuficient.

Després de 69 dies de procés, el material obtingut presentava un elevat grau de maduresa i estabilitat.

Es va dur a terme un estudi complet de les característiques de les lipases extretes del material durant el procés de compostatge. Per a l'estudi de l'estabilitat de les lipases a diferents pH i temperatures, s'emprà la tècnica del disseny d'experiments. L'Equació 4 mostra la funció obtinguda per descriure la relació entre l'activitat residual i els factors estudiats.

$$AR = 114{,}2 - 49{,}2T + 28{,}0\,pH - 42{,}9T^{2} - 35{,}6\,pH^{2} - 10{,}3TpH \quad \text{(Equació 4)}$$

on AR: activitat residual (%)
T: temperatura.

De l'anàlisi de l'expressió obtinguda es deriva que hi ha un efecte encreuat dels dos factors estudiats (terme d'interacció T-pH) i que les lipases són lleument més sensibles a l'efecte de la temperatura que al pH (coeficients per a T més grans que per a pH). Optimitzant la funció obtinguda, s'observà que la màxima estabilitat dels enzims extrets correspon a un valor de temperatura dins el rang mesòfil (38ºC) i a pH lleugerament alcalí (pH 8).

La mostra d'enzim es va purificar mitjançant cromatografia de bescanvi iònic, separant una submostra que presentava activitat lipolítica. Fent una electroforesi en gel de poliacrilamida es van observar dues bandes principals corresponents a pesos moleculars de 29 i 62 kDa. No va ser possible identificar les proteïnes corresponents a aquestes dues bandes mitjançant l'anàlisi de la seqüència terminal d'aminoàcids.

4. CONCLUSIONS FINALS

Les conclusions de la present tesi doctoral són:

S'ha dissenyat, construït i validat un sistema a escala laboratori i una planta pilot de compostatge en els laboratoris de l'EUPMA. Aquestes instal·lacions, en millora i ampliació continua, s'utilitzen actualment per dur a terme els treballs experimentals del grup de recerca en compostatge de l'EUPMA.

S'ha utilitzat la tècnica estadística del disseny d'experiments com eina sistemàtica per a l'estudi del procés de compostatge, obtenint la descripció del procés en els paràmetres estudiats. La metodologia emprada pot ser aplicada en l'estudi de la compostabilitat de diferents residus i és especialment útil per trobar les barreges òptimes en estudis de co-compostabilitat.

S'han utilitzat i analitzat diferents paràmetres com a eines de seguiment de l'activitat biològica en el procés de compostatge, concloent:

- L'índex respiromètric dinàmic resulta el paràmetre més adient com a mesura de l'activitat biològica en el procés de compostatge.
- L'índex respiromètric estàtic, determinat a temperatura d'operació, subestima l'activitat biològica en les primeres etapes del procés (etapa de descomposició termòfila) degut a limitacions en la difusió d'oxigen en la matriu sòlida durant l'assaig en condicions estàtiques. Tot i així es pot utilitzar com indicador de l'activitat biològica del procés de compostatge ja que la seva determinació és més econòmica que la de l'IRD.

- L'índex respiromètric estàtic, determinat a 37ºC, s'hauria d'utilitzar només com a mesura d'estabilitat en mostres procedents d'etapes mesòfiles finals de procés.
- El quocient respiratori és un paràmetre de control de les condicions aeròbiques del procés, però no es pot utilitzar com a paràmetre de monitorització de l'evolució de l'activitat biològica durant el procés de compostatge.

Algunes conclusions parcials obtingudes sobre els residus estudiats han estat:

- La compostabilitat d'una barreja fang:estructurant està influenciada pel volum d'operació, la mida de partícula i la proporció d'estructurant en el mateix ordre de magnitud. L'efecte d'aquestes tres variables està interrelacionat.
- Una barreja fang:encenalls en proporció volumètrica 1:1 i amb encenalls de mida de partícula 0-5 mm resulta òptima per a assaigs de compostabilitat a escala laboratori. Els efectes de compactació en aquesta barreja són negligibles a escala pilot.
- El co-compostatge dels fangs d'EDAR i la rapa produïts en la indústria vinícola és una alternativa viable per a la gestió sostenible d'aquests residus. El compost produït és una esmena orgànica d'excel·lent qualitat, aplicable a les vinyes.
- El compostatge de fangs en un sistema obert dinàmic, com una pila voltejada amb una freqüència elevada, es recomana com una alternativa òptima per al tractament d'aquest material orgànic, front a sistemes tancats estàtics, per a una millor homogenització, integració dels materials i control d'humitat.
- El fang biològic i el fang físico-químic del procés de destintat de la indústria paperera poden ser compostats de forma separada o co-compostats de forma conjunta, sense l'addició d'estructurant ni esmenes riques en nitrogen, a escala laboratori i pilot.
- És possible co-compostar fangs amb un contingut total de greixos fins al 50%, tot i que es recomana no excedir el 30% per a una millor eficàcia en la reducció del contingut en greix.

- A major quantitat de greix afegit a un fang, major durada de la fase termòfila. L'addició de greixos a materials amb baix contingut energètic pot ser una estratègia per assolir la completa higienització d'un material.
- Les lipases obtingudes durant el procés de co-compostatge de fangs i greixos presenten major estabilitat a temperatures mesòfiles i pH lleument alcalins.

Com a conclusió final, queda validat el co-compostatge com a eina de tractament i estabilització de residus orgànics, per a la producció d'un producte final estable.

5. ABREVIACIONS

Llista d'abreviacions emprades en la redacció d'aquesta memòria, i el seu equivalent en anglès, tal i com es troba a les publicacions presentades.

Abreviació	**Nom complet**	**Abreviació en anglès**	**Nom complet en anglès**
CRG	Calor Relativa Generada	RHG	*Relative Heat Generation*
EDAR	Estació Depuradora d'Aigües Residuals	WWTP	*Waste Water Treatment Plant*
EUPMA	Escola Universitària Politècnica del Medi Ambient		
FAL	Fracció d'Aire Lliure	FAS	*Free Air Space*
FB	Fangs Biològics	BS	*Biological Sludge*
FD	Fangs Digerits	ADS	*Anaerobically Digested Sludge*
FF	Fangs Frescos	RS	*Raw Sludge*
FFQ	Fangs Físico-Químics	PQS	*Physico-Chemical Sludge*
FORM	Fracció Orgànica de Residus Municipals	OFMSW	*Organic Fraction Of Municipal Solid Waste*
FP	Fangs de Paperera	PS	*Paper Sludge*
IRD	Índex Respiromètric Dinàmic	DRI	*Dynamic Respiratory Index*
		OUR	*Oxygen Uptake Rate*
IRE	Índex Respiromètric Estàtic	SRI	*Static Respiratory Index*
QR	Quocient Respiratori	RQ	*Respiratory Quotient*

6. ARTICLES PUBLICATS

A continuació es presenten els articles publicats que conformen la present tesi doctoral:

article I *Application of experimental design technique to the optimization of bench-scale composting conditions of municipal raw sludge*
Teresa Gea, Adriana Artola i Antoni Sánchez.
Compost Science and Utilization. 2003. Vol. 11(4). p. 321-329.

article II *Composting of wastes produced in the Catalan wine industry*
Teresa Gea, Adriana Artola, Xavier Sort i Antoni Sánchez.
Compost Science and Utilization. 2005. En procés de publicació.

article III *Composting of de-inking sludge from the recycled paper manufacturing industry*
Teresa Gea, Adriana Artola i Antoni Sánchez.
Bioresource Technology. 2005. Vol. 96(10). p. 1161-1167.

article IV *Monitoring the biological activity of the composting process: oxygen uptake rate (OUR), respirometric index (RI), and respiratory quotient (RQ)*
Teresa Gea, Raquel Barrena, Adriana Artola i Antoni Sánchez.
Biotechnology and Bioengineering. 2004. Vol. 88(4). p. 520-527.

article I

Application of experimental design technique to the optimization of bench-scale composting conditions of municipal raw sludge

Teresa Gea, Adriana Artola i Antoni Sánchez.

Compost Science and Utilization. 2003. Vol. 11(4). p. 321-329.

Application of experimental design technique to the optimization of bench-sca...
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Compost Science & Utilization; Autumn 2003; 11, 4; ProQuest Direct Complete
pg. 321

Compost Science & Utilization, (2003), Vol. 11, No. 4, 321-329

Application of Experimental Design Technique to the Optimization of Bench-Scale Composting Conditions of Municipal Raw Sludge

María Teresa Gea Leiva, Adriana Artola Casacuberta and Antoni Sánchez Ferrer
Escola Universitària Politècnica del Medi Ambient, UAB, Barcelona, Spain

Evaluation of the parameters affecting the compostability of dewatered raw sludge from a wastewater treatment plant has been carried out by means of a full factorial composite experimental design. The factors and their correspondent levels considered in the experimental design were: bulking agent:sludge ratio (from 1:1 to 4:1 by volume), bulking agent particle size (from 0-5 mm to 10-20 mm) and composting volume (in the range of typical laboratory-scale, from 1.5 to 25 L). Experimental design has permitted obtaining a polynomial second-order function that quantitatively describes the influence of the considered factors on the compostability of this waste in laboratory reactors. The function has been numerically optimized to find the optimal values for composting raw sludge resulting in a values of: 0-5 mm for bulking agent size, 1:1 for ratio bulking agent:sludge and a composting volume around 10 L. The use of this technique can be generalized and applied to the composting of other organic wastes and will permit comparison of composting performance of different wastes.

Introduction

The unique legal restriction to direct land application of sludge is given by its content on heavy metals and potentially toxic compounds. The European Union Commission is preparing a Directive on the biological treatment of organic wastes (European Commission 2001). According to this Directive, the hygienization of sludge before its application to land will be mandatory. Composting is one of the alternatives proposed by the Directive in order to achieve sludge hygienization.

The water content of sewage sludge is usually extremely high for direct composting even if a dewatering treatment is applied to this material. In a majority of cases, a bulking agent is needed to reach optimum water content, porosity and free air space (FAS) (Haug 1993). Many experiences are reflected in the literature referencing the research of optimum sludge-bulking agent mixtures for urban or industrial sludge composting. A long list of waste materials has been proposed as bulking agents in these experiences including, among others, green waste (Sesay *et al.* 1997), wheat or barley straw (Lasaridi *et al.* 2000; Miner *et al.* 2001), rice husks (Morisaki *et al.* 1989) or peat moss (Milne *et al.* 1998), although the most widely used materials are wood chips and sawdust (Atkinson *et al.* 1996; Larsen and McCartney 2000; Wong *et al.* 1997; Wong and Fang 2000). In addition to the type of bulking agent used, its particle size has been also emphasized as an important factor in sludge composting. Large bulking agent particles will provide an excess of FAS which will result in an oscillating temperature profile during composting (Jokela *et al.* 1997). The proportion of bulking agent in the final mixture has also been highlighted as a factor influencing the composting process (Morisaki *et al.* 1989; Jokela *et al.* 1997). Bulking agent:sludge volumetric ratios used in the reported experiences range from 0.5:1 to 6:1 depending mainly on the water content of the sludge (Wong *et al.* 1995; Larsen and McCartney 2000). An excess of bulking agent will lead to a low content on biodegradable organic matter in the final mixture. Sludge composting experiences are presented in some cases at different scales, laboratory and pilot plant (Sesay *et al.* 1997;

Jokela *et al.* 1997). The main difference observed at different scales is temperature evolution. The amount of heat generated and the heat retention capacity of the material increase as the volume of the treated material increases (Sherman-Hutton 2000).

If the real influence of such a number of factors on sludge composting has to be statistically determined, a detailed methodological study will be required including a large number of experiments.

The objective of this work is to examine the influence on relative heat generation (RHG) during raw sludge composting of the next three factors: bulking agent particle size, bulking agent:sludge volumetric ratio and operation volume (at laboratory scale). RHG is directly related to initial mixture water content because moisture determines the specific heat of the material (Haug 1993). Nevertheless, since the selected factors for this study (bulking agent:sludge volumetric ratio and bulking agent particle size) determine water content, the effect of this parameter is implicit in the obtained results.

A full composite factorial experimental design technique has been used to plan the experiments needed. The experimental design technique will be used to learn about the influence of these factors on the sludge composting process and to determine the relationship among them. The variable studied is the integration of temperature curve as a measure of relative heat generation. The feasibility of the experimental design technique has been widely demonstrated in other fields (Lay *et al.* 1999; Sanchez *et al.* 2000a; Ricou-Hoeffer *et al.* 2001). Its application to the sludge composting process will provide a methodology to face future studies on the compostability of other types of organic waste.

Materials and Methods

Materials

The waste material under study was dewatered raw sludge from the urban wastewater treatment plant of La Garriga (Barcelona, Spain). The dewatered raw sludge was obtained by centrifugation of activated sludge from the biological wastewater treatment. Wood chips from a local carpentry were used as bulking agent. The chips consist of a variable mixture of pine and beech tree wood. The characteristics of these materials are presented in Table 1 (presented as average values during sampling time). The parameters summarized in Table 1 were determined according to standard procedures (APHA-AWWA-WPCF 1992).

TABLE 1.
Average properties of dewatered raw sludge and wood chips used in the experiments (in brackets, standard deviation values).

Property	Dewatered Raw Sludge	Wood Chips
Water content (%)	73 (5)	5
Volatile solids content (%)	60 (4)	99.4
N-Kjeldhal content (%)	2.5 (0.4)	0.1
C/N ratio	13 (3)	500

Sludge and wood chip mixtures were handmade after screening of the bulking agent. A semi-industrial sieve (Filtra Vibración, FT-400) with a diameter of 400 mm was used for wood chip screening. Three different screens (20, 10 and 5 mm mesh) were available. Initial mixtures water content ranged from 79.6% to 47.3%.

Vessels

Three different vessels were used in the reported experiments: a 40-L reactor (working volume: 25L) and 4.5-L and 1.5-L Dewar® vessels. A schematic representation of the 40-L reactor is presented in Figure 1. The reactor is a cylindrical methacrylate tank with a perforated plate (at point 6) near the bottom that divides the vessel in two compartments. The upper compartment (A) over the perforate plate, contains the material to be composted while the empty compartment (B) under the perforated plate allows excess water content to leach providing at the same time an air distribution chamber when air supply is applied (air inlet at point 1). Leachate is recovered through a valve (2). The tank is top-covered with a methacrylate cover perforated in six points to allow exit of exhaust gas and monitoring of temperature (3) and oxygen concentration (4) in the tank. The vessel was thermo-insulated (5) with Cemiflex ® foam (Cemiflex, Spain).

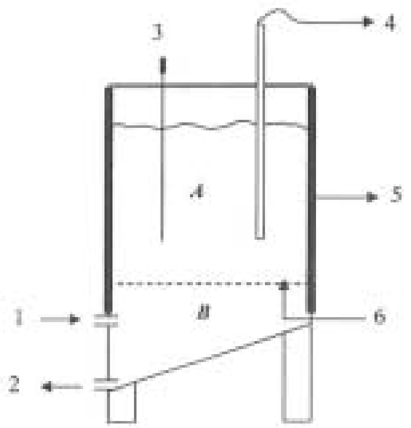

Figure 1. Scheme of the 40-L vessel used in composting at laboratory scale. 1-Air inlet, 2-Leachates outlet, 3-Temperature probe, 4-Air aspiration to oxygen sensor, 5-Thermal insulation, 6-Perforated plate, A-Composting volume, B-Leachates collection and air supply.

The Dewar vessels were conditioned for composting providing a stopper and placing a rigid wire net near the bottom to separate the material from a possible leachate. The stopper was perforated in two points for temperature monitoring and for air supply, if necessary.

Prior to the experiments with sludge, all vessels were validated for their use as composting tanks processing source separated organic fraction of municipal solid waste (water content: 78 %, volatile solids content: 75 %), (Gea 2001).

Temperature Monitoring

Pt-100 sensors were used for temperature monitoring connected to a data acquisition system (DAS-8000, Desin, Spain) which is connected to a standard PC. The system allows, by means of the proper software (Proasis® Das-Win 2.1, Desin, Spain), the continuos on-line visualization and registration of the value of different parameters (*i.e.* temperature and oxygen). Pt-100 sensors were placed in the material to have a measuring point at 1/3 of the height of the material in the vessel.

Oxygen Control

Oxygen concentration in intersticial air was monitored with an oxygen sensor (Sensox, Sensotran, Spain). In Dewar vessels, oxygen control was carried out manually, whereas the 40-L reactor is equipped with a feedback oxygen control which automatically supplies fresh air to the reactor when oxygen concentration is below 10%.

Experimental Design

Optimization of composting conditions was performed by means of a full composite factorial experimental design (Sánchez *et al.* 2000a; Sánchez *et al.* 2000b). Briefly, full factorial experimental design is based on the evaluation of the coefficients fitting a polynomial function (Y), which is proposed to describe the system under study (see, for instance, Equation 2 and 3). This polynomial function is an algebraic expression that combines the different factors (x_i) that have been taken into account and their polynomial coefficients (b_i). The factors considered were chosen according to the results obtained in previous experiments in sludge composting. Coefficients vector (B) of the function Y is calculated according to Equation 1 (Trochim 2002a; Trochim 2002b):

$$B = \left(M^T M\right)^{-1} M^T F_{obj} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where M is the matrix of experiments that includes the normalized levels of the considered factors, and F_{obj} is the proposed objective function (in this case, relative heat generation). A number of experiments were replicated to validate statistically the consistence of the proposed function representing the system.

Calculation of Equation 1 was carried out by a self-made software developed with Microsoft Fortran Powerstation® 4.0 (1994-1995).

Optimization of objective function was carried out by a quasi-Newton method using IMSL® libraries included in Microsoft Fortran Powerstation® 4.0 (1994-1995).

Results

Previous Experiments

Preliminary experiments (data not presented) showed that during dewatered raw sludge composting in static laboratory systems, the thermophilic range of temperature was not always reached, even when the same bulking agent:sludge ratio used in composting full-scale plants was chosen. Moreover, Haug (1993) observed a clear influence of the bulking agent particle size and the operation scale, especially in cases when wet substrates (such as raw sludge) were used.

The problems detected can be overcome by means of a systematic and statistical study on the influence of these factors on the performance of composting of the waste under study.

Experimental Design

The study of the factors affecting the composting process of dewatered raw sludge mixed with wood chip as bulking agent was carried out by means of a full composite factorial experimental design.

Bulking agent particle size, bulking agent:sludge volumetric ratio, and operation volume were selected as the main experimental factors that control the composting process. Bulking agent particle size and the bulking agent:sludge volumetric ratio directly determine Free Air Space (FAS) and water content (Haug 1993), which are directly related to heat retention capacity. On the other hand, a high amount of total composting mass implies a large thermal inertia, and thus, a higher hygienization potential (Sherman-Hunton 2000).

The levels of the factors considered in the experimental design are presented in Table 2. Wood chips were sieved to typical particle size values found in full-scale com-

TABLE 2.
Factors and levels considered in the experimental design (normalized values in parentheses).

Factor	Levels		
x_1 : operation volume (L)	1.5 (-1)	4.5 (-0.745)	25 (+1)
x_2 : bulking agent particle size (mm)	0-5 (-1)	5-10 (-0.333)	10-20 (+1)
x_3 : bulking agent:sludge volumetric ratio	1:1 (-1)	2:1 (-0.333)	4:1 (+1)

posting plants, whereas values of bulking agent:sludge volumetric ratio were selected among the typical referred values found elsewhere. The operation volumes were determined for the vessels available in the laboratory.

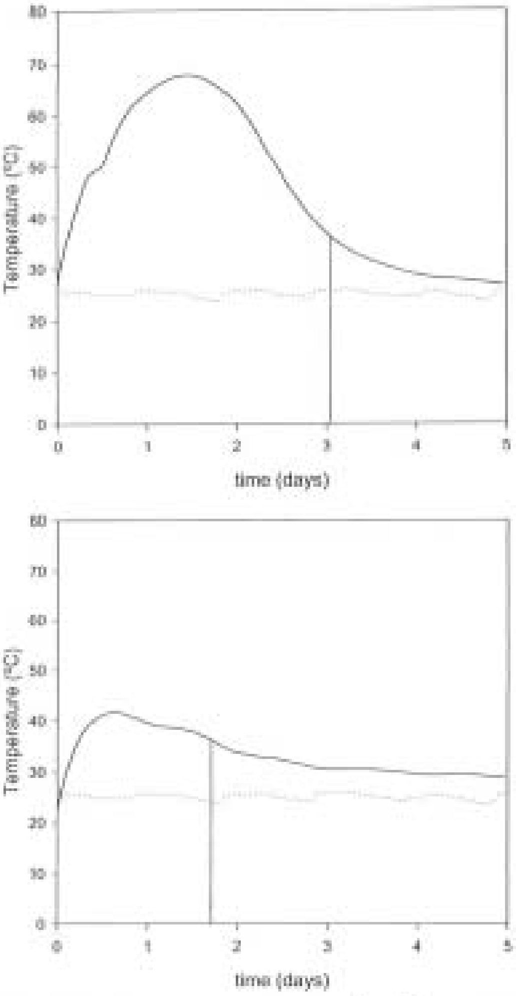

Figure 2. Evolution of temperature profiles for the experiment 1.5/5/2:1 (a) and 25/10/2:1 (b). ____ Material Temperature; .... Room Temperature; Vertical line: End Point for the integration.

The considered factors and their respective levels involved the realization of 27 (3x3x3) experiments, which were carried out following the evolution of temperature as a function of time in each case. Due to the high number of experiments, only the first thermophilic stage of the composting process was monitored, which in most cases corresponded approximately to 4 or 5 days. Experiments were carried out without air supply since it was previously observed that no oxygen limitation was found for any conditions tested in the experimental design (data not shown). To statistically validate the obtained results, 5 experiments were randomly chosen and carried out as replications to complete the experimental design, for a total of 32 experiments.

Once the factors to be studied and their levels were chosen, the next step in the experimental design was the selection of an objective function (F_{obj}). To evaluate the composting and hygienization potential of raw sludge:wood chips mixture, relative heat generation (RHG) based on the temperature achieved was chosen as the objective function. This parameter has been previously used in the composting field as a measure of composting potential (Larsen and McCartney 2000; Leth *et al.* 2001). In this work, RHG was defined as the area between the reactor and room temperature curves, as shown in Figure 2. The RHG value was obtained by the Simpson integration method, considering the end point of the process when reactor temperature dropped to a value lower than 10° C over room temperature. Values of the objective function obtained for each experiment are presented in Table 3.

The next step in the experimental design was the election of a polynomial function (Y) that properly fitted the experimental results. This function was obtained to describe the process under study and it was used to check the influence of the factors on the composting process of raw sludge. The quality of the fitting for all the tested functions was estimated from the value of the correlation coefficient R^2.

The simplest function proposed was a linear function considering all the factors studied and it is represented by Equation 2:

$$Y = b_0 + \sum_{i=1}^{3} b_i x_i \quad \text{.........................} \quad (2)$$

TABLE 3.
Experimental results for the full factorial experimental design.

Experiment	Operation Volume x_1 (L)	Bulking Agent Particle Size x_2 (mm)	Volumetric Ratio x_3	Objective Function (F_{obj}, RHG)
25/5/1:1	25	0 – 5	1:1	78.29
25/5/2:1	25	0 – 5	2:1	17.29
25/5/4:1	25	0 – 5	4:1	13.66
25/10/1:1	25	5 – 10	1:1	33.26
25/10/2:1	25	5 – 10	2:1	22.87
25/10/4:1	25	5 – 10	4:1	0
25/20/1:1	25	10 – 20	1:1	27.80
25/20/2:1	25	10 – 20	2:1	2.88
25/20/4:1	25	10 – 20	4:1	0
4.5/5/1:1	4.5	0 – 5	1:1	124.07
4.5/5/1:1-ii	4.5	0 – 5	1:1	100.99
4.5/5/2:1	4.5	0 – 5	2:1	152.18
4.5/5/4:1	4.5	0 – 5	4:1	16.25
4.5/5/4:1-ii	4.5	0 – 5	4:1	70.77
4.5/10/1:1	4.5	5 – 10	1:1	136.93
4.5/10/2:1	4.5	5 – 10	2:1	24.30
4.5/10/2:1-ii	4.5	5 – 10	2:1	39.12
4.5/10/4:1	4.5	5 – 10	4:1	10.51
4.5/10/4:1-ii	4.5	5 – 10	4:1	15.46
4.5/20/1:1	4.5	10 – 20	1:1	36.24
4.5/20/1:1-ii	4.5	10 – 20	1:1	28.70
4.5/20/2:1	4.5	10 – 20	2:1	11.17
4.5/20/4:1	4.5	10 – 20	4:1	5.58
1.5/5/1:1	1.5	0 – 5	1:1	86.27
1.5/5/2:1	1.5	0 – 5	2:1	87.32
1.5/5/4:1	1.5	0 – 5	4:1	16.25
1.5/10/1:1	1.5	5 – 10	1:1	63.91
1.5/10/2:1	1.5	5 – 10	2:1	25.09
1.5/10/4:1	1.5	5 – 10	4:1	0
1.5/20/1:1	1.5	10 – 20	1:1	32.83
1.5/20/2:1	1.5	10 – 20	2:1	20.68
1.5/20/4:1	1.5	10 – 20	4:1	0

A low correlation coefficient, $R^2 = 0.62$ was obtained in this case, which indicated a poor fitting of experimental data. In consequence, different new terms were included in the linear function (Equation 2), to take into account possible interactions between the considered factors. The values of R^2 showed that the best modeling function was a full second-order function including quadratic terms and interactions for x_1, x_2 and x_3 (Equation 3), where the value of R^2 was 0.77.

$$Y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=4}^{6} b_i x_i^2 + b_7 x_1 x_2 + b_8 x_1 x_3 + b_9 x_2 x_3 \quad \cdots\cdots\cdots\cdots\cdots (3)$$

The values of coefficients b_i for Equation 3 are presented in Table 4.

The quality of the fitting was statistically validated by means of an F-test. Experimental value of F-test was 8.10, higher than the tabulated value for a confidence of 99%, which was 3.35. Thus, it could be assumed that the contributions of the factors were more significant than experimental error in the fitting of the statistical model. Therefore, a statistically validated function described by Equation 3 could be used for the quantitative representation of the composting of raw sludge using wood chips as a bulking agent.

Moreover, analysis of b_i coefficients (Table 4) indicated that the three factors under study affected the composting process in a similar order of magnitude. Also, the interaction between the variables was as significant as the variables themselves since the interactions affected the process in a similar order of magnitude. Analysis of the obtained function (Y) allows the study of the influence and interactions between the factors. For instance, for a fixed volume (x_1=0), Figure 3 shows the combined influence of bulkinpg agent particle size (x_2) and bulking agent:sludge volumetric ratio (x_3) on objective function.

The fact that the variables are interrelated is of special relevance since the influence of these factors on composting performance is often considered separately or on the basis of adjusting a proper mixture water content or C:N ratio (Wong, *et al.* 1997; Milne *et al.* 1998; Kalyuzhnyi *et al.* 1999; Miner *et al.* 2001). However, this procedure does not ensure a correct Free Air Space value of the initial mixture.

TABLE 4.
Values of b_i coefficients obtained for Eq. 3.

Coeficient	Coeficient for	Value
b_0	-	49.68
b_1	x_1	-5.963
b_2	x_2	-22.73
b_3	x_3	-24.67
b_4	x_1^2	-43.56
b_5	x_2^2	17.00
b_6	x_3^2	8.353
b_7	$x_1 \cdot x_2$	8.755
b_8	$x_1 \cdot x_3$	5.745
b_9	$x_2 \cdot x_3$	11.73

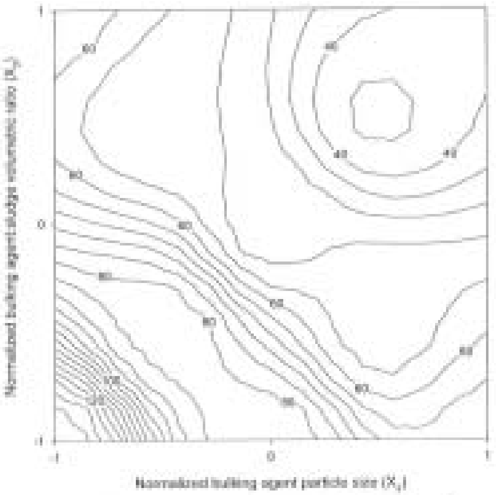

Figure 3. Surface response of Equation 3 for a fixed normalized value of X_1=0.

Optimization

Function Y obtained was used to determine the optimal conditions to carry out the composting process of a raw sludge:wood chips mixture under laboratory conditions, by means of an optimization program based on a quasi-Newton method. The results obtained were:

bulking agent particle size: 0 – 5 mm
bulking agent:raw sludge ratio: 1:1
operation scale: 10.49 L

The optimum mixture for the composting process carried out in laboratory scale vessels consisted of wood chips from 0 to 5 mm, mixed in a 1:1 ratio with raw sludge. The small particle size produced a very homogeneous mixture with small sized aggregates, with a high porosity and more accessible to microorganisms and air, breaking the typical compacted structure of a dewatered sludge. This effect could be observed when mixing the materials for the experiments, and it was also reflected in partial results since maximum Relative Heat Generation values corresponded to the optimal mixture and the 2:1 ratio mixture in the three scale levels studied (Table 3). It is worthwhile to mention that the small sized wood chips can also act as amendment for raw sludge and not only as a bulking agent, since some carbon source is available for microorganisms and contributes to balance the excess of nitrogen often found in raw sludge (Haug 1993).

The optimum value found for the operation volume indicated that the mixture 1:1 with 0-5 mm wood chips was suitable for laboratory scale operation when working with the studied materials. However, at full-scale operation, it seems evident that others factors such as compression, agitation, etc., can play an important role in the available Free Air Space (McCartney and Chen 2001).

The fact that the optimum value was near the lowest limit of the studied interval of volumes could be related to the different thermal insulation capacity of the used vessels. Thus, it seemed evident that the 1.5-L and 4.5-L Dewar vessels insulate the material and prevent heat loses in a more effective way than the 25-L reactor. Although the three vessels had been previously validated composting source-separated organic fraction of municipal solid waste (MSW), reaching temperatures above 70°C for extended periods of time, the 25 L reactor did not have enough heat retention for materials such as raw sludge. Laboratory experiences show that biodegradation rate in the first stages of sludge composting process is lower than MSW, even with the same water content in the initial mixture.

Therefore, it can be concluded that composting of raw sludge at laboratory scale requires a proper thermal insulation.

Nevertheless, the optimization of the function obtained from the factorial experimental design has permitted the determination of the optimal conditions for the composting of raw sludge, proving to be a useful technique that can be applied to other similar wastes.

Conclusions

The composting process of raw sludge from urban wastewater treatment plants has been systematically studied, determining the factors affecting the compostability of this waste. In this work, the quantification of the influence of bulking agent:sludge ratio and the particle size of the added bulking agent are of special interest.

Experimental design technique has proved to be a valid tool to determine the optimal initial operation conditions for the composting of raw sludge. Moreover, the systematic study of composting conditions by means of experimental design can be generalized and applied to the composting of other organic wastes and will permit the comparison of composting performance of different wastes.

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article II

Composting of wastes produced in the Catalan wine industry

Teresa Gea, Adriana Artola, Xavier Sort i Antoni Sánchez.

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Composting of Wastes Produced in the Catalan Wine Industry

Teresa Gea(1), Adriana Artola (1), Xavier Sort (2) and Antoni Sánchez *(1)

(1) Escola Universitària Politècnica del Medi Ambient
Universitat Autònoma de Barcelona
Rbla Pompeu Fabra 1, 08100-Mollet del Vallès (Barcelona, Spain)
(2) Miguel Torres, S.A.
C/ Comerç 22, 08720-Vilafranca del Penedès (Barcelona, Spain)

*Corresponding Author: Dr. Antoni Sánchez
Escola Universitària Politècnica del Medi Ambient
Rbla Pompeu Fabra 1, 08100-Mollet del Vallès (Barcelona, Spain)
Tel.: (34) 93 579 67 84
Fax: (34) 93 579 67 85
E-mail: asanchez@eupma.uab.es

Abstract

The wine industry in Catalonia (Spain) plays an important role in the economy of the region. In this framework, Miguel Torres S.A. is a well known industry specialized in the production of high-quality wines and brandy, which possesses its own vineyard. Two of the main solid wastes produced in this kind of industry are: stalk (waste from grape harvesting which is only produced during September and October) and wine sludge from the biological wastewater treatment plant which is steadily generated.

A composting process was proposed to treat these two organic wastes for recycling its organic matter content to the vineyard crops. Experiments at laboratory scale in static composting systems did not show positive results for different stalk:sludge mixtures due to the high moisture content of both wastes. Field composting experiments with windrow methods showed that the thermophilic range of composting could be achieved for a 2:1 stalk:sludge volumetric ratio resulting in a complete sanitation of the material with thermophilic temperature of over 55ºC for 28 days. The stability and maturity of the final compost was very high (Dewar self-heating test maturity grade V and static respirometric index 0.10 mg $O_2 \cdot g$ Total Organic Matter$^{-1} \cdot h^{-1}$). Due to its seasonal production, stalk storage was necessary. A study of the changes of stalk properties during one year is also presented. Bulk density and water holding capacity decreased with storage time while FAS (Free Air Space) and porosity increased. No remarkable changes in organic matter content were observed.

Co-composting of stalk and wine sludge generated in the wine industry is presented as a sustainable waste management strategy, which produces a sanitized fertilizer suitable for application in the vineyard, closing the organic matter cycle.

Introduction

The wine industry in Catalonia, Spain, plays an important role in the economy of the region. Spain is an European leader in the innovations in the area. Large companies' investments have been characteristic in the last years, which have supposed the improvement of the quality of wines and spirits and the image of the cellars (Miguel Torres S.A., 2004). Miguel Torres S.A. (Vilafranca del Penedès, Barcelona, Spain) is a well known industry specialized in the production of high-quality wines and brandy. During the production process significant amounts of liquid and solid wastes are generated. Miguel Torres has a wastewater treatment plant in its facilities for the treatment of wastewater and vinasse generated in the industry. Approximately, 80% of the total wastes generated are organic wastes or by-products. The characteristic of the wastes produced in Miguel Torres are grape pomace (50%), lees (20%), stalk (7.6%) and dewatered wine sludge from the wastewater treatment plant (7%). Recycling of grape pomace (grape peels and seeds) which is produced during the grape pressing, and lees, consisting of the sediments from the fermentation and the clarification operations, is already well established. The management of the other two important organic solid wastes, stalk and wine sludge, is at the moment ordered to an external company.

At present, there is a tendency towards the agricultural use of sludge. The unique legal restriction to direct land application of the sludge is given by its content on heavy metals and potentially toxic compounds. Therefore "clean" sludges coming from agricultural industries may be suitable for direct land application. Miguel Torres possesses its own vineyard in Spain, California and Chile. Given that Spanish soils are poor in organic matter, vineyard crops require organic fertilization. However, European Commission is preparing a new Directive on the biological treatment of organic wastes (European Commission, 2001). According to this Directive the sanitation of sludge before its application to land will be mandatory. The absence of pathogen microorganisms is also crucial in the vineyard cultivation. Composting is one of the alternatives proposed by the Directive in order to achieve sludge sanitation.

Main factors influencing the composting process are temperature, water content, oxygen concentration in the composting matrix, porosity and free air space (FAS). Temperature is both a consequence of the composting process (microbial metabolism) and a control parameter. Temperatures providing the maximum degradation velocity are in the range of 40-70ºC (Haug 1993). The water content of sewage sludge is usually too high for direct composting and a bulking agent is necessary to reach an optimum water content, porosity and FAS (Haug 1993). The optimization of the sludge-bulking agent mixtures for urban or industrial sludge composting has been extensively studied (Miner *et al.* 2001; Milne *et al.* 1998; Morisaki *et al.* 1989; Sesay *et al.* 1997). Also, the type of bulking agent used and its particle size has been empathized as an important factor in sludge composting (Jokela *et al.* 1997; Morisaki *et al.* 1989). Studies on sludge composting are presented at different scales, laboratory, pilot plant or field scale (Jokela *et al.* 1997; Sesay *et al.* 1997), being the temperature profile the main difference observed at the studied scales. Obviously, the amount of heat generated and the heat retention capacity of the material increase as the mass of the material increases.

Stalk appears to be an ideal bulking agent for sludge composting, due to physical properties such as porosity (provided by its branch-type structure) and resistance to biodegradation of the hard-wood fraction (Tuomela *et al.* 2000). Its chemical properties are also optimal. Stalk C/N ratio is high (around 39) and equilibrates the low C/N ratio of sludge (around 5). Co-composting wine sludge from the wastewater treatment plant with stalk as bulking agent would generate a stabilized fertilizer suitable for its application to the vineyard crops. Moreover the composting process allows treating different wood wastes that are generated sporadically in the wine industry, such as ground old oak barrels (Bertrán 2004).

Wine sludge from the wastewater treatment plant is generated steadily during the year and stalk is yearly produced during the grape harvest, a short period of 4-6 weeks around September. Stalk storage is necessary to use it in wine sludge composting during the rest of the year.

The objectives of this work are: i) to study the co-compostability of stalk with wine sludge to ensure a complete sanitation of the material, ii) to determine the stability and maturity of the final compost and iii) to monitor the properties of ground stalk during the storage time to study their influence on the optimum ground stalk:sludge volumetric ratio for composting.

Materials and methods

Materials

The waste materials studied were dewatered (by centrifugation) wine sludge from Miguel Torres wastewater treatment plant; and stalk from the wine production process after grape harvest. Wine sludge is steadily generated during the year and was sampled just after dewatering. Stalk is generated only during the grape harvest in September and October. Stalk was ground to 5 cm after the grape harvest and stored in a pile in the open air. These materials were monthly sampled for analysis. Composting experiments were carried out during the year following the grape harvest in September.

Pine wood chips were used as alternative bulking agent. Sawdust was used as drying agent. Both materials were obtained from a local carpentry.

Composting experiments

Laboratory-scale experiments were undertaken using 4.5-L Dewar® vessels conditioned for static composting providing a stopper and placing a rigid wire net near the bottom to separate the material from possible leachates. The stopper was perforated in three points for temperature and oxygen content monitoring and air supply. Wine sludge and stalk were used in the laboratory-scale experiments mixed in different volumetric ratios. In some experiments, wood chips were used as an alternative bulking agent, whereas sawdust was used as absorbent material to decrease the moisture content of the wine sludge. A complete description of the composting Dewar vessel can be found in Gea *et al*. (2003).

Field scale composting experiments were carried out under real conditions in Miguel Torres facilities using a dynamic windrow composting system. Two composting piles of approximately 150 m^3 (height: 2 m, width: 3 m) were built. The volumetric ratios (stalk:sludge) of the piles were 1:1 and 2:1 for Pile 1 and 2, respectively. Initial mixing and pile turnings were performed using a front end loader. Pile turning was performed weekly during the composting experiment. The piles were not covered and situated on a concrete floor to collect the possible leachates generated in the process. Temperature and interstitial oxygen of the piles were routinely monitored.

Oxygen, aeration control and temperature monitoring

Laboratory scale: Air was supplied to the vessels in aeration cycles where the total time of the cycle, aeration time and air flow were programmed and changed on the basis of the measured oxygen concentration, to ensure an oxygen concentration over 10%. Pt-100 sensors were used for temperature monitoring connected to a data acquisition system (DAS-8000, Desin, Spain) which was connected to a standard PC.

The system allowed, by means of the proper software (Proasis® Das-Win 2.1, Desin, Spain), the continuous on-line visualisation and registration of temperature. Pt-100 sensors were placed in the material to measure temperature at half of the height of the material in the Dewar vessel.

Field Scale: Temperature was measured with a portable Pt-100 sensor (Delta Ohm HD9214) at two different depths of pile, 40 and 90 cm, in at least 7 different points. Temperature values are presented as average values.

Oxygen concentration in interstitial air was measured with a portable O_2 detector (Oxy-ToxiRAE, RAE) connected to a portable aspiration pump, both in laboratory and field experiments. Oxygen and temperature were measured at the same points in the field scale experiments.

Respirometric tests

A static respirometer was built according to the original model described previously by Ianotti *et al.* (1993) and following the modifications and recommendations given by the U.S. Department of Agriculture and U.S. Composting Council (2001). Approximately 250 mL of a compost sample were placed in a 500 mL Erlenmeyer flask on a nylon mesh screen that allowed air movement under and through the solid sample. The setup included a water bath to maintain the temperature at 37ºC during the respirometric test. Prior to the assays, samples were incubated for 18 hours at 37ºC. During all the incubation period samples were aerated with previously humidified air at the sample temperature. The drop of oxygen content in the flask containing the compost sample was monitored with an oxygen meter (Lutron 5510, Lutron Co. Ltd., Taiwan) connected to a personal computer (RS232 communication protocol) with a proper software to register the oxygen values. The rate of respiration of a compost sample based on total organic matter content (TOM) was then calculated from the slope of oxygen level decrease according to the standard procedures (U.S. Department of Agriculture and U.S. Composting Council, 2001). Results of the static respirometric index (SRI) referred to total organic matter content are presented as an average of three replicates.

Germination tests

Relative Germination Rate and Relative Root Elongation of cucumber seeds were determined from a compost extract following the method 05.05-B 'In-Vitro Germination and Root Elongation' proposed by TMECC (Test Methods for the Examination of Composting and Compost, U.S. Department of Agriculture and U.S. Composting Council 2001).

Analytical methods

Different parameters were determined according to the standard procedures recommended by TMECC (Test Methods for the Examination of Composting and Compost, U.S. Department of Agriculture and U.S. Composting Council 2001) as follows: water and dry matter content were determined with the method 03.09-A 'Total Solids and Moisture at 70±5ºC', total organic matter (TOM) content with the method 05.07-A 'Loss On Ignition Method', bulk density, free air space (FAS), pore space (PS), water holding capacity (WHC) with the method 03.01-A 'Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water-Holding Capacity of Compost', and Dewar self-heating maturation test with the method 05.08-D 'Dewar Self-Heating Test'.

Results and discussion

Evolution of material properties

Figures 1 and 2 show the evolution of water and organic matter content of the materials under study. Wine sludge obtained after centrifugation in the wastewater treatment plant presented a high water content (average value 88%), and an average organic matter content of 70%. Ground stalk, stored outdoors, presented high water content (60-80%) due to the rainfall in winter and spring and the humidity of the area. Consequently, water content within the recommended range of 40-60% (Haug 1993; Saña and Soliva 1987) could not be achieved by mixing the two materials. Therefore it was decided to cover a part of the ground stalk storage pile to prevent the contact of the material with water from rainfall (covered stalk). As Figure 1 shows, this strategy produced an important reduction of the stalk water content.

Additionally, it has to be pointed that although certain biodegradation took place during storage time, leading to physical and structural changes that could be visually recognized, no significant reduction of the total organic matter content was observed in stalk when increasing the storage time (Figure 2). This was due to the fact that stalk is composed mainly by branches (hard-wood fraction) and the biodegradation only takes place in the soft-wood fraction which is a minor fraction of stalk (Tuomela *et al.* 2000). Wine sludge composition, on the other hand, presented steady values of water and organic matter content during the period of study.

Figure 3 shows the reduction of bulk density and water holding capacity and the increase of FAS and porosity during stalk storage time. This was the result of the biodegradation of the soft-wood fraction of stalk, which consisted of small size particles that filled the void spaces in the structure of the branches. The reduction in stalk bulk density (or FAS increase) can make more difficult the integration with wine sludge when acting as bulking agent. The water holding capacity of stalk also decreases with time and thus the capacity of absorbing excess water from the wine sludge. Consequently an increment of the stalk:sludge volumetric ratio required for composting was expected for long storage times. This fact will probably result in an excessive FAS in the final mixture (Haug 1993).

Composting experiences at laboratory scale

To evaluate the composting potential of the wastes under study, different ground stalk:sludge mixtures with volumetric ratios of 1:1, 2:1 and 3:1 were tested in 4.5-L composting vessels. Experiments were undertaken at different dates during the monitored period of stalk storage. Table 1 shows the initial water content and the maximum temperature reached in these experiments. Due to the high water content of the original materials, the mixtures resulted in water contents within 75-80% which were higher than those considered as optimal (Haug 1993). The mixtures presented poor activity and only in a few cases temperatures over 40ºC were reached. None of the experiments reached the thermophilic range of temperatures (over 45ºC) necessary to obtain a sanitized material suitable for land application (European Commission, 2001). Figure 4 shows the temperature profile obtained for three of the mixtures tested.

When the stored stalk was covered, its water content was reduced to about 20% (Figure 1). Different experiments were carried out with covered stalk and other alternative bulking agents such as wood chips, but the resulting mixtures again presented an excessive water content and the material did not reach temperatures above 40ºC (Table 2).

Since the studied wine sludge was dewatered by centrifugation, one possible strategy to reduce water content in the centrifugation operation might be the addition of polyelectrolyte to the wine sludge. To simulate this, sawdust was added to wine sludge as an absorbent of moisture before mixing with stalk. This strategy improved the results of the composting process. The temperature reached values near 50ºC and the thermophilic range was retained for 3.5 days, as can be seen in Figure 5. Adding sawdust in moderate amounts had a positive effect in the composting process because sawdust absorbed excess water from the wine sludge, although the reduction in the total water content of the mixture was low (75.5%, Table 2). Moreover, sawdust produced a very homogeneous mixture with wine sludge breaking the typical aggregated structure of a dewatered sludge and improving its integration with stalk by reducing the size of aggregates. This effect was mainly due to the small particle size of sawdust.

Field experiments

Piles 1 and 2 (stalk:sludge volumetric ratios of 1:1 and 2:1, respectively) were built during the grape harvest period (September) and monitored during the composting process. Due to the high water content of the fresh stalk, the water content of the initial mixtures of both piles was high, 81% for Pile 1 and 75% for Pile 2. In Pile 1, the material practically had no porosity and consequently temperature did not reach the thermophilic range, remaining under 40ºC (data not shown) and it was decided to stop the composting experiment after two weeks. On the other hand, in Pile 2 the composting process reached average temperatures over 50ºC in one week and over 60ºC in two weeks (Figure 6). Values of temperatures over 55ºC were measured for 4 weeks (days 14-42). As pile turnings were carried out once a week, it can be concluded that the totality of the material was exposed to temperatures in the thermophilic range. Besides, according to U.S. Environmental Protection Agency Rule 503, total sanitation of biosolids is obtained at 55ºC for 15 days and turned 5 times, which was the case of wine sludge composting (U.S. Environmental Protection Agency 1995). On the other hand, aerobic conditions were maintained in the first layer of the pile (40 cm depth), but oxygen content dropped under 8% when approaching the center of the pile (90 cm). Because of water evaporation due to the high temperatures reached, the water content of Pile 2 was reduced from 75 to 68% in 11 days in spite of the rainfall. However, values of moisture in Pile 2 were always in the high range of the composting process (average value of 70%).

On the other hand, other monitored parameters correlated well with those found in traditional biosolids composting (Haug 1993). For instance, total organic matter content decreased from an initial value of 77% to a final value of 63%, whereas pH value ranged from 8.2 to 9.3. Finally, an important reduction of total volume was observed in a percentage of 43% in a total composting period of 120 days.

The excellent results obtained for Pile 2 contrast with the laboratory scale experiments carried out with the same volumetric ratio 2:1 (Figure 4, Table 1) where the thermophilic range was not achieved. As mentioned before, the water content and the FAS of the mixture were critical for the proper composting development. However, in large scale experiments the thermal inertia of the material compensated the problems presented with FAS and excess of water. Furthermore, frequent turning of the material contributed to the complete higienization of the material and to the disintegration of stalk.

In addition to stalk:sludge volumetric ratio, bulking agent particle size and operation scale are interrelated and can affect the composting process in the same order of magnitude (Gea *et al*. 2003). The particle size of ground stalk (5 cm) may result too big for composting in 4.5L vessels. On the other hand, as the bulk density of stalk decreased within the storage time, the amount of stalk required for adjusting the properties of the mixture with wine sludge (*i.e.* water content) increased, leading to high requirements of bulking agent volumetric ratio and to an excessive FAS. This excess of FAS can be reduced when operating at higher scales because of the compaction phenomena of the composting material (McCartney and Chen 2001). Moreover, the bigger the organic matrix is, the higher heat generation and retention occurs, which may improve the temperature profiles obtained (Sherman-Huntoon 2000). On the other hand, open dynamic systems such as turned piles produce higher losses of water by evaporation due to the high surface area available.

Final compost properties

One of the most important characteristics to determine in sludge compost intended for vineyard application is the stability and the maturity of the material obtained. Therefore, the Dewar self-heating test and the respirometric index were measured for the compost obtained. These tests are popularly used in predicting compost stability and maturation (Weppen, 2002; Adani *et al.*, 2003) and are recommended in standard procedures and regulations (U.S. Department of Agriculture and U.S. Composting Council, 2001; European Commission, 2001). Static respirometric index (SRI) obtained was 0.10 ± 0.02 mg $O_2 \cdot g\ TOM^{-1} \cdot h^{-1}$, which can be classified in the range of highly stable materials (California Compost Quality Council, 2001). Also, the Dewar self-heating test resulted in the maximum maturity grade (V) with temperature increments below 2ºC.

Finally, the germination indexes obtained for the final compost were 90% and 100% for the Relative Root Elongation and the Relative Germination Rate, respectively. These indexes indicated that there was no evidence of the presence of plant toxic compounds.

Conclusions

From this work, it can be concluded that: 1) Bulk density and water holding capacity of stalk decrease during the storage time as biodegradation of soft-wood fraction takes place, while FAS and porosity increase. Thus, the optimum volumetric ratio of stalk:sludge for composting should be increased during the year. 2) High water content of stalk and wine sludge was the main problem in the composting process studied under laboratory scale conditions. The addition of some adsorbent material or the improvement of the dewatering system are the two strategies proposed to reduce the water content. 3) At field scale, a highly stable and mature final compost was obtained when a 2:1 stalk:sludge volumetric ratio was used. Windrow composting with frequent turning appear to be an optimal technology for the composting of this waste from the wine industry.

In conclusion, co-composting of stalk and wine sludge generated in the wine industry is presented as a sustainable waste management strategy, which produces a sanitized fertilizer for application in the vineyard, closing the organic matter cycle.

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Tables

Table 1. Initial water content and maximum temperature achieved in laboratory scale composting experiments with different mixtures stalk/sludge.

Date	Volumetric Ratio		Initial Water Content (%)	Maximum Temperature (ºC)
	Stalk	Sludge		
17Sept	1	1	83.1	46.5
17Sept	1.5	1	83.2	39.1
17Sept	2	1	82.1	43.9
17Sept	2.5	1	82.0	38.9
17Sept	3	1	81.9	42.9
3Oct	1	1	83.2	30.3
3Oct	2	1	79.3	38.1
3Oct	3	1	76.8	36.7
17Oct	2	1	83.6	34.0
17Oct	3	1	80.5	30.5
14Nov	2	1	83.4	29.4
14Nov	3	1	80.8	31.2
16Jan	1	1	83.3	27.7
13Feb	2	1	73.2	38.5

Table 2. Initial water content and maximum temperature reached in laboratory scale composting experiments using an alternative bulking agent and amendment.

Date	Volumetric Ratio					Initial Water Content (%)	Maximum Temperature (ºC)
	Stalk	Covered stalk	Wood Chips	Sawdust	Sludge		
14Nov	0	0	1	0	1	76.6	38.6
16Jan	1	0	0	1	1	82.2	27.9
6Feb	0	0	1	0	1	80.2	32.5
6Feb	0	1	0	0	1	79.8	37.7
13Feb	1	0	0	1	1	70.5	38.7
13Feb	0	0	1	1	1	68.6	44.2
24Feb	1	0	0	3	3	73.3	49.5
24Feb	1	0	0	6	3	70.6	38.8
6Mar	0	2	0	0	1	77.6	33.3
6Mar	0	3	0	0	1	77.1	36.4
6Mar	0	6	0	1	3	75.5	28.0
6Mar	0	8	0	1	4	70.8	31.9

Legend to figures

Figure 1. Water content evolution of the materials under study: stalk (circle), covered stalk (triangle) and wine sludge (square). Time scale (in months) ranges from September (grape collection) to the end of the study.

Figure 2. Total Organic Matter content evolution of the materials under study: stalk (circle), covered stalk (triangle) and wine sludge (square). Time scale (in months) ranges from September (grape collection) to the end of the study.

Figure 3. Evolution of physical properties of stalk during storage time: Bulk density (kg/m^3) (circle), Pore Space (%) (triangle), Free Air Space (%) (square) and Water Holding Capacity (% volume basis) (diamond). Time scale (in months) ranges from September (grape collection) to the end of the study.

Figure 4. Example of temperature profiles obtained for three stalk and sludge mixtures in different dates and for different stalk:sludge volumetric ratios: 17 Sept/3:1 (solid line), 17 Sept/2:1 (dotted line), 17 Sept/1:1 (dashed-dotted line), room temperature (dashed line).

Figure 5. Temperature profile for the mixture stalk:sawdust:sludge in 6:1:3 volumetric ratio: composting temperature (solid line) and room temperature (dotted line).

Figure 6. Temperature and oxygen profiles for the field experiment with 2:1 (stalk:sludge) volumetric ratio: composting temperature (circle), oxygen (square) and pile turning (triangle).

Fig 1; Gea et al

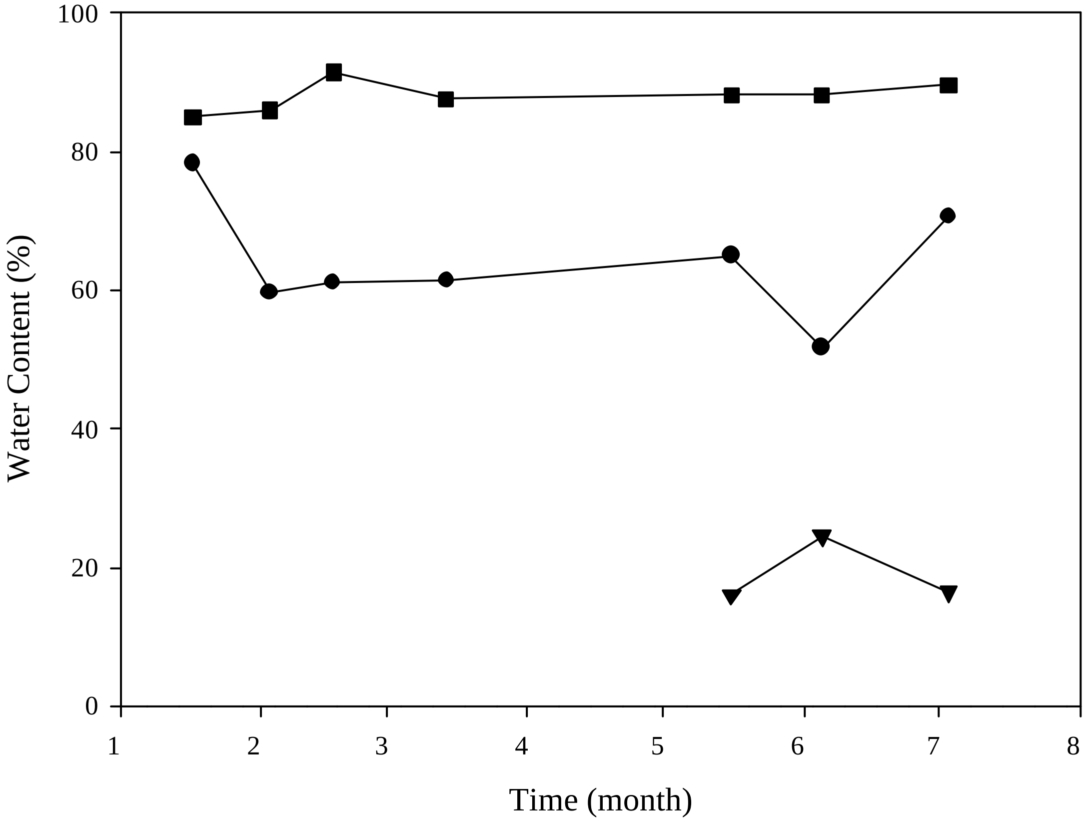


Fig 2; Gea et al

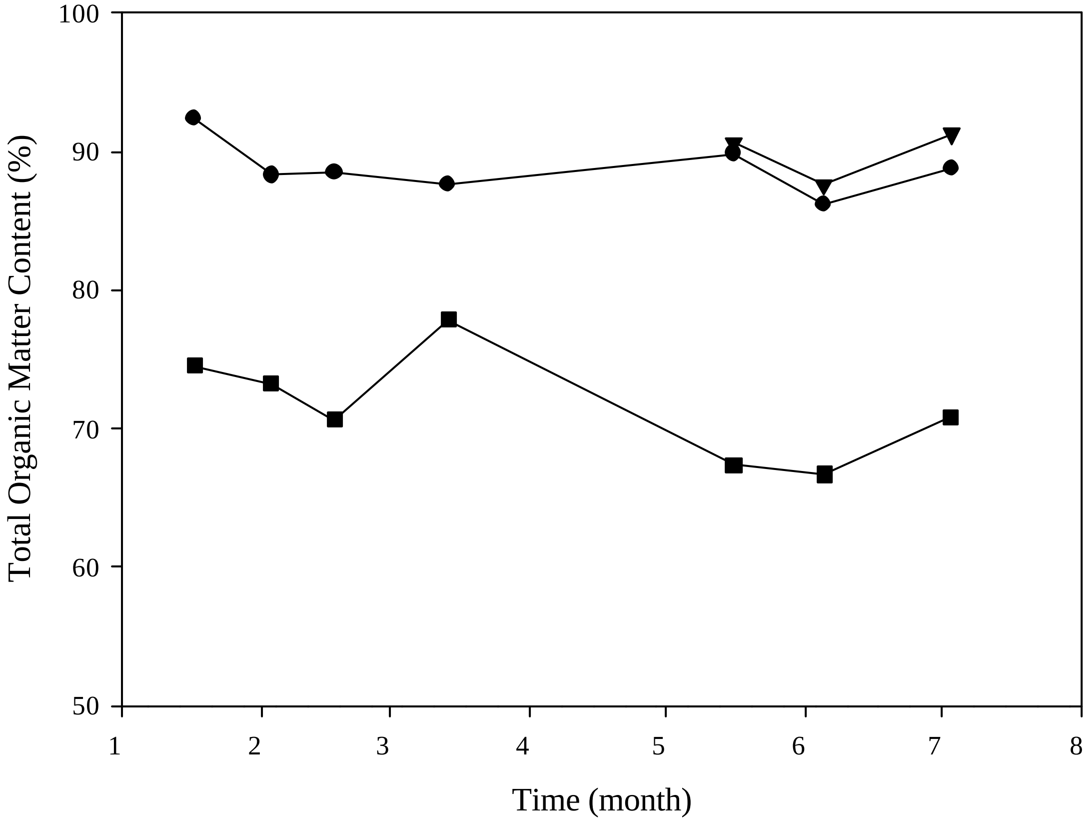
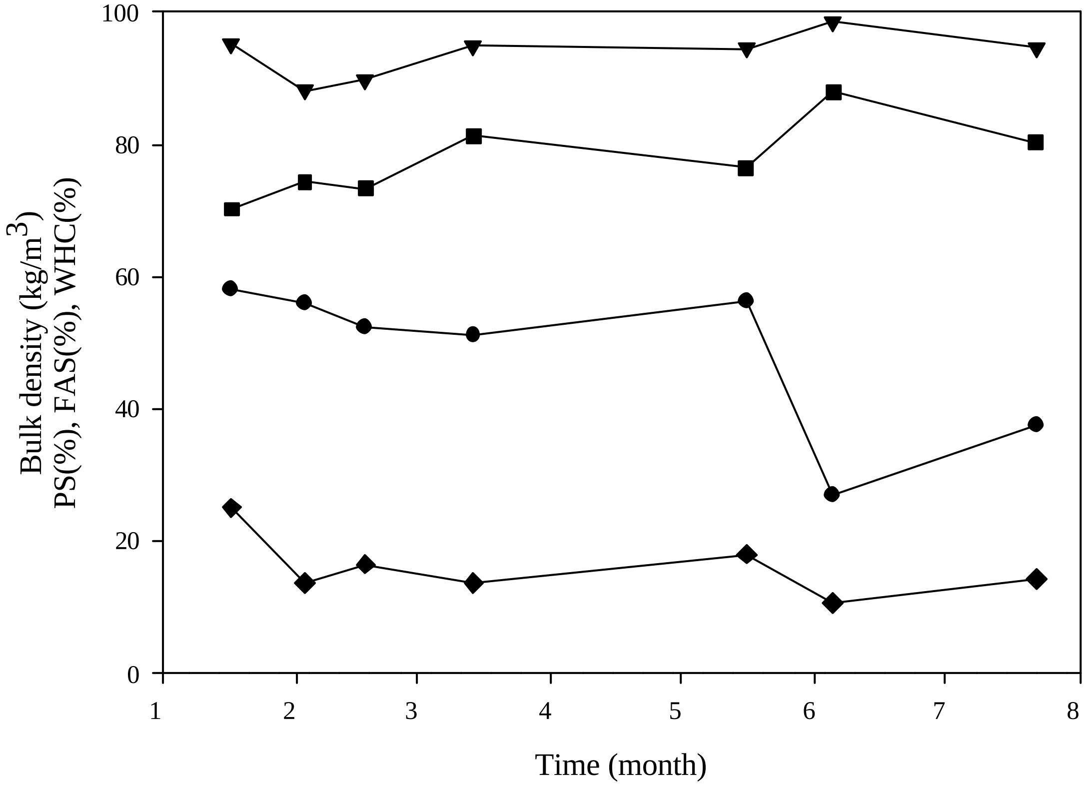


Fig 3; Gea et al



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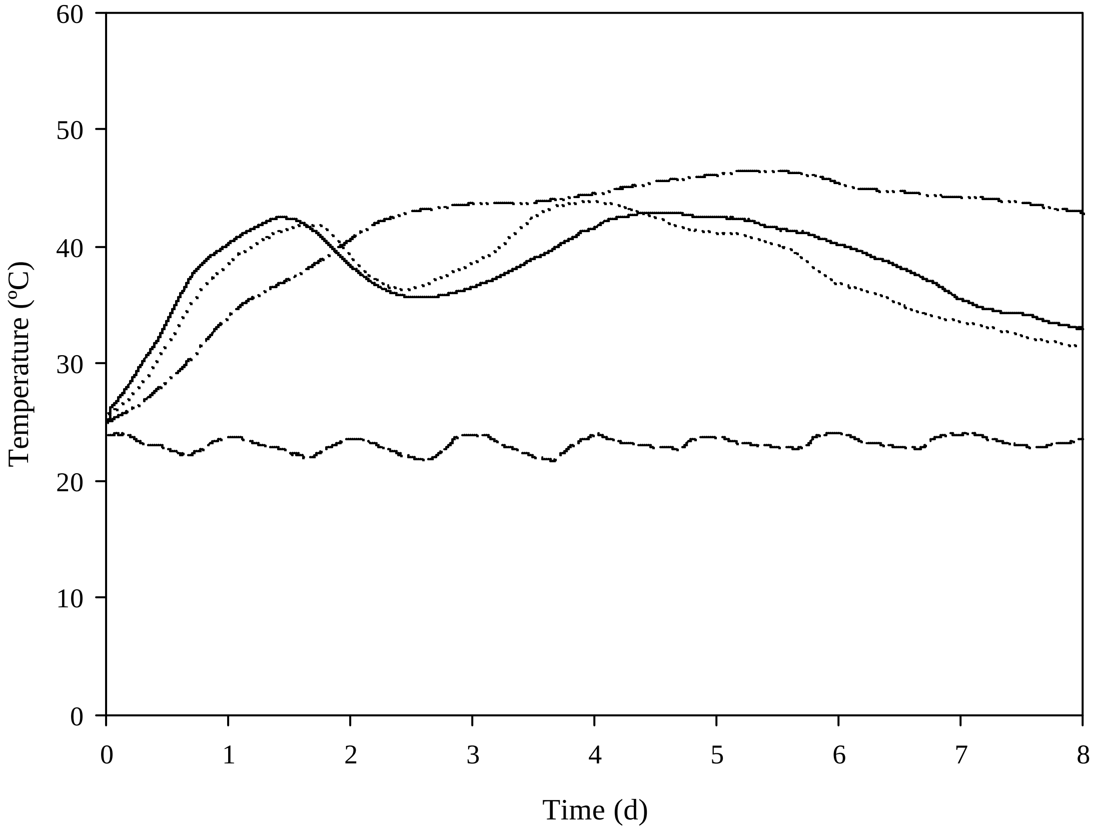

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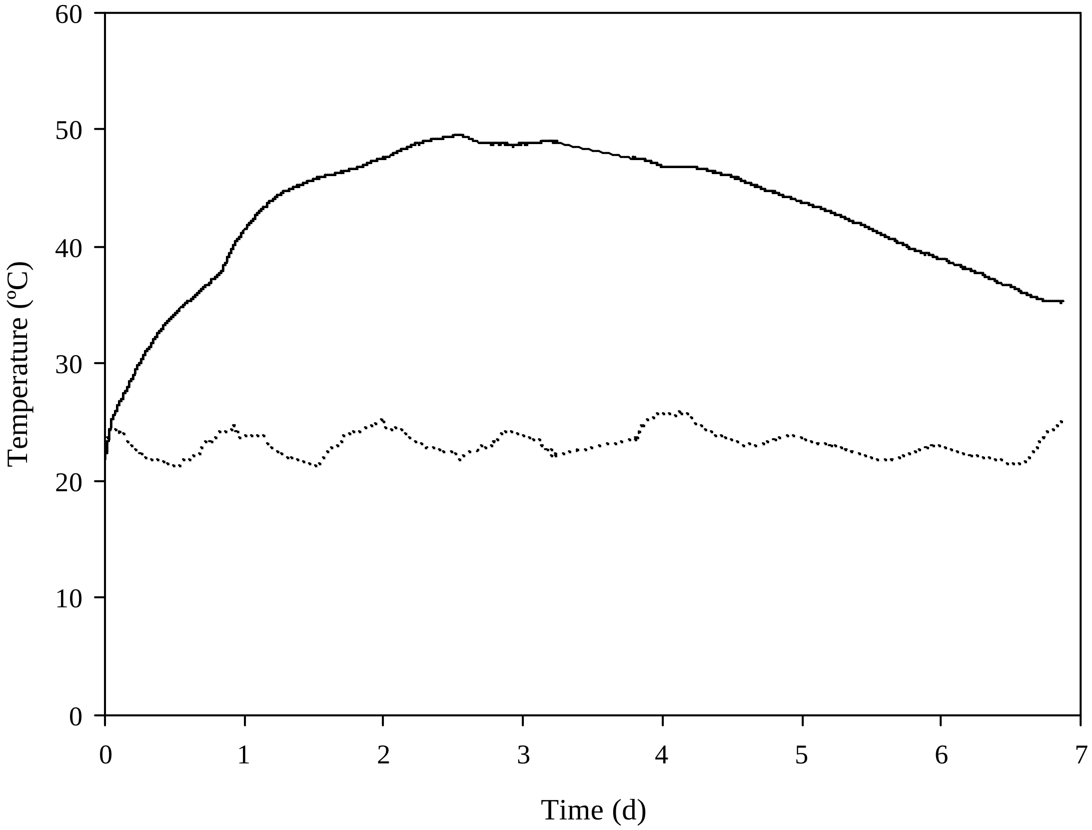

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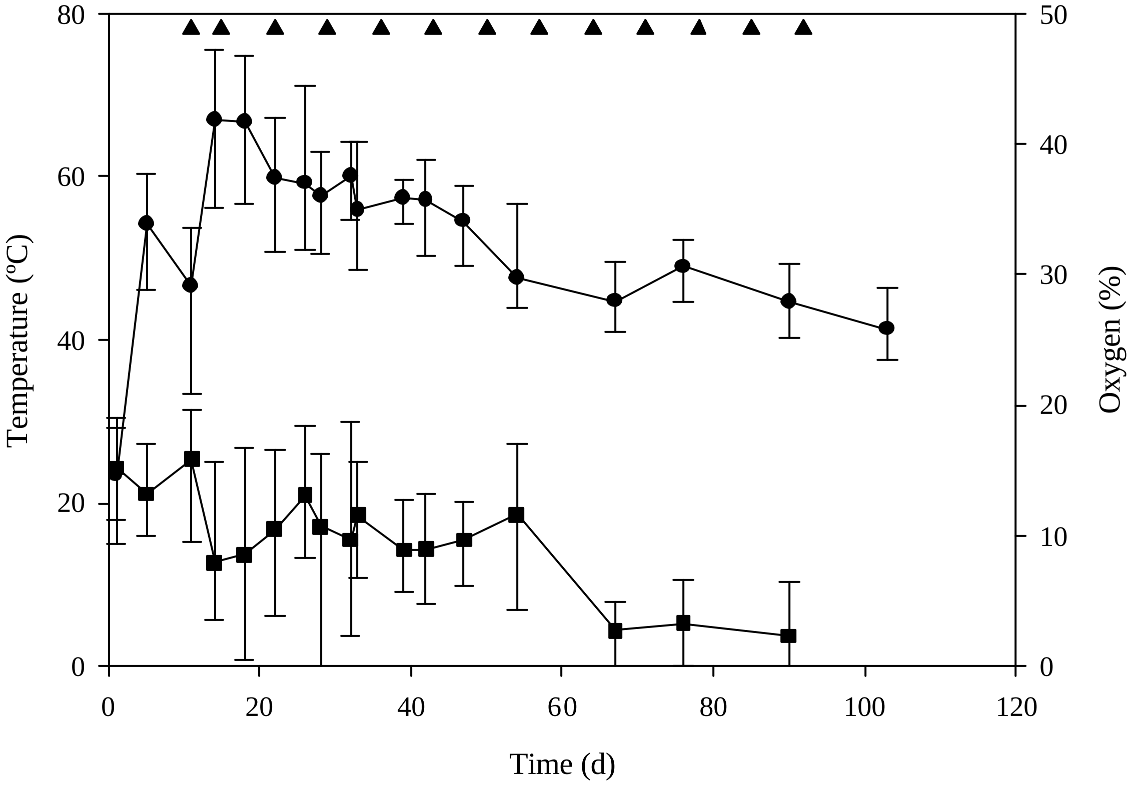

article III

Composting of de-inking sludge from the recycled paper manufacturing industry

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Composting of de-inking sludge from the recycled paper manufacturing industry

Teresa Gea, Adriana Artola, Antoni Sánchez *

Escola Universitària Politècnica del Medi Ambient, Universitat Autònoma de Barcelona, Rbla Pompeu Fabra 1,
08100-Mollet del Vallès, Barcelona, Spain

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Abstract

Composting of two different types of sludge from the recycled paper manufacturing industry was carried out at laboratory scale. Physico-chemical sludge (PCS) from the de-inking process and biological sludge (BS) from the wastewater treatment plant were composted and co-composted with and without addition of a bulking material. Despite its poor initial characteristics (relatively high C/N ratio, low organic content and moisture), PCS showed excellent behaviour in the composting process, reaching and maintaining thermophilic temperatures for more than 7 days at laboratory scale, and therefore complete hygienization. Pilot scale composting of PCS was also studied, and a respiratory quotient of 1.19 was obtained, indicating a full aerobic biological process. Respiration tests showed a complete stabilization of the material, with final values of the static respiration index in the range of 1.1 mg $O_2\,g\,TOM^{-1}\,h^{-1}$. Composting is proposed as a suitable technology for the effective recycling of this type of sludge from the recycled paper manufacturing industry.

Keywords: C/N ratio; Composting; Hygienization; Recycled paper manufacturing sludge; Respiratory quotient

1. Introduction

In recent years, new legislation in the European Union and the United States has promoted the utilization of recycled fibres in newsprint. This fact, together with the implementation of source-separated waste paper collection programs, has changed the raw materials in the paper manufacturing industry. In Spain, some industries are solely accepting waste paper to transform it into recycled paper.

Recycled paper industries remove inks, clay filters and coatings of used paper by a de-inking process and recycle the wood fibres by using physico-chemical treatments. However, some wood fibres are rejected from this process and constitute a sludge with some organic content. Moreover, this type of industry usually generates biological sludge from the biological treatment of wastewater.

Since the majority of sludge from paper manufacturing industries is landfilled or incinerated, alternative methods to treat this waste are being developed. Composting is one of the most promising technologies to treat paper sludge in a more economical way (Das et al., 2002a). It is defined as the biological decomposition and stabilization of organic substrates, under controlled conditions (Haug, 1993). The composting process permits the hygienization of the product by reaching thermophilic temperatures and reducing mass and volume, which makes compost suitable for agricultural applications.

Previous works have studied the feasibility of the composting of sludge from different pulp and paper manufacturing industries. Jokela et al. (1997) studied

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* Corresponding author. Tel.: +34 93 5796784; fax: +34 93 5796785.
E-mail address: asanchez@eupma.uab.es (A. Sánchez).

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the aerobic and anaerobic digestion of pulp and paper-mill sludge, concluding that in the case of de-inking sludge composting some urea addition is necessary to adjust the initial C/N ratio. In fact, C/N ratio appears to be one of the most crucial parameters to adjust in the composting of lignocellulosic wastes. Thus, nitrogen-rich amendments such as chicken broiler floor litter or poultry manure (Charest and Beauchamp, 2002) or chemicals such as ammonium nitrate (Das et al., 2002b), ammonium sulphate (Paul et al., 1999) or urea (Jokela et al., 1997) are often added to the sludge in order to decrease the initial C/N ratio. However, some recent works have pointed out that in some cases the composting of paper and pulp manufacturing sludge can be successfully carried out at C/N ratios higher than those currently used with other wastes (e.g. organic fraction of municipal solid waste or sludge from wastewater treatment plants). Besides, in these cases the amendment with nitrogen-rich wastes may not be necessary (Larsen, 1998; Charest and Beauchamp, 2002).

Other works have focused on particular aspects of paper sludge composting such as the optimization of decomposition rate (Ekinci et al., 2002) or the microbial activities during composting of pulp and paper-mill primary solids, revealing a particular microbial community in the biodegradation of such wastes (Atkinson et al., 1997). The application of composts from paper and pulp manufacturing wastes has also been studied and validated in soil and crops (Hackett et al., 1999; Baziramakenga and Simard, 2001; Rantala and Kuusinen, 2002).

This paper describes an investigation of the possibility of composting and co-composting the most typical wastes produced in the recycled paper manufacturing industry, PCS (physico-chemical sludge) and BS (biological sludge). The work consisted of an initial set of laboratory scale experiments to explore the compostability of different mixtures of paper sludges and a second pilot scale experiment where biological indices were determined for the optimal mixture. This methodology can be generalized for the study of similar organic wastes for which few data are available.

2. Methods

2.1. Sludge and bulking agent

PCS and BS were collected from a recycled paper manufacturing industry in Spain. PCS was obtained after centrifugation of the liquid fraction of the waste paper de-inking process. BS was obtained after the centrifugation of the biological sludge generated in the wastewater treatment plant of the recycled paper manufacturing industry. In this particular industry, PCS is produced in significantly larger amounts than BS. The main parameters of PCS and BS collected in the industry are presented in Table 1.

Wood chips from a local carpentry were used as bulking agent. The chips consisted of a variable mixture of pine and beech tree wood.

2.2. Composting experiments

Laboratory scale experiments were undertaken using 4.5-l Dewar® vessels conditioned for composting and previously validated in the composting of organic fraction of municipal solid waste and wastewater sludge (Gea et al., 2003). A perforated lid was fitted for temperature monitoring and air supply and a rigid wire net was placed near the bottom of the vessel to separate the composting material from possible leachates.

Pilot tests were undertaken in an old 100-l refrigerator adapted for use as a static composter. The recipient was placed horizontally with a slight inclination to allow its opening from the top and to permit the collection of leachates. A plastic mesh was fitted at the bottom of the recipient to support the material and separate it from possible leachates. Several holes were perforated through the walls of the vessel to permit air movement, leachate removal and the insertion of different probes. Air was supplied to the composter by means of control software to maintain an O_2 concentration over 10%.

2.3. Temperature, O_2 and CO_2 monitoring

2.3.1. Laboratory scale

Pt-100 sensors were used for temperature monitoring in the 4.5-l Dewar® vessels placed in the material to have a measuring point at 1/2 of the height of the material in the vessel. Temperature sensors were connected to a data acquisition system (DAS-8000, Desin, Spain) which was connected to a standard personal computer. The system allowed, by means of the proper software (Proasis® Das-Win 2.1, Desin, Spain), the continuous on-line monitoring and recording of the temperature. O_2 content was measured with a portable O_2 detector (Oxy-ToxiRAE, RAE) with a frequency of 3–7 times during 1 day.

2.3.2. Pilot scale

Four Pt-100 sensors (Desin mod. SR-NOH) inserted at different points inside the 100-l tank were used for monitoring the temperature in the pilot scale composting experiments. Temperature was recorded every 30 min. Interstitial air was pumped out of the reactor every 10 min and sent for O_2 and CO_2 measurement to an oxygen sensor (Sensox, Sensotran, Spain) and a CO_2 infrared detector (Sensontran I.R., Sensotran, Spain) respectively. All sensors were connected to a specially-made data acquisition system. Oxygen was controlled by means of a feedback oxygen control which

Table 1
Main characteristics of PCS and BS

Parameter	PCS	BS
Dry matter (%)	63.3	47.3
Water content (%)	36.7	52.7
Total organic matter (%, dry matter basis)	33.7	58.8
pH (water extract 1:5)	7.50	6.80
Electrical conductivity (dS/m, water extract 1:5)	1.92	3.60
Total N Kjeldahl (%, dry matter basis)	0.43	1.07
C/N ratio	34.0	23.7
N-NH_4 (%, fresh matter basis)	0.08	0.17
Total P (%, dry matter basis)	<0.10	0.37
Total K (%, dry matter basis)	<0.10	0.13

automatically supplied fresh air to the reactor (flow rate 20 l/min) to maintain an oxygen concentration over 10%. Measures of temperature and O_2 and CO_2 content showed a high level of reproducibility in laboratory and pilot experiments, with a deviation of less than 1%.

2.4. Respiratory quotient (RQ)

RQ was calculated as the quotient of CO_2 produced and O_2 consumed as indicated in Eq. (1)

$$\mathrm{RQ} = \frac{\mathrm{CO}_{2,\mathrm{out}}}{20.9 - \mathrm{O}_{2,\mathrm{out}}} \tag{1}$$

where RQ, respiratory quotient (dimensionless); $CO_{2,out}$, carbon dioxide concentration in the exhaust gases (%); $O_{2,out}$, oxygen concentration in the exhaust gases (%). CO_2 percentage in inlet air was considered negligible and O_2 concentration in inlet air was 20.9%. RQ is presented as an average of 10 values taken over 100 min of measurement.

2.5. Analytical methods

Water content, total organic matter (TOM), pH, electrical conductivity, total nitrogen (Kjeldahl method), N-NH_4 and compost maturity grade (Dewar self-heating test) were determined according to the standard procedures (US Department of Agriculture and US Composting Council, 2001). Cellulose content was determined according to the method proposed by Rivers et al. (1983).

Total weight of the material was monitored on-line using a semi-industrial scale BACSA mod. I200.

2.6. Respiration tests

A static respirometer was built according to the original model described by Ianotti et al. (1993) and following the modifications and recommendations given by the US Department of Agriculture and US Composting Council (2001). Approximately 250 ml of compost samples were placed in 500 ml Erlenmeyer flasks on a nylon mesh screen that allowed air movement under and through the solid samples. The setup included a water bath to maintain the temperature at 37 °C during the respiration test. Prior to the assays, samples were incubated for 18 h at 37 °C. Samples were aerated with previously humidified air at the sample temperature throughout the incubation period. The drop of oxygen concentration in each flask containing a compost sample was monitored with a dissolved oxygen meter (Lutron 5510, Lutron Co. Ltd., Taiwan) connected to a data logger. The rate of respiration of the compost sample (Oxygen Uptake Rate, OUR or Respiration Index, RI) based on total organic matter content, TOM was then calculated from the slope of oxygen level decrease according to the standard procedures (US Department of Agriculture and US Composting Council, 2001). Results of the static respiration index referred to total organic matter content are presented as averages of three replicates.

3. Results and discussion

Composting of different paper sludges and bulking agent mixtures was studied in two steps.

3.1. Laboratory scale experiments

The objective of these experiments was to investigate the optimal mixture in paper sludge composting when temperature was selected as process variable. Table 2 presents a summary of the results obtained on composting different mixtures of PCS, BS and bulking agent (wood chips) at laboratory scale (4.5 l). In all the experiments, the moisture content was maintained within the optimal range for composting (40–60%) (Haug, 1993). In Table 2, the maximum temperatures achieved and the times for which temperature was over the thermophilic range threshold (>45 °C) are presented as average values of at least two experiments resulting in a total number of 18 runs. Maximum temperature is a good indicator of the composting possibilities of each

Table 2
Summary of the results obtained in the composting and co-composting of different sludges and mixtures

Sludge volumetric ratio			Average maximum temperature (°C)	Thermophilic time (d + h)
PCS	BS	Bulking agent		
1	0	0	65.5	7 + 14
1	0	1	60.1	1 + 17
1	0	2	52.3	1 + 15
2	1	0	55.0	5 + 1
1	1	0	49.8	1 + 23
2	2	1	34.0	–
2	1	1	34.9	–
1	1	2	38.6	–

mixture, since it determines if the thermophilic range of temperatures is reached and hence sanitation of the material achieved. From the results obtained in Table 2, it could be stated that

- PCS by itself showed the best potential for composting, since it reached the highest temperature (65.5 °C) and maintained thermophilic temperatures (over 45 °C) for the longest period (7 days + 14 h).
- The addition of an inert bulking agent (wood chips) did not improve the composting of PCS at either volumetric ratio tested (1:1 and 2:1). Therefore, the inherent porosity of PCS can be considered as adequate for composting. When an inert highly-porous bulking agent such as wood chips was added to the mixture, temperature values were lower than those obtained in the composting of PCS alone (65.5 °C without bulking agent vs. 60.1 °C and 52.3 °C using increasing ratios of bulking agent).
- The mixtures of PCS and BS at different ratios reached the thermophilic range, however, maximum temperature was lower than that achieved in the PCS composting, and thermophilic conditions were maintained for shorter times. Characteristics of the two sludges (Table 1) seemed to indicate that they were complementary in aspects such as C/N ratio or organic matter content. In practice, however, it was very difficult to mix the two sludges homogenously, and the final product mainly contained unmixed parts of both sludges.
- The addition of a bulking agent to the mixtures of PCS and BS produced a negative effect in the composting process, and the thermophilic range was not achieved. This effect had been already observed in the composting of PCS alone.

Moreover, as PCS is produced in much larger amounts than BS, PCS composting without the addition of BS or bulking agent was selected for the pilot study.

3.2. Composting of PCS at pilot scale

At this point, composting of PCS was studied with the objective of determining the biological indices (RQ and RI) to validate temperature profiles obtained at laboratory scale. Temperature profile is presented in Fig. 1. The thermophilic range of temperature was reached within 2 days, and was maintained for more than 2 weeks, which implied a full sanitation of the material. Other values of temperature registered at different points of the composter showed similar profiles (data not shown). The decrease in the temperature at day 8 corresponded to a failure in the aeration system. These results are in agreement with other works undertaken with similar sludges (Charest and Beauchamp, 2002; Das et al., 2002a).

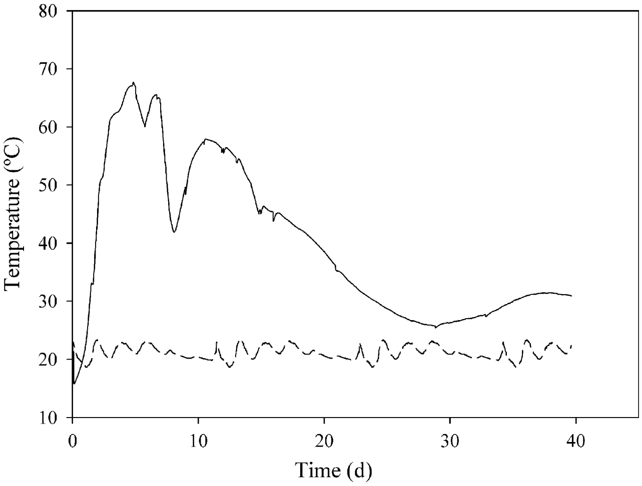

Fig. 1. Temperature profiles in the composting of PCS at pilot scale. Composting temperature (central probe, solid line) and room temperature (dashed line).

On the other hand, oxygen and carbon dioxide concentrations (Fig. 2) showed a typical profile in the composting process. Initially, oxygen was consumed at a high rate (air flow rate up to 1 l/s) and CO_2 was produced in large amounts, reaching extremely high values (over 20%). Oxygen concentration fell below 5% from day 2 to 5, however no evidence of anaerobic conditions was observed (malodours, presence of organic acids, etc.). This period corresponds to the thermophilic phase (Fig. 1).

CO_2 and O_2 can be related by means of the respiratory quotient (RQ). This parameter is defined as the ratio between CO_2 produced and O_2 consumed, and has been routinely used in the biotechnological field (Atkinson and Mavituna, 1983) but, to the authors' knowledge, it is rarely measured in composting processes. Its value is approximately equal to 1 under aerobic condi-

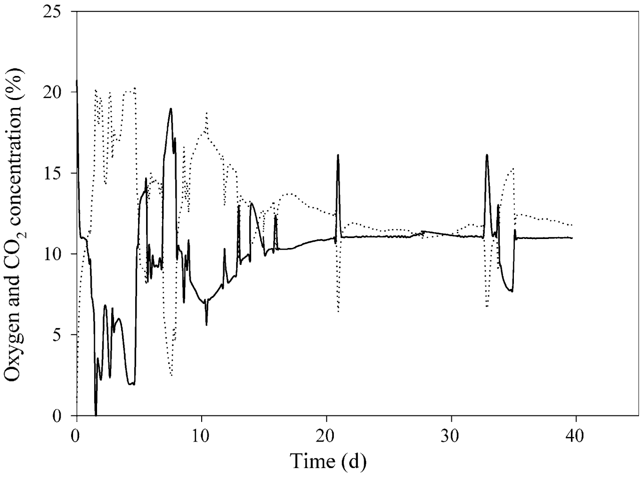

Fig. 2. Oxygen (solid line) and carbon dioxide (dotted line) profiles in the composting of PCS at pilot scale.

tions, although this obviously depends on the state of oxidation of the organic material. For instance, Smars et al. (2001) reported a value of 1.02 in the composting of source-separated household waste. Other authors (Mönnig et al., 2002) reported similar values for the composting of municipal solid wastes. The range of RQ for PCS was between 0.96 and 1.31 (average value 1.19), which clearly indicated that PCS was composed of organic material with a moderate degree of oxidation. Since RQ is a characteristic value directly related to organic waste composition, RQ can be used in the control and monitoring of the composting process of PCS to predict air requirements and CO_2 production. RQ value can also be used to compare PCS with other wastes.

Other typical parameters of the composting process remained practically steady throughout the experiments. Initial and final values of such parameters are presented in Table 3. For instance, the pH of compost material only increased slightly from 7.6 to 8.0. A similar pattern was observed for the total nitrogen profile, which only decreased from 0.43% to 0.30% during the composting period. This fact, together with the high organic matter decomposition, implied that C/N ratio decreased significantly to reach a final value of 26.0. Although this C/N ratio value could not be compared to the typical values for stabilized compost of below 15 (Haug, 1993), it could be considered satisfactory since no nitrogen amendments had been used, and it was in accordance with other studies (Das et al., 2002a). Other forms of nitrogen, such as ammonium nitrogen, were not detected during the composting process. Finally, electrical conductivity decreased slightly from an initial value of 1.92 dS/m to a final value of 1.31 dS/m, which has been also observed in the composting of paper residues (Jokela et al., 1997; Das et al., 2002b).

Moisture content and organic matter content profiles are shown in Fig. 3. It is evident from Fig. 3 that moisture and organic matter followed similar profiles. Thus, the presence of easily biodegradable compounds provoked the temperature increase and water evaporation. Once the thermophilic phase was reached, values of both organic matter and moisture content reached plateaus. Overall reductions in total weight, dry matter, moisture and organic matter content are also presented in Table 3. As a consequence of the rigid aggregated structure of PCS, no considerable compaction was observed during the composting time, and the volume reduction can be considered as negligible. Total nitrogen losses were only 13% (Table 3), which was probably caused by the high C/N ratios observed throughout the composting time. Additionally, ammonia in exhaust gases was not detected. However, as no leachates were collected the only possible fate for this nitrogen is its release as ammonia emissions in exhaust gases. More significant losses were observed for moisture (37.5%) and organic matter (33.5%), which contributed to the observed decrease in C/N ratio. Compared to other de-inking paper sludges, the C/N ratio of PCS is low (Charest et al., 2004). However, the organic matter content of PCS is also very low (33.7%, Table 1). This fact accounts for a low C/N ratio since other paper sludges present a higher organic matter content.

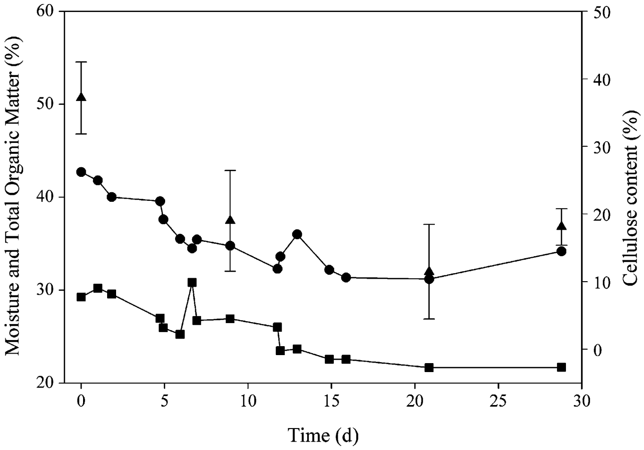

Fig. 3. Moisture (circles), total organic matter (squares) and cellulose (triangles) profiles in the composting of PCS at pilot scale.

Table 3
Reduction of different parameters in the composting of PCS at pilot scale

Parameter	Initial value	Final value	Reduction (%)
Total weight (kg)	73.0	57.0	22.0
Dry matter content (%)	57.3	65.8	10.3
Moisture content (%)	42.7	34.2	37.5
Organic matter (%, dry matter basis)	29.3	21.7	33.5
pH (water extract 1:5)	7.6	8.0	–
Elec. cond. (dS/m, water extract 1:5)	1.92	1.31	–
C/N ratio	34	26	–
Total nitrogen (%, dry matter basis)	0.43	0.30	13.0
Cellulose (%, organic matter basis)	37.2	18.1	67.7

Among all the organic compounds present in PCS, cellulose was expected to be one of the major components involved in material decomposition. Fig. 3 shows the evolution of cellulose content during PCS composting at pilot scale. Cellulose and total organic content profiles were very similar, presenting a high initial decomposition rate during the thermophilic phase and a plateau during the final mesophilic phase. Cellulose content decreased from an initial value of 37.2% to a final value of 18.1% (both expressed as a percentage of total organic matter) resulting in a cellulose reduction of 67.7% (Table 3). When this value was compared to total organic material reduction (Table 3), it could be concluded that cellulose corresponded to the 75% of the total organic matter decomposed. This fact confirmed that cellulose was the main organic material degraded during composting of PCS. Other studies

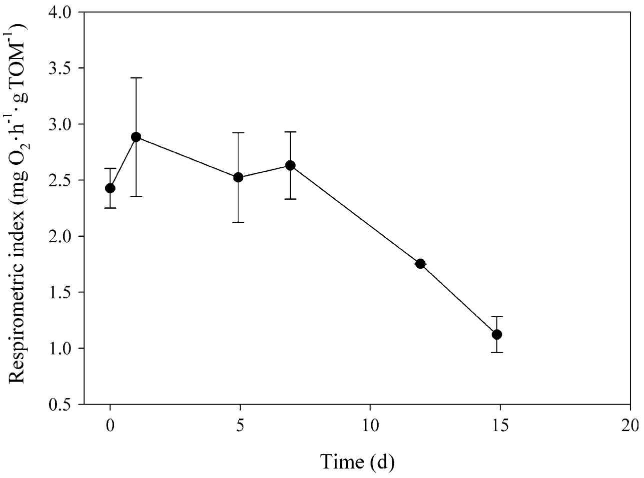

Fig. 4. Static respiration index profile in the composting of PCS at pilot scale.

reported similar results in the composting of de-inking paper sludge, showing that the cellulose breakdown is more rapid than that of the hemicellulose, whereas lignin fractions can be considered as resistant to biodegradation (Charest and Beauchamp, 2002).

Finally, the results obtained from the respiration tests (Fig. 4) indicated that a real stabilization of the organic matter occurred for PCS. Final values of the respiration index were in the range of 1.1 mg O_2 g $TOM^{-1} h^{-1}$, which are in the range of stable compost according to the international standards (California Compost Quality Council web site, 2001). Besides, other maturity tests such as the Dewar self-heating test resulted in the maximum maturity grade (V).

These results confirm that PCS can be successfully composted with a high biological activity to obtain a stabilized organic material.

4. Conclusions

Composting of two types of sludge from the recycled paper manufacturing industry was studied at laboratory and pilot scale. Biological sludge (BS) composted similarly to other biosolids from wastewater treatment, although no bulking agent was necessary when it was co-composted with PCS. Physico-chemical sludge (PCS) from the de-inking process, which is the major waste produced in this type of industry, was successfully composted without the addition of amendments or bulking agents, which implied an important cost reduction. Although the moisture and organic matter content in PCS were low, the composting material reached a fully thermophilic temperature that permitted its sanitation. Oxygen and carbon dioxide profiles, together with respiratory quotient, indicated a complete decomposition of the material. In addition, respiration index determination showed a high level of organic matter stabilization, which is a key factor in the application of composts from such sludges. The methodology used in this work can be generalized to the study of similar organic wastes for which few data are available.

The composting of this type of sludge, which is predicted to be produced in increasing amounts in the following years, is an innovative sustainable technology for the recycling of paper manufacturing wastes, which are currently landfilled or incinerated.

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article IV

Monitoring the biological activity of the composting process: oxygen uptake rate (OUR), respirometric index (RI), and respiratory quotient (RQ)

Teresa Gea, Raquel Barrena, Adriana Artola i Antoni Sánchez.

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Monitoring the Biological Activity of the Composting Process: Oxygen Uptake Rate (OUR), Respirometric Index (RI), and Respiratory Quotient (RQ)

Teresa Gea, Raquel Barrena, Adriana Artola, Antoni Sánchez

Escola Universitària Politècnica del Medi Ambient, Universitat Autònoma de Barcelona, Rbla Pompeu Fabra 1, 08100-Mollet del Vallès, Barcelona, Spain;
telephone: (34) 93 579 67 84; fax: (34) 93 579 67 85;
e-mail: asanchez@eupma.uab.es

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Abstract: Composting of several organic wastes of different chemical composition (source-separated organic fraction of municipal solid waste, dewatered raw sludge, dewatered anaerobically digested sludge and paper sludge) was carried out under controlled conditions to study the suitability of different biological indexes (oxygen uptake rate, respirometric index, and respiratory quotient) to monitor the biological activity of the composting process. Among the indexes tested, oxygen uptake rate (also referred to as dynamic respirometric index) provided the most reliable values of microbial activity in a compost environment. On the other hand, values of the static respirometric index measured at process temperature, especially in the early stages of the composting process, were significantly lower than those of the dynamic index, which was probably due to oxygen diffusion limitations present in static systems. Both static and dynamic indexes were similar during the maturation phase. Static respirometric index measured at 37°C should not be used with samples obtained during the thermophilic phase, since it resulted in an underestimation of the respiration values. Respiratory quotient presented only slight variations when changing the process temperature or the waste considered, and its use should be restricted to ensure aerobic conditions in the composting matrix. © 2004 Wiley Periodicals, Inc.

Keywords: biological activity; composting; organic wastes; oxygen uptake rate (OUR); respiratory quotient (RQ); respirometric index (RI)

INTRODUCTION

In recent years, the increasing amounts of organic solid wastes generated by municipalities, industries, or agricultural activities have become a worldwide problem. Among the available technologies to treat and recycle organic wastes, composting is presented as one of the most promising options to recycle organic materials into a valuable organic fertilizer popularly known as compost.

Composting is a biotechnological process by which different microbial communities decompose organic matter into simpler nutrients. Composting is an aerobic process, which requires oxygen to stabilize the organic wastes, optimal moisture, and porosity (Haug, 1993). Temperature is often selected as the control variable in the composting process because it is an indicator of the biological activity of the material. Also, as the composting process is usually carried out within the thermophilic range of temperature, it permits hygienization of the final product (Salter and Cuyler, 2003).

There is abundant literature related to different aspects of composting, such as microbiological studies (Gamo and Shoji, 1999; Tiquia et al., 2002), changes of chemical composition (Pichler et al., 2000), technical and operational considerations (Qiao and Ho, 1997; Wong and Fang, 2000), emission of pollutants (Eitzer, 1995; He et al., 2001), or process modeling (López-Zavala et al., 2004). However, there is scarce information about the monitoring of biological activity of composting processes in comparison with other biotechnology fields, such as fermentation technology or wastewater treatment.

Oxygen uptake rate (OUR) has been traditionally used in aerobic processes to estimate on-line the biological activity, especially in wastewater treatment. In the composting field, OUR is often referred to as the dynamic respiration index (DRI). OUR or DRI can also be estimated off-line and without continuous aeration by using a respirometric technique known as the static respiration index (SRI, or simply RI), which is commonly used to determine compost stability (Ianotti et al., 1993; Chica et al., 2003). Both parameters are indicators of the biological activity of a composting process. Ideally, DRI and SRI would be identical in an aerobic environment but significant differences have been found between both indexes in

Correspondence to: Antoni Sánchez
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composting experiments. Concretely, the use of SRI results in an underestimation of the biological activity of a compost sample, which is usually attributed to oxygen diffusion problems in the determination of the respirometric index in solid static samples (Scaglia et al., 2000; Adani et al., 2003).

The respiration quotient (RQ), representing the relationship between CO_2 produced and O_2 consumed, is approximately equal to 1 under aerobic conditions (Atkinson and Mavituna, 1983), although it depends on the biochemical composition of the organic material. This parameter is, to our knowledge, rarely measured in composting processes. Since it is a characteristic value directly related to organic waste composition and active microbial communities, RQ can be used in the monitoring and control of the composting process (Atkinson et al., 1997) and to predict air requirements and CO_2 production (Smars et al., 2001). Given a defined waste, the value of RQ in a composting process can be considered steady under different conditions of aeration rate or moisture (Klauss and Papadimitriou, 2002; Mönning et al., 2002). In other works, some authors have found some differences in RQ when composting wastes amended with fats (Weppen, 2001), or when composting the same waste under different temperature regimes (Nakasaki et al., 1985). Both facts have been correlated with metabolic effects associated with the growth of different microbial communities using different organic substrates under different conditions.

In the present work, different wastes were composted under controlled conditions with the following objectives: 1) to determine the values of OUR-DRI, SRI, and RQ related to the composting of different wastes of different biochemical composition; 2) to study the suitability of these indexes to monitor the biological activity of the composting process; 3) to compare the values of DRI and SRI; and 4) to study the factors affecting the RQ value.

MATERIALS AND METHODS

Composted Materials

Four wastes were used in the composting experiments: source-separated organic fraction of municipal solid waste (OFMSW) amended with vegetal wastes from the municipal composting plant of Sant Cugat del Vallès (Barcelona, Spain); dewatered raw sludge (RS) composed of primary and activated sludge from the urban wastewater treatment plant of La Garriga (Barcelona, Spain); dewatered anaerobically digested sludge (ADS) from the urban wastewater treatment plant of Granollers (Barcelona, Spain), and paper sludge (PS) from a recycled paper manufacturing industry (Zaragoza, Spain). Table I presents the main characteristics of composted materials. In the case of wastewater sludge (RS and ADS), wood chips from a local carpentry were used as bulking agent in a volumetric ratio 1:1, which was optimal for sludge composting (Gea et al., 2003).

Table I. Main characteristics of the composted materials.

Parameter	OFMSW	ADS	RS	PS
Moisture (%)	36.1	67.7	72.7	36.7
Dry matter (%)	63.9	32.3	27.3	63.3
Total organic matter (% dry basis)	52.3	52.8	60.4	33.7
N-Kjeldhal (% dry basis)	2.2	2.6	2.5	0.43
C/N ratio	20	8	12	34
pH	6.1	7.6	7.1	7.5

Composting Experiments

Composting experiments were undertaken in a 100-L static composter (Fig. 1). A plastic mesh was fitted at the bottom of the recipient to support the material and separate it from possible leachates. Several holes were perforated through the walls of the vessel to permit air movement, leachates removal, and the insertion of different probes. The composter was placed on a scale (BACSA mod. I200) for on-line waste weight monitoring. Four Pt-100 sensors (Desin mod. SR-NOH) inserted at different points inside the 100-L tank were used for monitoring temperature in the composting experiments. Temperature average values are presented. After aspiration from the sample, oxygen and CO_2 concentration in interstitial air were monitored with an oxygen sensor (Sensox, Sensotran, Spain) and an infrared detector (Sensontran I.R., Sensotran, Spain), respectively. All sensors were connected to a home-made data acquisition system. Oxygen was controlled by means of a feedback oxygen control which automatically supplied fresh air (room temperature) to the reactor by means of a flow meter (Sensotran mod. MR3A18SVVT) to maintain an oxygen concentration over 10%.

Moisture content was initially adjusted and maintained between 40–60% during all the experiences (adding tap water when necessary), since it is considered optimal for composting (Haug, 1993).

Monitored Parameters

OUR or DRI was determined on-line using:

$$\mathrm{OUR} = \frac{\mathrm{F} \cdot (20.9 - \mathrm{O}_{2,\mathrm{out}})}{\mathrm{M} \cdot 100} \cdot \frac{\mathrm{P} \cdot 32 \cdot 60}{\mathrm{R} \cdot \mathrm{T} \cdot \mathrm{DM} \cdot \mathrm{TOM}} \quad (1)$$

where OUR (g $O_2 \cdot$Kg $TOM^{-1} \cdot h^{-1}$); *F*, air flow into the reactor ($L \cdot min^{-1}$); $O_{2,out}$, oxygen concentration in the exhaust gases (%, mol $O_2 \cdot mol^{-1}$); M, total mass of waste in the reactor (kg); P, atmospheric pressure at the elevation of measurement (atm); 32, oxygen molecular weight (g $O_2 \cdot$mol O_2^{-1}); 60, conversion factor from minutes to hours; 20.9, percentage of oxygen in inlet air; R, ideal gas constant (0.08206 $L \cdot atm \cdot mol^{-1} \cdot K^{-1}$); *T*, temperature (K); DM, fraction of dry matter of a parallel sample aliquot (kg $DM \cdot kg^{-1}$); TOM, fraction of total organic matter of a parallel sample aliquot in dry basis (kg TOM·kg DM^{-1}).

SRI was determined off-line using a static respirometer based on the model previously described by Ianotti et al.

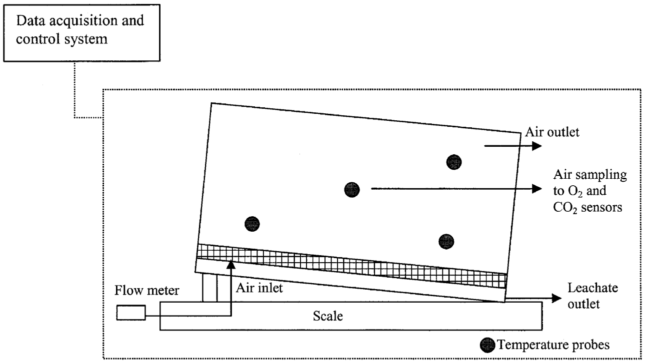

Figure 1. Scheme of the composting vessel.

(1993, 1994), following the modifications and recommendations given by the U.S. Department of Agriculture and U.S. Composting Council (2001). Briefly, the drop of oxygen content in a flask containing a compost sample was monitored with an oxygen meter (Lutron 5510, Lutron, Taiwan) connected to a personal computer (RS232 communication protocol) with the proper software to register oxygen values. The setup included two water baths for carrying out experiments at two different temperatures simultaneously. Temperatures assayed were a fixed value of 37°C (U.S. Department of Agriculture and U.S. Composting Council, 2001) and the in situ temperature of the composter at the moment of sampling. Prior to the assays, samples for experiments at 37°C were incubated for 18 h at this temperature, while samples for experiments at in situ temperatures where incubated for 4 h at this temperature. During all the incubation periods samples were aerated with previously humidified air at the sample temperature. Once the incubation period was finished, the O_2 level was then recorded every 15 sec for 90 min. In all experiments three replicates were used. After oxygen measurement, the total volume of free air space in each sample flask was determined. The SRI of the compost sample related to total organic matter content was calculated from the slope in a linear segment on the chart O_2 (%) versus time using:

$$\mathrm{SRI} = \frac{\mathrm{V} \cdot \mathrm{P} \cdot 32 \cdot \mathrm{s} \cdot 60}{\mathrm{R} \cdot \mathrm{T} \cdot \mathrm{X} \cdot \mathrm{DM} \cdot \mathrm{TOM}} \tag{2}$$

where SRI (g $O_2 \cdot$ kg $\mathrm{TOM}^{-1} \cdot \mathrm{h}^{-1}$); *V*, volume of air in the flask (L); s, slope of change in O_2 percentage per minute divided by 100 (mol $O_2 \cdot \mathrm{mol}^{-1} \cdot \mathrm{min}^{-1}$); X, wet weight of compost test aliquot (kg).

RQ was determined on-line using:

$$\mathrm{RQ} = \frac{\mathrm{CO}_{2,\mathrm{out}}}{20.9 - \mathrm{O}_{2,\mathrm{out}}} \tag{3}$$

where RQ (dimensionless); $CO_{2,\mathrm{out}}$, carbon dioxide concentration in the exhaust gases (%); $O_{2,\mathrm{out}}$, oxygen concentration in the exhaust gases (%). CO_2 percentage in inlet air was considered negligible.

Analytical Methods

Moisture, Dry Matter (DM), Total Organic Matter (TOM), N-Kjeldhal, and pH were determined according to the standard procedures (U.S. Department of Agriculture and U.S. Composting Council, 2001). The composter material was manually homogenized prior to sampling and the volume of sample was 1 L to ensure a representative portion of the material.

RESULTS AND DISCUSSION

Composting Experiments

Composting of different wastes was performed under controlled conditions. Relevant parameters of the composting experiments are presented in Figures 2–5 for OFMSW, ADS, RS, and PS, respectively. In the case of OFMSW, ADS, and RS the thermophilic range of temperatures was quickly achieved and maintained for 3–5 days (Figs. 2a, 3a, 4a). This period was followed by a longer mesophilic maturation phase, which corresponded to a typical composting temperature profile at laboratory scale. In the case of PS, the thermophilic phase was longer and the air requirements were higher (Fig. 5a). This was probably due to the combination of organic compounds found in paper sludge, which consists of an easily biodegradable cellulose fraction and a more recalcitrant fraction of lignin responsible for the prolongation of the composting process and the possible presence of a specialized microbial community (Charest et al., 2004).

An oxygen-based control was used in the composting experiments. Using this control, oxygen in the material was maintained under strictly aerobic conditions (no oxygen limitation) by adjusting the air flow. As can be seen in Figures 2b, 3b, 4b, and 5b, the oxygen content in interstitial air was over 10%, except in some sporadic occasions that corresponded to compressor failures or sampling. On the other hand, temperature profiles for all the wastes permitted the hygienization of the material.

Static Respirometric index (SRI)

The respirometric index has been proposed both as a biological activity indicator and a stability index (Ianotti et al., 1993; Adani et al., 2000). Among the different strategies used for the determination of the respirometric index, one of the most popular, simple, and low-cost techniques is the static respirometric index (SRI, or simply RI), which is carried out off-line by incubating a compost sample and calculating the oxygen consumption. RI is often determined at temperatures ranging from 30–37°C (Ianotti et al., 1993; U.S. Department of Agriculture and U.S. Composting Council, 2001; Lasaridi and Stentiford, 1998), which are adequate to predict compost stability. However, if the objective is to estimate the biological activity of the composting process in the initial thermophilic phase, the RI

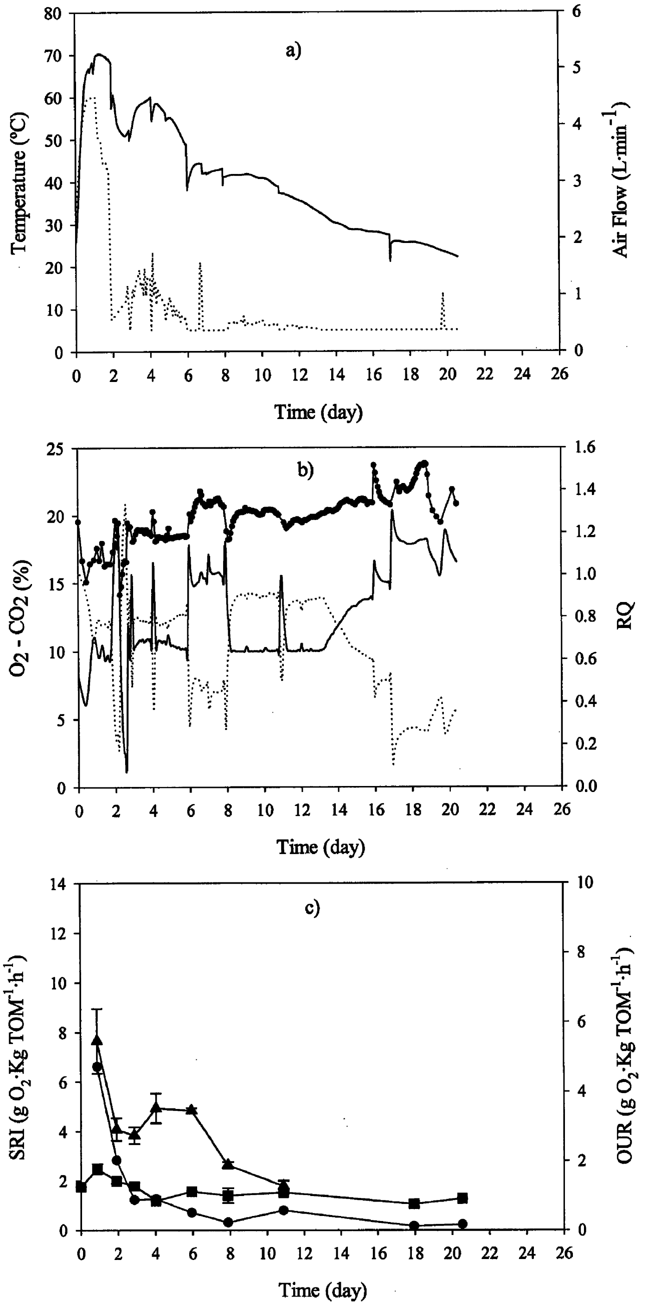

Figure 2. Composting of OFMSW. **a:** Temperature (continuous line) and air flow (dotted line). **b:** Oxygen content (continuous line), carbon dioxide content (dotted line), and respiratory quotient (circles). **c:** Oxygen uptake rate (circles), static respirometric index at process temperature (triangles) and SRI at 37°C (squares).

should be determined at the process temperature (Liang et al., 2003; Mari et al., 2003).

In Figures 2c, 3c, 4c, and 5c, the profiles obtained in the determination of SRI for several samples both at 37°C and process temperature are shown. Table II presents a summary of some of the results obtained for the SRI and other biological parameters. As can be seen, SRI determined at process temperature was higher than that of 37°C. As expected, differences between both indexes were more significant in the thermophilic phase (Table II, maximum values of SRI) than in the final compost, when the temperature was close to 37°C (mesophilic phase, Table II, minimum values of SRI). It is likely that in the determination of SRI at 37°C, the thermophilic microorganisms only exhibit a limited growth, whereas the mesophilic population is scarce. At process temperature, SRI is determined at the in situ composting conditions and the microbial populations present in the material are fully active. It was also found that changes in the SRI determined at 37°C during the composting process were minimal, whereas SRI profiles determined at process temperature

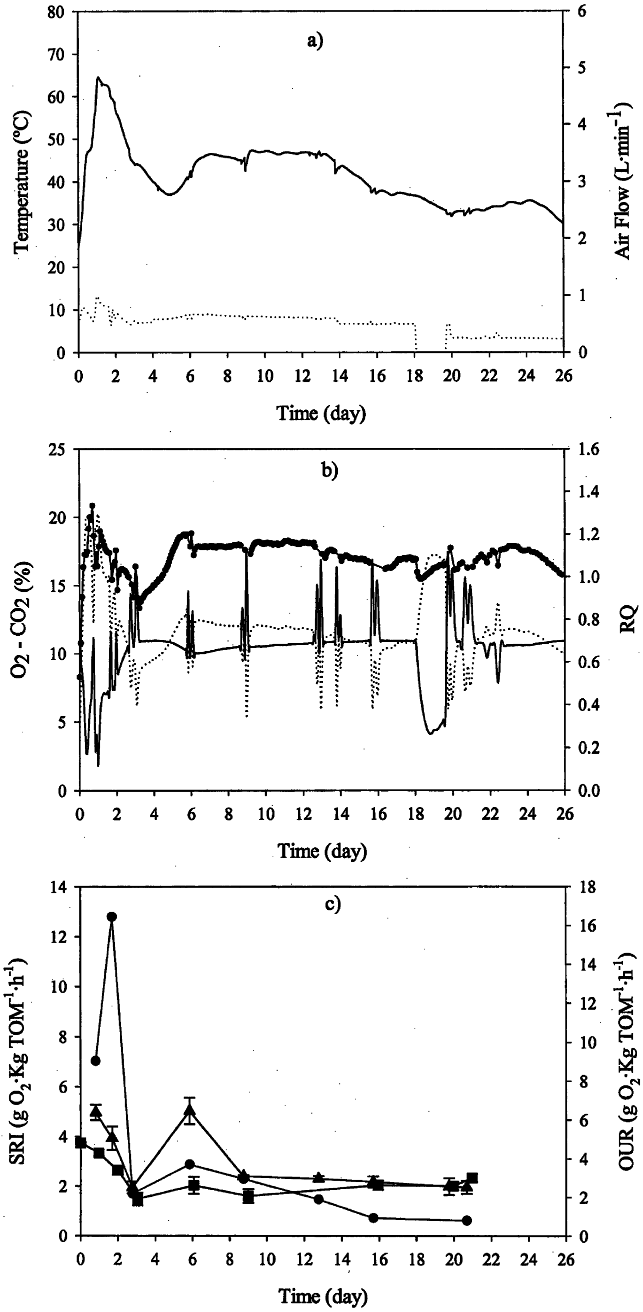

Figure 3. Composting of ADS. Same legend as Figure 2.

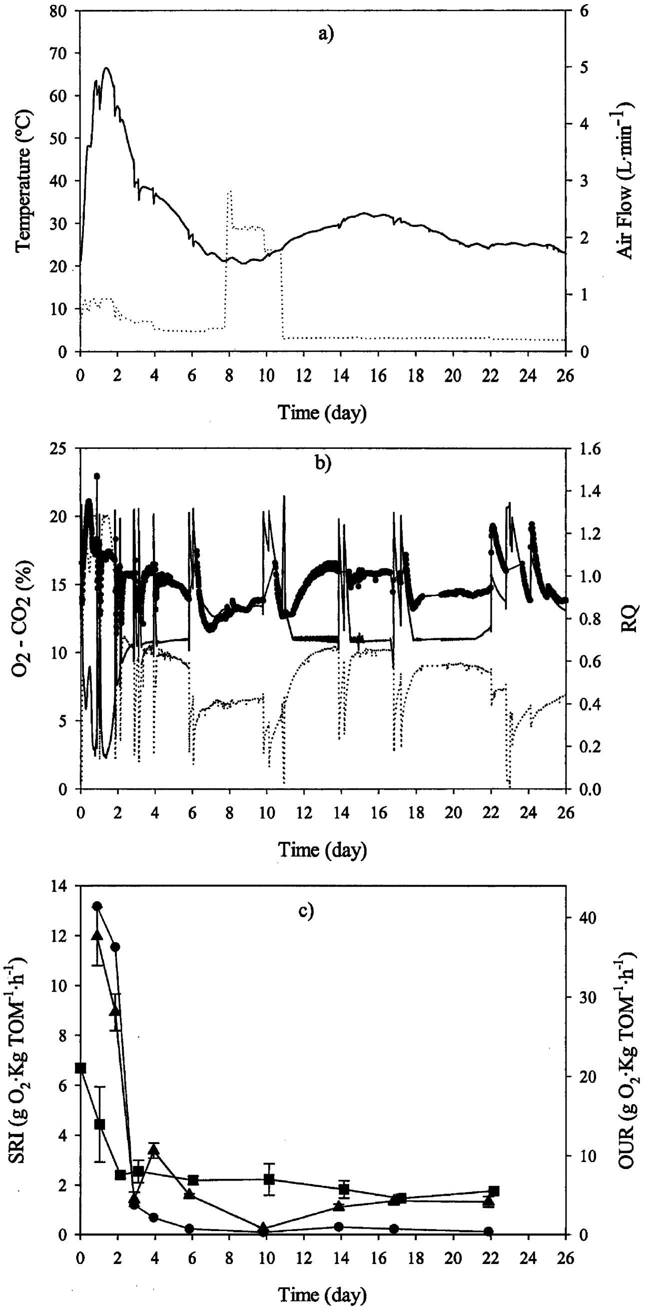

Figure 4. Composting of RS. Same legend as Figure 2.

correlated well with process temperature. On the other hand, the values of SRI at process temperature were initially higher for OFMSW and RS than for ADS and PS (Table II), which was in accordance with the biodegradability of the wastes and the presence of more labile organic compounds in "fresh" wastes such as OFMSW and RS.

In conclusion, it can be stated that SRI can be used for monitoring the biological activity of the composting process; however, it should be determined at the same conditions of the process material, especially temperature. Although the SRI determination is usually conducted at mesophilic temperatures, these values should be exclusively used for compost material in the maturation stage.

Oxygen Uptake Rate (OUR)

Microbial respiration is typically expressed as OUR in the biotechnological field. Although OUR is a general term which does not presuppose any specific conditions in its determination, in the composting field it is usually reserved for on-line measurements of oxygen consumption. Indeed, OUR is often referred to as dynamic respirometric index (DRI) and it is determined in pilot-scale composters with high levels of instrumentation (Adani et al., 2000, 2001).

In the present study, OUR was determined on-line for the different wastes studied in several stages of the composting process. Some representative values of OUR are

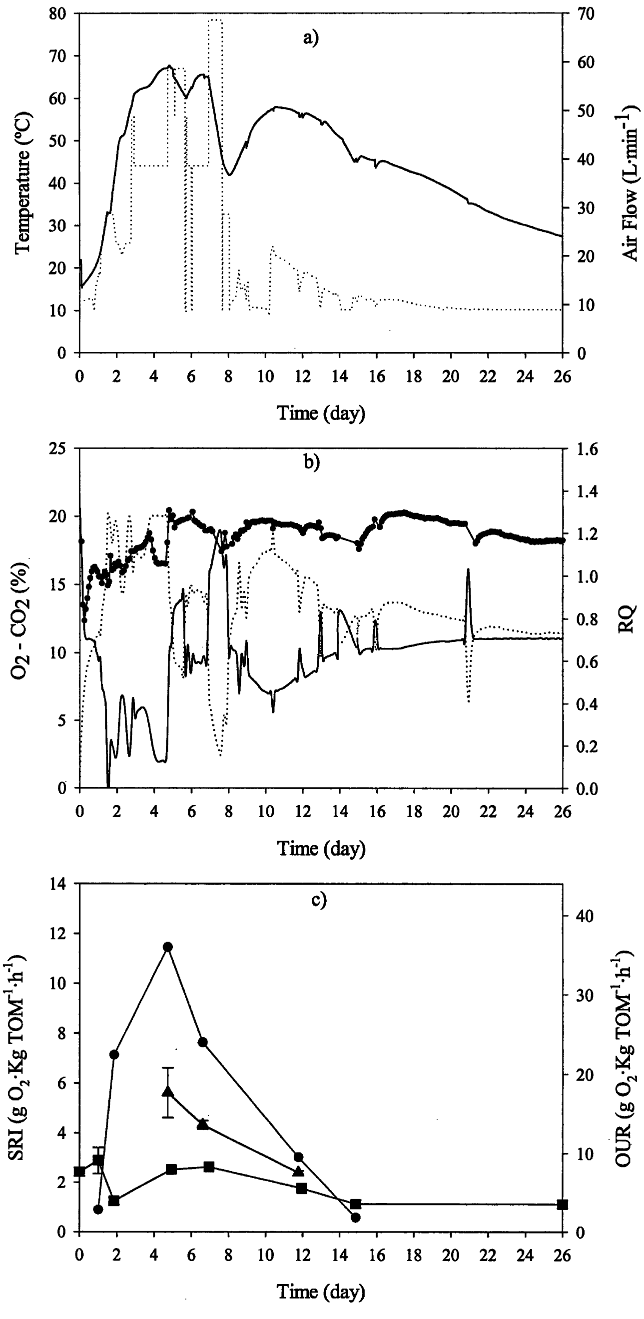

Figure 5. Composting of PS. Same legend as Figure 2.

Table II. Summary of the results obtained in the monitoring of composted wastes.

Parameter	OFMSW	ADS	RS	PS
RQ (average and standard deviation)	1.24 ± 0.09	1.09 ± 0.08	1.00 ± 0.08	1.17 ± 0.09
Maximum OUR ($g\ O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$)	4.73	16.45	41.38	35.98
Minimum OUR ($g\ O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$)	0.11	0.79	0.27	1.80
Maximum SRI (T)* ($g\ O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$)	7.64	5.02	11.96	5.61
Minimum SRI (T)* ($g\ O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$)	1.77	1.95	0.23	2.40
Maximum SRI (37°C) ($g\ O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$)	2.48	3.74	6.68	2.88
Minimum SRI (37°C) ($g\ O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$)	1.05	1.48	1.46	1.11

*Determined at composting process temperature.

shown in Figures 2c, 3c, 4c, and 5c. In general, evolution of OUR was well correlated with the composting activity (temperature) and an important decrease in OUR values was observed during the composting process. Additionally, when the different wastes were compared in terms of initial OUR, values for RS were largely higher (41.38 g $O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$) than that of ADS (16.45 g $O_2 \cdot Kg\ TOM^{-1} \cdot h^{-1}$), according to the high biodegradability of RS. Both aspects confirmed that OUR can be very useful in the monitoring of the biological activity of the process. However, as OUR determination requires a closed reactor and a level of instrumentation that is not found in most of the composting plants (e.g., windrow composting), the values of OUR were compared to those of SRI at process temperature, as OUR is inherently determined at process temperature. As can be seen in Figures 2c, 3c, 4c, and 5c and Table II, values of OUR were much higher than those of SRI, especially in the initial stages of the process. These important differences may be caused by the insufficient oxygen diffusion in static samples, which limited biological activity. In dynamic systems, forced aeration may contribute significantly to the oxygen supply in all the biologically active areas of the material. These results had been previously observed in the measurement of the biological activity of compost samples by static and dynamic approaches and the discrepancies between the static and dynamic index had been quantified (Scaglia et al., 2000; Adani et al., 2003). Practically, some authors propose that the dynamic index can be estimated by multiplying the static index by a factor of 2 (Scaglia et al., 2000). The differences between the dynamic and the static index found in our work were, however, more important, especially at the initial stages of the process. For instance, in the case of ADS, RS, and PS, the dynamic index was ~ 4– 5 times higher than the static index, which could be due to a more efficient air supply in the composting reactor or the fact that the majority of referred RIs were obtained from samples of mature compost. OFMSW was the only waste where dynamic and static indexes were similar, which was probably due to the high level of porosity of this material and the consequent absence of diffusional problems.

At the final maturation phase, values of OUR and SRI determined at process temperature and 37°C were relatively similar (Figs. 2c, 3c, 4c, and 5c). In this case, it was probable that a reduced biological activity had become the limiting step in the oxygen consumption instead of oxygen diffusion, which tended to equilibrate static and dynamic indexes. From this point of view, OUR (DRI) is the best biological activity correlating parameter in the composting process, since the SRI at process temperature provides an underestimated value of the biological activity in the early stages of the composting process. Nevertheless, the predicted patterns of biological activity seem correct when using both indexes (Figs. 2c, 3c, 4c, and 5c).

Finally, it is worthwhile noting that although temperature values are similar for all the wastes considered, biological activity indexes for each waste studied are significantly different according to its organic matter composition. Additionally, temperature is not always useful for monitoring biological activity; for instance, large amounts of material with high thermal inertia may exhibit high temperatures when biological activity has ceased (Haug, 1993). This confirms the suitability of these indexes to monitor biological activity in the composting process.

Although some of these results had been previously observed in the measurement of the biological activity of compost samples, this is, to our acknowledge, the first work where the different approximations of OUR used in the composting field, namely, DRI, SRI at process temperature and 37°C, are compared as biological activity monitors in the whole process of composting of wastes of different composition.

Respiratory Quotient (RQ)

The microbial RQ (moles of carbon dioxide produced per mol of oxygen consumed) is known to be different when wastes of different organic composition are degraded under aerobic conditions (Atkinson and Mavituna, 1983). In general, the values of RQ increase as the material becomes more oxidized. For instance, it is reported that the value of RQ decreases from 0.95 to 0.87 when some fats, a low-oxidized organic material, are mixed with OFMSW (Weppen, 2001). In Figures 2b, 3b, 4b, and 5b, values of oxygen and carbon dioxide contents, and the calculated value of RQ for the considered wastes, are shown. Table II presents the average values of RQ for the studied wastes.

Although RQ is commonly used in the biotechnological field usually using pure cultures, it is rarely determined in the composting process. From our results, it can be seen that only slight differences were observed when changing a waste. As expected, values of RQ for RS (Table II, 1.00) were lower than that of ADS (Table II, 1.09) because it is likely that RS contained more labile nonoxidized organic compounds. However, the value obtained for OFMSW (Table II, 1.24) is probably higher than expected, since it is a fresh material. Other authors have reported values of RQ for different wastes under composting conditions. For instance, values of RQ for MSW were between the range of 1.02 (Smars et al., 2001) to 0.95 (Weppen, 2001), whereas values for paper sludge were 0.92 (Atkinson et al., 1997). Other studies observed slight differences of RQ when temperature was changed, which was attributed to the dominance of catabolism or anabolism at different temperatures (Nakasaki et al., 1985). All these values are quite similar to our results. However, comparison with these values may not be possible, since the presence of anaerobic conditions is not routinely quantified in composting experiments. The production of methane and several nitrogen oxides emissions during composting has been extensively studied (He et al., 2001; Beck-Friis et al., 2003). Thus, since part of the material can obviously be anaerobically degraded, the resulting RQ will undergo a dramatic change.

More interesting is the fact that RQ values are quite stable during composting of different wastes, even when there is a transition between a thermophilic and a mesophilic phase (Figs. 2b, 3b, 4b, and 5b) and the waste composition is changing. This fact has been confirmed by other studies, when deviations found in RQ during a composting process are usually below 10%, even when aeration strategies and oxygen control were changed (Klauss and Papadimitriou, 2002).

This seems to indicate that biodegradation of organic matter in a composting environment may not be a sequential process, including different steps with different microbial communities involved in a complex structure of organic matter degradation, similar to the anaerobic degradation of organic matter. It can be hypothesized that organic matter degradation in composting is a more straightforward process, in which communities of microorganisms able to degrade some substrates coexist and are sustained and the changes in microbial communities are more gradual. This fact could have important implications in the future modeling of the composting process.

Another possible explanation might be, nevertheless, a lack of sensibility in the determination of the RQ, or the presence of not-considered anaerobic or anoxic zones (He et al., 2001). The determination of the role of RQ in the composting field will, of course, be the subject of future studies; however, from the scope of the present work, it is obvious that it cannot be used for monitoring the microbial activity of the process.

CONCLUSIONS

From the results obtained, it can be concluded that:

1) OUR (DRI) provided the most suitable values of microbial activity in a compost environment.
2) SRI measured at process temperature, especially in the early stages of the composting process, was significantly lower than DRI, which was probably due to oxygen diffusion limitations in static systems. Both values of static and dynamic indexes were similar during the maturation phase.
3) SRI measured at 37°C should not be used with samples obtained during the thermophilic phase, since it resulted in an underestimation of the respiration values.
4) RQ presented only slight variations when changing the process temperature or the waste considered, and its utility should be restricted to ensure aerobic conditions in the composting matrix or future modeling studies.

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7. DOCUMENTACIÓ COMPLEMENTÀRIA

Els articles, actualment en procés de revisió, que es presenten són:

article V *Sanitation of sludge from wastewater treatment through composting*
Teresa Gea, Adriana Artola i Antoni Sánchez.
Process Biochemistry. Enviat: juliol 2004.

article VI *Co-composting of sewage sludge:fats mixtures and characteristics of the lipases involved*
Teresa Gea, Pau Ferrer, Gregorio Álvaro, Francisco Valero, Adriana Artola i Antoni Sánchez.
Journal of Biotechnology. Enviat: gener 2005.

article V

Sanitation of sludge from wastewater treatment through composting

Teresa Gea, Adriana Artola i Antoni Sánchez.

Process Biochemistry. Enviat: juliol 2004.

Sanitation of sludge from wastewater treatment through composting

María Teresa Gea, Raquel Barrena, Adriana Artola* and Antoni Sánchez

Escola Universitària Politècnica del Medi Ambient

Universitat Autònoma de Barcelona

Rbla Pompeu Fabra 1

08100-Mollet del Vallès (Barcelona), Spain.

* Corresponding author: Adriana Artola

Escola Universitària Politècnica del Medi Ambient

Universitat Autònoma de Barcelona

Rbla Pompeu Fabra 1, 08100-Mollet del Vallès (Barcelona), Spain

Phone: 34-93-5796790

Fax: 34-93-5796785

E-mail address: aartola@eupma.uab.es

Abstract

Composting of two types of sludge produced in wastewater treatment plants, raw sludge (RS) and anaerobically digested sludge (ADS), has been systematically studied by means of the experimental design technique. The results have been analyzed using a full factorial experimental design and the surface response method in order to determine the optimal conditions for composting such sludges in terms of bulking agent particle size and bulking agent:sludge volumetric ratio, two of the key parameters to ensure an optimal performance of the composting process. The objective function selected was the death kinetics of *Salmonella*, which was chosen as a model pathogen microorganism to represent the sanitation of the material. For both types of sludge, optimal values were found at 5 mm bulking agent particle size and 1:1 bulking agent:sludge volumetric ratio when a Gaussian function was fitted to the experimental data. Pilot scale experiments using optimal values obtained were successfully undertaken and confirmed a full sanitation of the sludge by means of the composting process.

Keywords: Anaerobically Digested Sludge, Composting, Experimental Design, Raw Sludge, *Salmonella*, Sanitation.

Abbreviations and Notations

ADS: Anaerobically Digested Sludge.

FAS: Free Air Space.

OUR: Oxygen Uptake Rate (mg $O_2 \cdot$g TOM$^{-1} \cdot$h^{-1}).

RQ: Respiratory Quotient.

RS: Raw Sludge.

TOM: Total Organic Matter (% of dry matter).

WWTP: Wastewater Treatment Plant.

a, b, c, X_{10} and X_{20}: Coefficients of Gaussian function.

C: Pre-exponential factor (min^{-1})

E_D: Inactivation energy (Kcal/mol).

F_{obj}: Objective function.

k_D: Thermal inactivation coefficient (min^{-1}).

n: Viable pathogen population.

r: Coefficient of correlation.

T: Temperature (ºC or K).

t: Time (min or day).

X_1: Bulking agent particle size (mm).

X_2: Bulking agent:sludge volumetric ratio.

X_i: Independent variables of experimental design.

1. Introduction

Handling and disposal of large quantities of sludge is a problem derived from the growing environmental awareness, which is leading to an increasing number of wastewater treatment plants entering in operation [1]. At present, there is a tendency towards an agricultural application of sludge in order to reuse its organic matter and nutrient content. The unique legal restriction to direct land application of the sludge is given by its content of heavy metals and potentially toxic compounds. European Commission is preparing a new Directive on the biological treatment of organic wastes, which intends to regulate among other quality parameters the degree of sludge sanitation before its application to land [2]. The two alternatives proposed by the Directive to ensure effective pathogen elimination are thermophilic anaerobic digestion and composting. Sewage sludge composting can effectively decompose organic matter of sewage sludge into a stable end product. In addition, the high temperature reached due to the metabolic heat generated during the thermophilic phase of the composting process is effective in destroying the pathogens allowing the final product to be safely used as fertiliser or soil conditioner [3]. For sludge composting, in the draft of the above mentioned Directive different combinations of temperature and time are indicated in order to reach the proper sanitation of the final product [2].

Main factors influencing the composting process are temperature, water content, oxygen concentration in the composting matrix, porosity and free air space (FAS). Temperature is simultaneously a consequence of the composting process (microbial metabolism) and a control parameter. Temperatures providing the maximum degradation rate are in the range 40-70ºC [4]. The optimum water content has been established within

40-60%. Microbial activity begins to decrease if the water content is lower than 40%. If moisture increases over 60-70%, water will fill part of the porous of the composting matrix interfering in the oxygen exchange between the air provided to the system and microorganisms responsible of the organic matter degradation. Oxygen concentration within the composting matrix should not be lower than 5-7%. Proper aeration of the composting material will only be possible if enough porosity and FAS are provided. Free air space has been defined as the void volume that air can occupy with respect to the total volume of composting material. Optimum values of FAS are around 30% [4].

The water content of sewage sludge is usually too high for direct composting even if a dewatering treatment is applied to this material. In a majority of cases the addition of a bulking agent is needed. A bulking agent is a material that provides FAS and regulates the water content of the waste to be composted. Common bulking agents are fibrous carbonaceous materials with low moisture content [5-8]. A long list of waste materials have been proposed as bulking agents although the most widely used materials are wood chips and sawdust [9-12]. These materials are not significantly degraded under composting conditions because their high lignin content and hence they can be recycled to the composting process. In addition to the type of bulking agent used, its particle size and the proportion of bulking agent in the final mixture have also been empathised as important factors in sludge composting [7,10,13].

The objective of this work is to examine the influence of the bulking agent particle size and bulking agent:sludge volumetric ratio on the composting process of two different types of sewage sludge: dewatered raw sludge (RS) and dewatered anaerobically digested sludge (ADS). The full composite factorial experimental design technique will be applied to plan the experiments needed to learn on the influence of the mentioned factors on the sludge composting process and to determine the relationship among them. The variable monitored is the temperature profile reached in a composting experiment. As stated above, the combination of the two parameters (temperature and time) will determine the degree of sludge sanitation. *Salmonella* was selected as a model pathogen to calculate the sanitation degree using a first order decay model, since this microorganism is widely used in sludge contamination studies [14,15].

2. Materials and Methods

2.1 Sludge and bulking agent

The waste materials studied were dewatered raw sludge (composed of primary and activated sludge) from the urban wastewater treatment plant of La Garriga (Barcelona, Spain) and dewatered anaerobically digested sludge from the urban wastewater treatment plant of Granollers (Barcelona, Spain). Wood chips from a local carpentry were used as bulking agent. The chips consist of a variable mixture of pine and beech tree wood. The characteristics of these materials are presented in Table 1.

Sludge-wood chips mixtures were handmade after screening of the bulking agent. All mixtures presented moisture content of 50-75%. A semi-industrial sieve (Filtra Vibración, FT-400) was used for wood chips screening. Three different screens (20, 10 and 5 mm mesh) were available.

2.2 Composting experiments

Laboratory-scale experiments were undertaken using 4.5-L Dewar® vessels conditioned for composting. A perforated cork lid was conditioned for temperature monitoring and air supply and a rigid wire was placed near the bottom of the vessel to separate the composting material from possible leachates. In previous experiments, Dewar vessels performed similarly to other laboratory composters [16].

Pilot tests were undertaken in a 100-L static composter. The recipient was placed horizontally with a slight inclination to allow its opening from the top and to permit the collection of leachates. A plastic mesh was fitted at the bottom of the recipient to support the material and separate it from possible leachates. Several holes were perforated through the walls of the vessel to permit air movement, leachates removal and the insertion of different probes. An O_2-content based control provided the air for the composting process.

2.3 Temperature, O_2 and CO_2 monitoring

Laboratory scale: Pt-100 sensors were used for temperature monitoring in the 4.5-L Dewar vessels placed in the material to have a measuring point at 1/2 of the height of the material in the vessel. Temperature sensors were connected to a data acquisition system (DAS-8000, Desin, Spain) which is connected to a standard personal computer. The system allows, by means of the proper software (Proasis® Das-Win 2.1, Desin, Spain), the continuous on-line visualisation and registration of the temperature. O_2 content was measured with a portable O_2 detector (Oxy-ToxiRAE, RAE).

Pilot scale: Four Pt-100 sensors (Desin mod. SR-NOH) inserted at different points inside the 100-L tank were used for monitoring the temperature in the pilot scale composting experiments. After aspiration from the sample, oxygen and CO_2 concentration in interstitial air were monitored with an oxygen sensor (Sensox, Sensotran, Spain) and an infrared detector (Sensontran I.R., Sensotran, Spain) respectively. All sensors were connected to a self-made data acquisition system. Oxygen was controlled by means of a feedback oxygen control which automatically supplied fresh air to the reactor to maintain an oxygen concentration over 10%.

2.4 Analytical methods

Water content, total organic matter (TOM), pH, electrical conductivity and total nitrogen (Kjeldahl method) were determined according to the standard procedures [17].

2.5 Respirometric tests

A static respirometer was built according to the original model described previously [17] and following the modifications and recommendations given by the U.S. Department of Agriculture and U.S. Composting Council [17]. Approximately 250 mL of compost samples were placed in 500 mL Erlenmeyer flasks on a nylon mesh screen that allowed air movement under and through the solid samples. The setup included a water bath to maintain the temperature at 37ºC during the respirometric test. Prior to the assays, samples were incubated for 18 hours at 37ºC. During all the incubation period samples were aerated with previously humidified air at the sample temperature. The drop of oxygen content in a flask containing a compost sample was monitored with a dissolved oxygen meter (Lutron

5510, Lutron Co. Ltd., Taiwan) connected to a data logger. The rate of respiration of the compost sample (Oxygen Uptake Rate, OUR, based on total organic matter content, TOM) was then calculated from the slope of oxygen level decrease according to the standard procedures [18]. Results of the static respirometric index referred to total organic matter content are presented as an average of three replicates.

2.6 Numerical procedures

Surface response graphs and fitting of equations were performed with Sigmaplot® for Windows 8.0 (SPSS Inc., 1986-2001).

2.7 Optimization of composting conditions

Optimization of composting conditions was performed by means of a full composite factorial experimental design [19]. The feasibility of the experimental design technique has been widely demonstrated in other related [20] and non-related fields [21].

The experimental design was carried out as follows:

1) Bulking agent particle size (X_1) and bulking agent:sludge volumetric ratio (X_2) were selected as the main factors affecting the compostability of RS and ADS. Other important factors affecting the composting process (*e.g.* water content and oxygen supply) were not considered in the experimental design since they were initially adjusted and maintained throughout the experiment (water content in the range of 40-60% and air supply to ensure aerobic conditions). As stated above, FAS is a function of bulking agent particle size and bulking agent:sludge volumetric ratio. Two bulking agents of different particle size applied in different volumetric ratios to the same sludge, could lead to different mixtures with a similar resulting FAS. Although resulting in the same void volume for air, the integration of

the bulking agent with the sludge would be different and thus would have a different behaviour when composting. For that reason the experimental design consider the two factors, bulking agent size and volumetric ratio instead of FAS.

2) The levels of the factors considered were selected according to usual composting operation [4,7,10,13]:

- Bulking agent particle size (X_1): level 1: 0-5 mm; level 2: 5-10 mm; level 3: 10-20 mm.
- Bulking agent:sludge volumetric ratio (X_2): level 1: 1:1; level 2: 2:1; level 3: 4:1.

3) Material sanitation was selected as the objective function. Usually, time/temperature conditions are given for the destruction of each pathogen. Temperatures within the range of 55-65ºC and times from few to 60 min are usually recommended [2,4]. First order decay models are normally used to estimate heat inactivation kinetics, according to Eq. 1:

$$\frac{dn}{dt} = -k_D n \qquad \text{Eq. 1}$$

where n is the viable cell population of a given pathogen and k_D is the thermal inactivation coefficient (min^{-1}). Integration of Eq. 1 permits to calculate the logarithmic reduction of a pathogen population (Eq. 2):

$$\log_{10}\left(\frac{n_0}{n_t}\right) = \frac{1}{2.303}\int_0^t k_D dt \qquad \text{Eq. 2}$$

where k_D is an exponential function of temperature (T):

$$k_D = C\exp(-E_D/RT) \qquad \text{Eq. 3}$$

being C a constant value and E_D the inactivation energy for each pathogen. Both values can be obtained for several pathogens from data found in literature [4]. For instance, U.S. EPA regulations for Class A sludges recommend a temperature of 60ºC held for 30 min to provide a 6$\log_{10}$ reduction of *Salmonella*. This microorganism was selected as a model pathogen in the composting experiences, since is commonly used as an indicator of pathogen contamination [22,23].

From composting experiments, when a curve temperature/time was obtained, k_D and $\log_{10}$ reduction were calculated. The minimum temperature to perform calculations of sanitation was 45ºC, corresponding to the beginning of the thermophilic range, where most of the pathogen microorganisms do not survive.

3. Results and discussion

3.1 Laboratory scale experiments

Laboratory scale composting experiments were carried out in 4.5-L Dewar vessels following the full factorial experimental design, which implied to perform all the experiments for the totality of levels and factors. In this case, nine experiments (3^2) for RS and ADS were respectively undertaken. Table 2 summarizes the results obtained for all the experiments. Several replications randomly selected were also carried out under different conditions of the experimental design, resulting in a total value of 14 experiments.

Figure 1 shows two examples of the determination of k_D, that is a function of process temperature and is needed for the determination of the objective function for different initial conditions. Figure 1a corresponds to 5/1:1 conditions for RS composting (0-5 mm bulking agent particle size and 1:1 bulking agent:sludge volumetric ratio), where the thermophilic temperature range was completely achieved (maximum temperature: 62ºC) and the objective function ($\log_{10}$) value was very high (2339), whereas Figure 1b corresponds to 5/2:1 (0-5 mm bulking agent particle size and 2:1 bulking agent:sludge volumetric ratio) for ADS composting, where the thermophilic temperature was reached for a shorter time (maximum temperature: 47ºC) and the objective function value was lower (16).

Results in Table 2 show that there was a common tendency for RS and ADS to produce the best results in terms of objective function when the low particle sizes and the low bulking agent:sludge ratio were selected (0-5 mm and 5-10 mm; 1:1 and 2:1). Therefore, it can be concluded that adjusting the values of the factors studied, RS and ADS can be fully sanitized by means of composting.

Quantitative results can be obtained by selecting a function that fits the whole set of experimental values. In the composting experiments with RS and ADS, a Gaussian function was selected (Eq. 4):

$$F_{obj} = a \exp(-0.5\left(\left(\frac{X_1 - X_{10}}{b}\right)^2 + \left(\frac{X_2 - X_{20}}{c}\right)^2\right)) \qquad \text{Eq. 4}$$

Function coefficient values obtained for RS and ADS are presented in Table 3. Both functions describing RS and ADS were relatively similar (coefficients with the same signal and order of magnitude) and correlation quality (r) was excellent for both cases.

Objective function obtained in Table 3 for both RS and ADS was numerically optimized to obtain the optimal conditions to perform the composting process. Bulking agent particle size within 0-5 mm and a bulking agent:sludge volumetric ratio of 1:1 for both RS and ADS were the results obtained in the optimization of F_{obj} using a quasi-Newton method within the experimental domain. These results were in agreement with those presented in Table 2, and corresponded to the high values of the objective function.

In terms of composting process, the conclusion is that a low particle size of bulking agent is preferred to give the material an adequate porosity instead of using large quantities of bulking agent. Small bulking agent particles create a real porous structure inside the material that acts as an efficient oxygen diffuser. Also, they act as effective water absorbers, which is interesting in the composting of wet wastes such as WWTP sludge [4]. On the other hand, water content of the initial mixture needs to be adjusted to values within 40-70% [4], which discards the possibility of working at bulking agent:sludge volumetric ratios lower than 1:1 (as stated before, moisture content values obtained for the initial mixtures were within 50-75%). However, these results should be validated in industrial scale composting to investigate the effect that may produce the utilization of small particle bulking agent in the compaction of the material and the consequent loss of FAS [4,24].

3.2 Comparison between RS and ADS

Figure 2 shows the surface responses (F_{obj}) obtained for RS and ADS. A similar behaviour was observed for both types of sludge. In general, values of objective function for RS were higher (Figure 2a) than those obtained for ADS (Figure 2b). This was in accordance to the fact that the organic matter content of ADS was lower than RS (Table 1) and, consequently, organic matter suitable for degradation was more abundant in RS.

The temperature profiles obtained in composting experiments suggested that thermophilic conditions and effective sanitation could be assumed for values of objective function higher than 10 (equivalent to a 10^{10} reduction of pathogens), which implied a restricted zone in the case of RS and ADS (Figure 2) and demonstrated that it was crucial to select an optimal initial mixture to produce an effective sanitation of the material. Optimal composting conditions were found for small particle size of bulking agent and low bulking agent: sludge volumetric ratio, and the best results were produced when bulking agent size was 0-5 mm and volumetric ratio was 1:1 (normalized values: X_1=-1, X_2=-1).

3.3 Pilot scale experiments

Two experiments were undertaken in a 100-L composting reactor to corroborate the results obtained from the experimental design technique as optimal conditions for sludge composting in 4.5-L vessels. Each experiment correspond to one of the studied sludges (RS and ADS) mixed with 0-5 mm wood chips in a sludge-wood chips volumetric ratio of 1:1. The results obtained (temperature profiles, O_2 concentration in the compost matrix and CO_2 concentration in exhaust gases) are presented in Figure 3 (3a for RS and 3b for ADS). The results of the respirometric assays in terms of OUR and respiration quotient (RQ, CO_2 produced/O_2 consumed) are also included. Peaks in the CO_2 and O_2 profiles corresponded to the entrance of fresh air to the composter during material sampling. The strong variation in %O_2 profile in Figure 3b (ADS) between days 18 and 20 was due to a mechanical failure in the aeration system.

Values of the objective function calculated for the two pilot scale experiments were 1626 and 628 for RS and ADS respectively (25 days of composting approximately), which were well correlated with 4.5-L experiments. As can be seen in Figure 3, the maximum temperature reached for the two types of sludge was approximately the same, slightly higher for RS. From objective function values and temperature and O_2 profiles plotted in Figure 3 it could be stated that the porosity supplied by the bulking agent added was sufficient to overcome possible compression effects at pilot scale.

The results obtained from the respirometric tests indicate that a real stabilisation of the organic matter present in the composting matrix occurred for the two types of sludge. Initial values of OUR for RS (6.68 mg O_2·g TOM^{-1}·h^{-1}) were clearly higher than those for ADS (3.74 mg O_2·g TOM^{-1}·h^{-1}) as was expected according to the nature of organic matter present in the two different types of sludge. However, values obtained from day 3 to day 23 were very close to final OUR values of 1.76 and 2.00 mg O_2·g TOM^{-1}·h^{-1} for RS and ADS respectively. These values were slightly higher than those established in the standards for mature composts (0.5-1.5 mg O_2·g TOM^{-1}·h^{-1}) [25] and correspond to a material at the end of the thermophilic composting period. OUR values lower than 1 mg O_2·g TOM^{-1}·h^{-1} can be expected after a maturation period.

The respiration quotient (RQ), representing the relationship between CO_2 produced and O_2 consumed, is approximately equal to 1 under aerobic conditions [9]. As can be seen if Figures 3a and 3b are compared, values of RQ obtained for ADS were slightly higher than those obtained for RS in concordance with the degree of oxidation of the organic matter present in the two types of sludge, with ADS containing more degraded substances. However, RQ values for the two types of sludge were close to 1 (average RQ value for ADS=1.10 and for RS=0.96) reflecting the aerobic conditions existing during the composting experiments. The value of RQ was steady during the complete composting process. RQ has been routinely used in the biotechnological field [26] but, to our knowledge, it is rarely measured in composting processes. Since it is a characteristic value directly referred to organic waste composition, RQ can be used in the monitoring and control of the composting process of different wastes and to predict air requirements and CO_2 production.

4. Conclusions

Optimization of composting conditions permitted to obtain the optimal values of bulking agent particle size: 0-5 mm and bulking agent:sludge volumetric ratio: 1:1 for both RS and ADS. Under these conditions, the temperature and time required to destroy *Salmonella* were reached.

Pilot scale experiments under the optimal conditions obtained from the experimental design confirmed that the conditions to obtain the sanitation of the material were present. Moreover, an effective stabilization of the material took place, which was confirmed by respirometric analysis (OUR).

The respiratory quotient for RS and ADS was determined. This value can be useful in future composting experiments to monitor and control the process and to predict air requirements and CO_2 production. Additionally, it can be used to characterize a complex organic waste.

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Tables

Table 1: Properties of sewage sludge (ADS and RS) and wood chips used in the experiments

Parameter	Anaerobically Digested Sludge (ADS)	Raw Sludge (RS)	Wood Chips
Water Content (%)	67.7	72.7	5.0
Total Organic Matter (% dry basis)	52.8	60.4	99.4
N-Kjeldhal (% dry basis)	2.6	2.5	0.1
C/N ratio	8	12	500
pH	7.6	7.1	not determined
Conductivity (μS/cm)	2100	1800	not determined

Table 2: Values of the objective function for the experimental design for RS and ADS. Normalized values of X_1 and X_2 are shown in parentheses

Bulking Agent Particle Size (X_1) (mm)	Bulking:sludge volumetric ratio (X_2)	F_{obj} (RS)	F_{obj} (ADS)
0 – 5 (-1)	1:1 (-1)	2339	522
0 – 5 (-1)	2:1 (-0.333)	not determined	16
0 – 5 (-1)	4:1 (+1)	14	4
5 – 10 (-0.333)	1:1 (-1)	522	303
5 – 10 (-0.333)	2:1 (-0.333)	0	1
5 – 10 (-0.333)	4:1 (+1)	0	0
10 – 20 (+1)	1:1 (-1)	0	0
10 – 20 (+1)	2:1 (-0.333)	0	0
10 – 20 (+1)	4:1 (+1)	0	0

Table 3: Parameters of the equation fitting the results of experimental design for RS and ADS

Parameter	Raw Sludge	Anaerobically Digested Sludge
X_{10}	-0.779	-0.909
X_{20}	-1.082	-1.038
a	652.4	2487.9
b	-0.371	-0.329
c	-0.272	-0.173
r	0.99990	0.99998

Legends to Figures

Figure 1: Examples of determination of the objective function for 4.5-L experiments for a) RS: 0-5 mm and 1:1; b) ADS: 0-5 mm and 2:1. Composting temperature (dashed line), k_D (solid line). Horizontal dotted line corresponds to 45ºC, limit of *Salmonella* deactivation.

Figure 2: Surface response for the fitted objective function. Objective function is plotted for the whole range of normalized values of the factors considered (X_1: bulking agent particle size; X_2: bulking agent: sludge volumetric ratio) for a) RS; b) ADS.

Figure 3: Evolution of control parameters during composting time for 100-L scale experiments for a) RS; b) ADS. Upper graph: Temperature (solid line) and OUR (triangles); lower graph: %O_2 (solid line), %CO_2 (dotted line) and RQ (circles).

Fig 1.a)

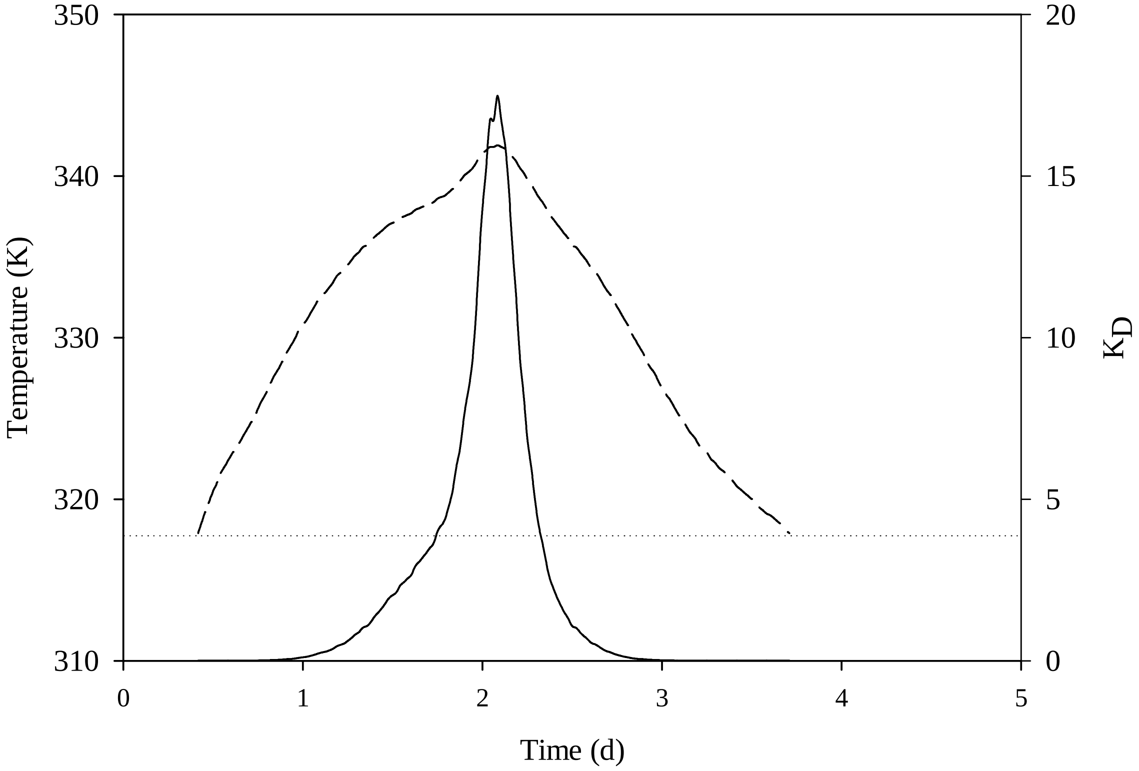


Fig. 1.b)

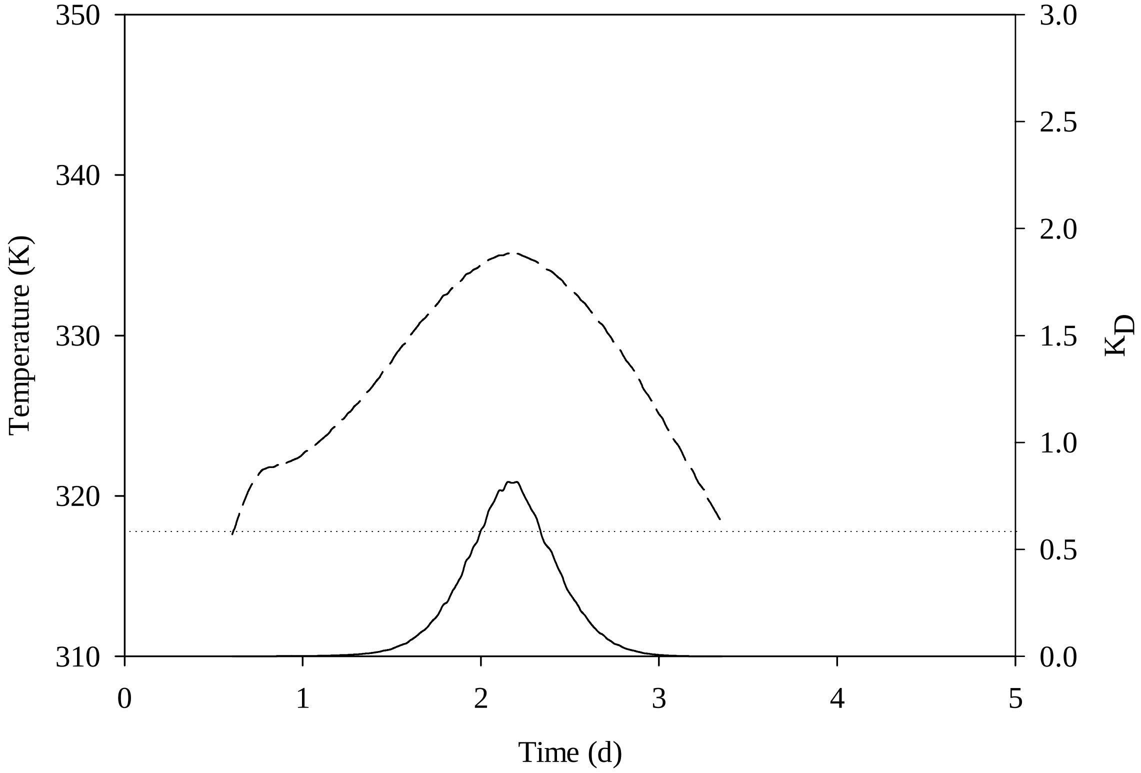

350
340
330
320
310
Temperature (K)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
K_D
0
1
2
3
4
5
Time (d)

Fig. 2.a)

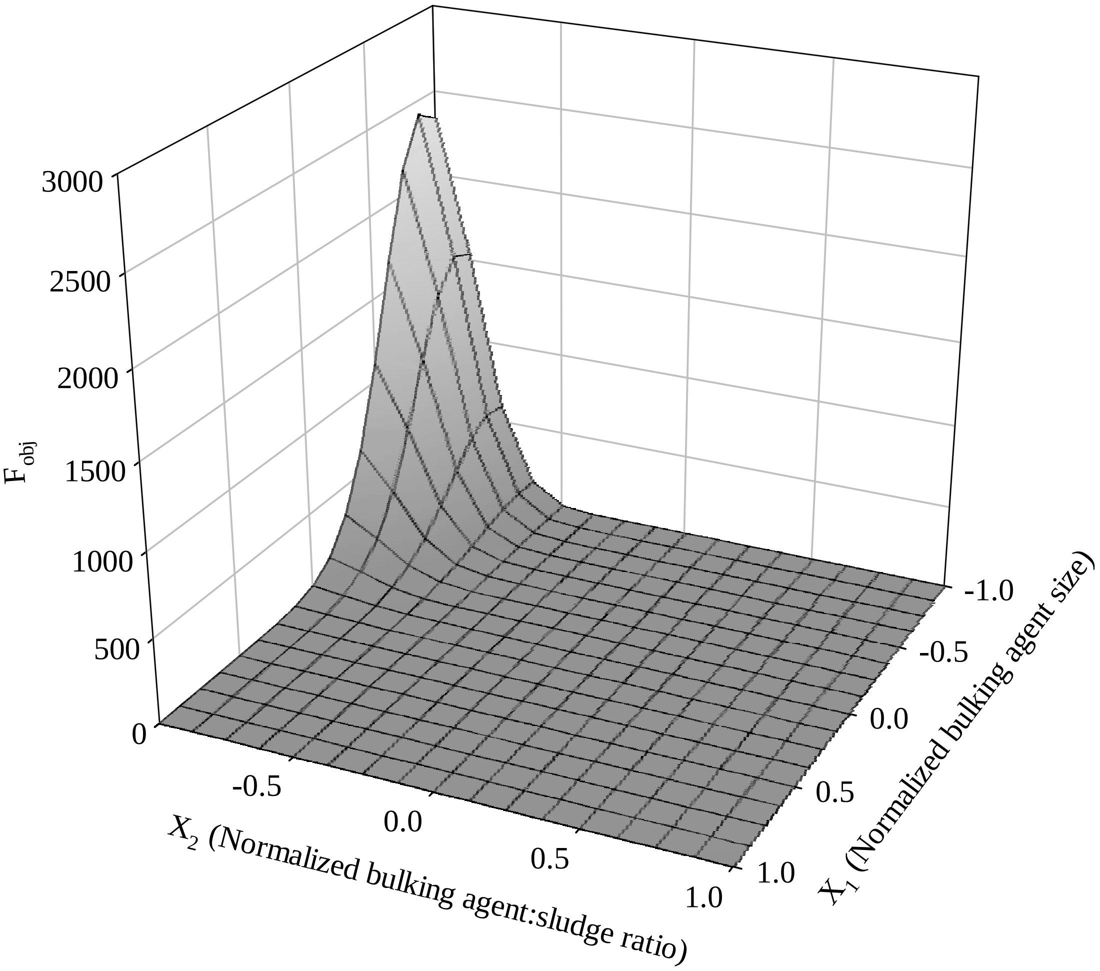

3000
2500
2000
1500
1000
500
0
F_{obj}
-0.5
0.0
0.5
1.0
X_2 (Normalized bulking agent:sludge ratio)
-1.0
-0.5
0.0
0.5
1.0
X_1 (Normalized bulking agent size)

Fig. 2.b)

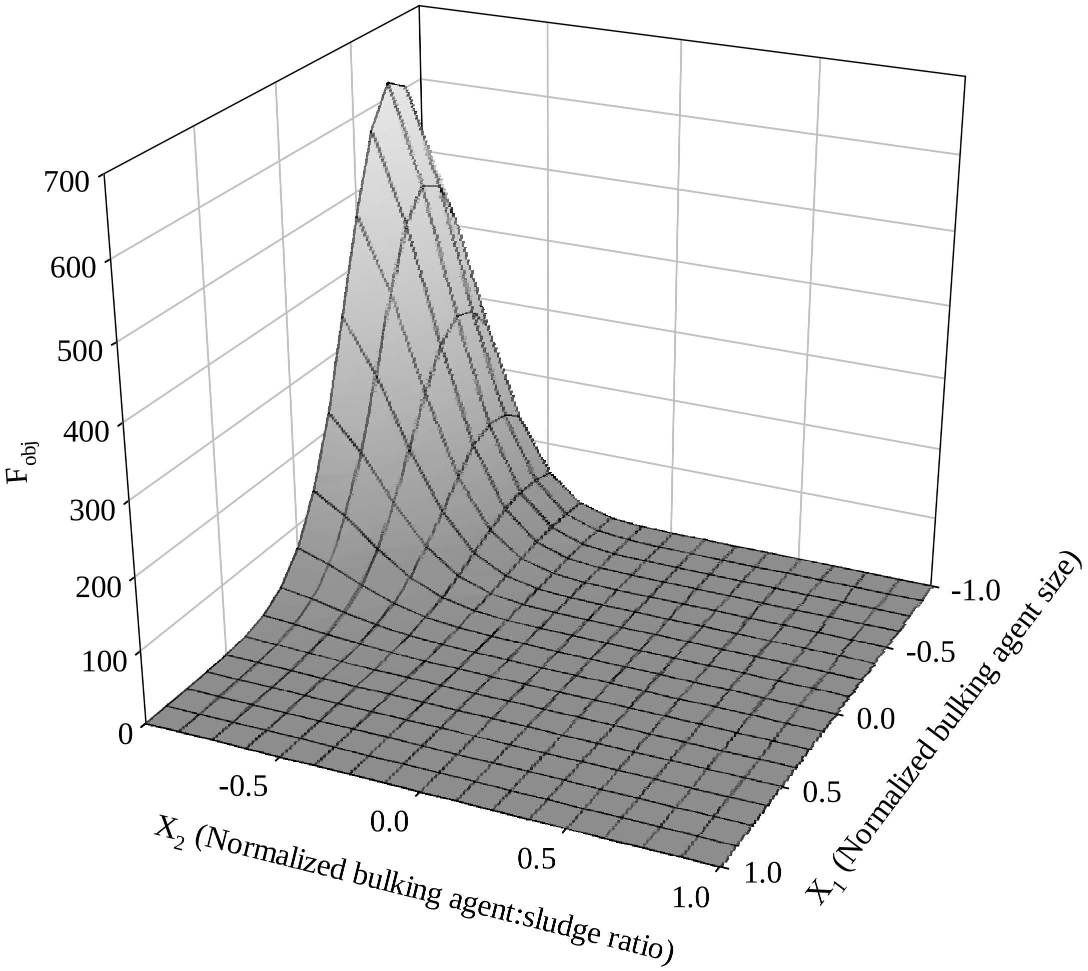

700
600
500
400
300
200
100
0
F_{obj}
-0.5
0.0
0.5
1.0
X_2 (Normalized bulking agent:sludge ratio)
-1.0
-0.5
0.0
0.5
1.0
X_1 (Normalized bulking agent size)

Fig. 3.a)

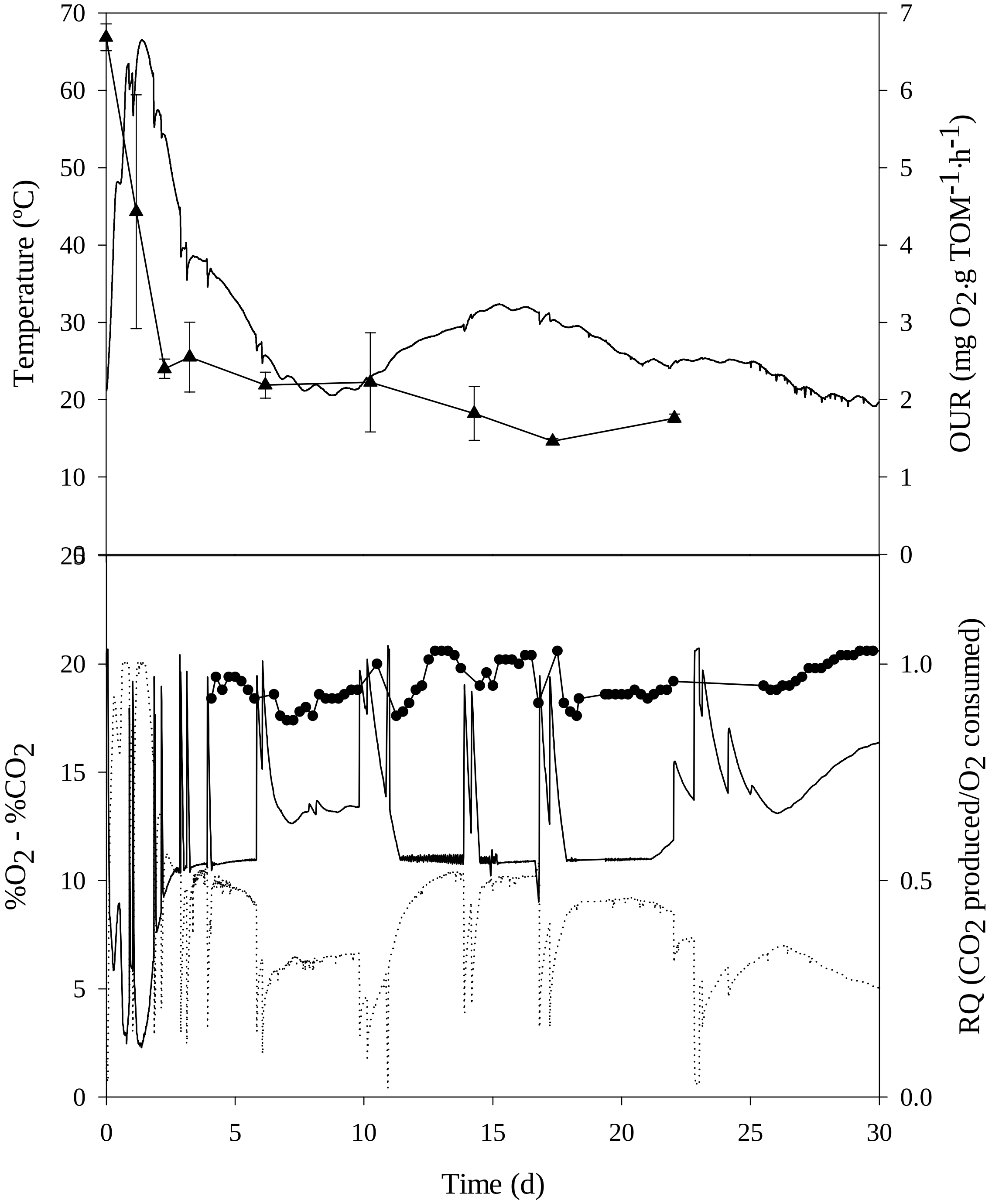
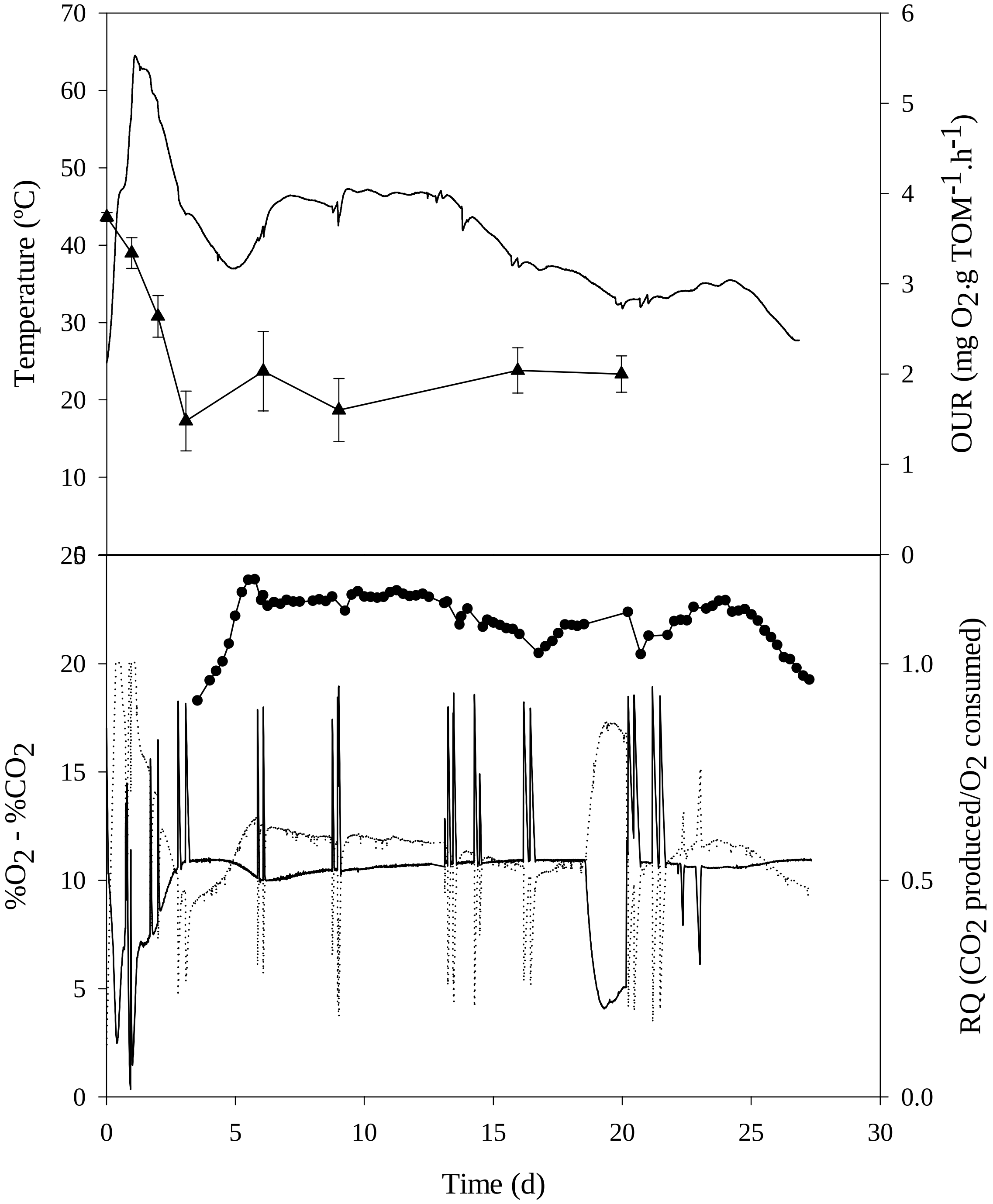

70
60
50
40
30
20
10
0
Temperature (ºC)
7
6
5
4
3
2
1
0
OUR (mg O2·g TOM-1·h-1)
25
20
15
10
5
0
%O2 - %CO2
1.0
0.5
0.0
RQ (CO2 produced/O2 consumed)
0
5
10
15
20
25
30
Time (d)

Fig. 3.b)


Temperature (ºC)
70
60
50
40
30
20
10
0
OUR (mg O_2·g TOM^{-1}.h^{-1})
6
5
4
3
2
1
0
%O_2 - %CO_2
25
20
15
10
5
0
RQ (CO_2 produced/O_2 consumed)
1.0
0.5
0.0
0
5
10
15
20
25
30
Time (d)

article VI

Co-composting of sewage sludge:fats mixtures and characteristics of the lipases involved

Teresa Gea, Pau Ferrer, Gregorio Álvaro, Francisco Valero, Adriana Artola i Antoni Sánchez.

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Co-composting of sewage sludge:fats mixtures and characteristics of the lipases involved

Teresa Gea[1], Pau Ferrer[2], Gregorio Alvaro[2], Francisco Valero[2], Adriana Artola[1] and Antoni Sánchez[1]*

[1]Escola Universitària Politècnica del Medi Ambient. Universitat Autònoma de Barcelona

Rbla Pompeu Fabra 1, 08100-Mollet del Vallès (Barcelona, Spain).

[2]Departament d'Enginyeria Química, ETSE, Universitat Autònoma de Barcelona

08193-Bellaterra (Barcelona, Spain).

*Corresponding Author: Dr. Antoni Sánchez

Escola Universitària Politècnica del Medi Ambient. Universitat Autònoma de Barcelona

Rbla Pompeu Fabra 1, 08100-Mollet del Vallès (Barcelona, Spain).

Tel.: (34) 93 579 67 84; Fax: (34) 93 579 67 85

E-mail: asanchez@eupma.uab.es

Abstract

Co-composting of sewage sludge and animal fat mixtures was studied in order to determine the possibility of using this technology to recycle fat-enriched wastes. A maximum fat content of 30% in fat:sludge mixtures is recommended to achieve the international sanitation requirements on compost quality and to avoid an excessive thermophilic composting time. Under these conditions a fat content reduction of 85% was achieved. Biological activity was highly dependent on the moisture content as shown by the respiratory quotient values. Moisture content is a critical control factor because of the hydrophobic nature of fats and should be maintained above 40% in the composting of fats. Biological indices of the compost obtained after 69 days of process (Maturity Grade: IV; Respiration Index: 1.1 mg O_2 consumed per g organic matter and hour) indicated a high stability and maturity degree of the material. Lipases responsible for fat hydrolysis were monitored during the composting process and a sample from the thermophilic period was characterized in terms of stability in front of pH and temperature. Optimal conditions for lipase stability were found at 38.3ºC and pH 7.97, however the maximum lipolytic activity was observed at thermophilic temperatures. Lipases from the thermophilic period were purified by anion exchange chromatography and visualised by SDS-PAGE. Two major bands were observed at molecular weights of 29 and 62 kDa. These bands could not be identified precisely by N-terminal sequence analysis.

Key words: Composting, Fat, Lipase, Respiration Index, Respiratory Quotient, Sewage sludge.

1. Introduction

Among the available technologies to recycle organic solid wastes, composting is often presented as a low-technology and low-investment process to convert organic solid wastes to an organic fertilizer known as compost. Composting is a biotechnological process by which different microbial communities initially degrade organic matter into simpler nutrients and, in a second stage, complex organic macromolecules such as humic acids are formed (Hsu and Lo, 1999). Composting is an aerobic process, which requires oxygen for microbial biodegradation, optimal moisture and porosity (Haug, 1993). Temperature, oxygen and moisture content are often selected as the control variables in the composting process, jointly with other chemical, biochemical or microbiological properties (Scaglia et al., 2000; Liang et al., 2003; Saviozzi et al., 2004; Tang et al., 2004).

Fats and oils are essentially triglycerides consisting of straight-chain fatty acids attached, as esters, to glycerol. Fatty acids profile can vary considerably since they can differ in chain length and may be saturated or unsaturated (Wakelin and Forster, 1997). The biodegradation of a fat begins with an enzymatic hydrolysis of the ester bond carried out by lipases, followed by the consumption of glycerol and the beta-oxidation of fatty acids (Lalman and Bagley, 2000). Fats are, on the other hand, one of the major components of organic matter in wastewater (Saatci et al., 2001) and solid wastes, especially those coming from food industry (Galli et al., 1997; Mari et al., 2003).

Different works report biological treatment of fats, however, biodegradability of lipids is generally considered as largely limited by unfavourable physico-chemical properties, such as their insolubility in water (Lefebvre et al., 1998). The biological treatment of fats under thermophilic conditions is expected to be advantageous due to favourable changes in the physical properties of these hydrophobic compounds (Beker et al., 1999). In this sense, composting, which is characterized by the rapid development of a thermophilic initial stage, can be an alternative to treat effectively fats and oils. Composting of fats is nevertheless difficult due to the nutritional lacks of fats, especially low contents of nitrogen and phosphorous in relation to a high carbon content. This usually implies that a co-substrate is necessary to compensate the C/N ratio of the initial mixture, in a process called co-composting. Among the substrates co-composted with fats, different types of sludge are used, because of its typical low C/N ratio. Thus, olive mill wastewater (Vlyssides et al., 1996; Mari et al., 2003), sewage sludge (Wakelin and Forster, 1997) and municipal solid wastes (Lemus and Lau, 2002a) have been used in the co-composting of fats or fat-enriched wastes.

Several aspects in the composting of wastes with high fat content have been studied. In general, routine composting parameters show that co-composting of these wastes is possible with low percentages of fats (5-15%) in the initial mixture (Filippi et al., 2002; Lemus and Lau, 2002b; García-Gómez et al., 2003), causing a longer thermophilic phase that is attributed to the high chemical energy content of fats (Nakano and Matsumura, 2001). On the other hand, values of lipid degradation reported are usually high, within 80-90% (Lemus and Lau, 2002b; García-Gómez, 2003; Baeta-Hall et al., 2005). Other authors have reported studies on the physico-chemical parameters showing the importance of the control of pH (Sasaki et al., 2003), whereas in other works some microbiological studies are carried out, concluding that there is not a clear correlation between the microbial populations isolated during the different phases of the process with their role in the degradation of fats (Galli et al., 1997; Baeta-Hall et al., 2005) and that probably some global biological activity indicators such as respiration profiles are more useful (Mari et al., 2003).

However, other crucial aspects such as the presence and properties of lipases involved in the composting of fat-enriched wastes have been only qualitatively studied or remain unclear. To our acknowledge, although some few works have reported qualitative values of enzymatic activities during composting (Wong and Fang, 2000; Tiquia et al., 2001; Tiquia et al., 2002; Ryckeboer et al., 2003; Charest et al., 2004; Peláez et al., 2004), the characteristics and role of lipases in the composting of high-content fat wastes have not been studied.

The objectives of this work are: i) to study the behaviour of a co-composting process at laboratory scale using sewage sludge as a basic substrate (a commonly composted waste) amended with different ratios of fats in order to determine the optimal ratio fat:sludge in terms of composting time and efficiency, ii) the determination and evaluation of biological indices in the co-composting process at pilot scale for the optimal fat:sludge ratio, iii) to study the properties of the lipases implied in the hydrolysis of fats, especially those related to their stability and optimal conditions for enzymatic activity, and iv) to identify the main microbial lipases involved in the co-composting of sewage sludge and fats.

2. Materials and Methods

2.1. Composting Materials

Animal fat (Trg Sebo Fancy, KAO Corporation S.A., Spain) coming from a cow slaughterhouse was added to anaerobically digested sewage sludge from the wastewater treatment plant of Granollers (Barcelona, Spain) in different amounts. Wood chips from a local carpentry were used as bulking agent added to the mixture sludge-fat in a volumetric ratio 1:1, which was previously found as optimal for sewage sludge composting at laboratory scale (Gea et al., 2003). Main characteristics of composted materials are summarized in Table 1. The long chain fatty acid (LCFA) profile of animal fat is presented in Table 2.

2.2. Composting experiments at laboratory scale

Laboratory-scale experiments were undertaken using 4.5-l Dewar® vessels conditioned for composting. A perforated lid was conditioned for temperature monitoring and air supply and a rigid wire net was placed near the bottom of the vessel to separate the composting material from possible leachates.

Pt-100 sensors were used for temperature monitoring placed in the material to have a measuring point at 1/2 of the height of the material in the vessel. Temperature sensors were connected to a data acquisition system (DAS-8000, Desin, Spain) which was connected to a personal computer. The system allows, by means of the proper software (Proasis® Das-Win 2.1, Desin, Spain), the continuous on-line visualisation and registration of the temperature. Oxygen content was measured with a portable oxygen detector (Oxy-ToxiRAE, RAE) with a frequency of 3-7 times a day.

For the co-composting experiments with fat and sludge two replicates were undertaken. As differences observed between the two replicates were lower than 10% for any of the parameters analyzed, only the results of one replicate are presented in the Results section.

2.3. Composting experiments at pilot scale

Pilot experiment was undertaken in a 100-l static composter. A plastic mesh was fitted at the bottom of the recipient to support the material and separate it from possible leachates. Several holes were perforated through the walls of the vessel to permit air movement, leachates removal and the insertion of different probes. The composter was placed on a scale (BACSA mod. I200) for on-line waste weight monitoring.

Four Pt-100 sensors (Desin mod. SR-NOH) inserted at different points inside the 100-l tank were used for monitoring the temperature. Temperature average values are presented. Oxygen and CO_2 of interstitial air was measured with an oxygen sensor (Sensox, Sensotran, Spain) and a CO_2 infrared detector (Sensontran I.R., Sensotran, Spain) respectively. All sensors were connected to a self-made data acquisition system. Oxygen was controlled by means of a feedback oxygen control which automatically supplied fresh air (room temperature) to the reactor by means of a flow meter (Sensotran mod. MR3A18SVVT) to maintain an oxygen concentration above 10%.

2.4. Respiratory Quotient (RQ)

RQ was calculated as the quotient of CO_2 produced and O_2 consumed as indicated in Equation 1:

$$RQ = \frac{CO_{2,out}}{20.9 - O_{2,out}} \qquad \text{(Eq. 1)}$$

where: RQ, respiratory quotient (dimensionless); $CO_{2,out}$, carbon dioxide concentration in the exhaust gases (%); $O_{2,out}$, oxygen concentration in the exhaust gases (%).

CO_2 percentage in inlet air was considered negligible and average O_2 concentration in inlet air was 20.9%. RQ is presented as an average of 10 values (100 minutes of measurement).

2.5. Lipolytic activity

Lipolytic activity was determined using a commercial kit (Roche/Hitachi LIP num. 1821792) as described by López et al. (2002). Briefly, lipases were extracted from 5 g samples using 50 ml of 400 mM Tris-HCl buffer with 10 mM $CaCl_2$ and adjusted at pH 8.0, and adding 5% (w/w) Triton X-100 (Panreac, Barcelona, Spain) to ensure a quantitative extraction of lipases (Gessesse et al., 2003). After 30 minutes of extraction using a magnetic stirrer, supernatant was centrifuged (30 min, 3500g) and filtered (0.45 µm) to remove biomass and solids. This sample was then used for lipolytic activity determination. Standard lipolytic activity assays were run at 30ºC although other temperatures were used in some occasions. One activity unit was defined as the quantity of enzyme necessary to release 1 µmol of fatty acid per min under the described conditions. Lipolytic activity was expressed as activity units per gram of dry matter.

2.6. Long chain fatty acids (LCFA)

50 ml of heptane (99% purity) were added to 5 g of sample and mixed for 30 min to extract LCFA. The suspension was then centrifuged (30 min, 3500g) and the resulting supernatant filtrated through

a Millipore Millex-FGS filter (0.2 µm). This extract was used for free LCFA determination by gas chromatography. A Perkin-Elmer AutoSystem XL Gas Chromatograph with a flame ionization detector (FID) and a HP Innowax 30 m x 0.25 mm x 0.25 µm column was used. The carrier gas was Helium and a split ratio of 13 was used. An initial temperature of 120ºC was kept for 1 min; then, it was increased to 250ºC at 8ºC min^{-1}, and maintained at that temperature for 7 min. The system was calibrated with different dilutions of a standard mixture of LCFA of concentrations in the range of 0-100 mg l^{-1}.

2.7. Effect of temperature and pH on lipase stability

A full composite factorial experimental design was carried out to study the combined effect of pH and temperature on the stability of the lipases extracted from the composting samples. The experimental design consisted of 16 experiments (12 experiments and 4 replications in central points). The levels selected for both variables were: 3 levels for pH: 5.0, 7.0 and 9.0; and 4 levels for temperature, 30, 45, 60 and 75ºC. Residual activity after 1 hour of incubation was selected as the objective function and as a measure of lipase stability. Buffers used for the incubation at the selected pH were: Tris-HCl 1M, pH 9.0; Tris-HCl 1M, pH 7.0; Acetic acid-sodium acetate 1M, pH 5.0.

2.8. SDS-PAGE

Polyacrylamide gel electrophoresis (PAGE) was performed in 12% polyacrylamide gels in denaturing conditions as described by Laemmli (1970). A vertical slab Mini Protean II cell (Bio-Rad, Richmond, CA) was used for electrophoresis. Proteins were visualised by Coomassie staining according to standard procedures. Broad range molecular weight markers for electrophoresis were from Bio-Rad (Richmond, CA, USA).

2.9. Anion exchange chromatography (AEC)

All chromatographic steps were run on a Pharmacia FPLC system. Lipase sample was concentrated and dialyzed with an Ultrafree®-MC filter unit (Millipore), 10 kDa cut-off, using a 5 mM Tris-HCl buffer (pH 8). The concentrated sample was loaded to a Source® 15Q HR5/5 column equilibrated with 20 mM Tris-HCl (pH 8), at a flow rate of 1 ml min^{-1} and 2 ml fractions were collected. To elute proteins, NaCl concentration was increased from 0 to 1 M in 40 minutes. Lipolytic activity was determined in the fractions collected using the procedure previously described.

2.10. Amino terminal sequence determination of proteins

The protein samples were separated by SDS-PAGE and electrotransferred to Immobilon PVDF membranes (Bio-Rad) using a MiniProtean II blotting unit (Bio-Rad). The proteins were visualized by Coomassie following manufacturers instructions. The protein bands of interest were excised from the membrane and subjected to N-terminal sequence determination using a Beckman LF3000 sequencer.

2.11. Analytical methods

Moisture content, total organic matter, pH, conductivity and compost maturity grade (Dewar self-heating test) were determined according to the standard procedures (U.S. Department of Agriculture and U.S. Composting Council, 2001). Lipid content was measured using a standard Soxhlet method using n-heptane as organic solvent (U.S. Environmental Protection Agency, 1998).

Respiration Index was determined in a static respirometer built according to the original model described by Ianotti et al. (1993) and following the modifications and recommendations given by the U.S. Department of Agriculture and U.S. Composting Council (2001).

3. Results and Discussion

3.1. Laboratory scale experiments

Preliminary co-composting experiments were carried out using sewage sludge as basic substrate and increasing amounts of fat ranging from 0 to 80% of total dry weight. In general, fat content could be increased up to 50% with a thermophilic period maintained for longer times (data not shown). This is a positive effect in low energetic sludges such as anaerobically digested sludge, since it permits to fulfil international regulations on compost sanitation (U.S. Environmental Protection Agency, 1995; European Commission, 2001). For higher amounts of fat content, however, oxygen diffusion was severely hampered. Moreover, moisture content could not be maintained within the optimal range for composting (40-60%). Therefore, a fat content of 50% (dry basis) was considered as the upper limit for the co-composting process.

Fat and sludge co-composting process was carried out at laboratory scale to determine the optimal fat content of the mixture (within 0 to 50% dry basis) in terms of process duration and fat content reduction. Lipolytic activity during the process was also studied. Composting experiments were undertaken for 36 days: a control experiment with no fat added to the sludge; sludge with 30% of total fat content (L30); and sludge with 50% of total fat content (L50).

The initial moisture content, total fat content, pH and conductivity of the mixtures sludge:fat with bulking agent are presented in Table 3. Moisture content was within the recommended range for composting (40-60%) (Haug, 1993) during the process for all the mixtures.

Figure 1 shows the temperature profiles, the evolution of total fat content and the lipolytic activity during the composting process of different laboratory scale experiments. Missing data on days 19, 27 and 33 correspond to system failures, with no oxygen control and no data acquisition. As can be observed, control experiment did not reach the thermophilic range of temperatures in the whole composting period which can be attributed to a low chemical energy content found in anaerobically digested sludge (Gea et al., 2004). However, temperatures in L30 and L50 experiments reached the thermophilic range (temperatures above 45ºC), and temperatures above 55ºC were maintained for an average period of 6.6 days for L30 and 10 days for L50. Although the international requirements on compost sanitation were not completely fulfilled for L30 and L50 experiments (U.S. Environmental Protection Agency, 1995; European Commission, 2001) it is likely that composting of these mixtures at large scale will ensure a complete sanitation of compost, because of the thermal inertia effect found in large composting masses (Haug, 1993). The strategy of co-composting a high energy waste such as fats or fat-enriched wastes with a low energy waste such as certain sludges can be recommended in order to achieve the sanitation of compost. As expected, thermophilic phase duration was significantly longer for L50 (Fig. 1c). Thus, although mixtures with fat content up to 50% (dry basis) can be composted, a lower fat content can be recommended if excessively long composting periods are to be avoided (e.g. in full-scale facilities).

A significant fat reduction was observed in all the experiments (Fig. 1) although a remaining fat fraction was found in all the final products. It is possible that fat present in sludge contents a recalcitrant fraction of non biodegradable lipidic substances. Nevertheless, a detailed chemical composition of sludge was not available. Table 3 shows total dry weight and total fat reductions. As can be observed, dry weight reduction was similar for L30 and L50 (near 40%), and higher than that of the control experiment (14%). The higher fat content reduction was for L30 (85%) while L50 presented a lower efficiency in fat degradation (68%). It must be pointed that these results are obtained with an initial fat content much higher than those previously reported (Filippi et al., 2002; Lemus and Lau, 2002b; García-Gómez et al., 2003).

Lipolytic activity (Fig. 1) was higher for L30 experiments, which is also in accordance with a higher fat content reduction. In all experiments lipolytic activity tended to increase at the beginning of the process and to reach a final plateau (Fig. 1a to 1c).

The concentration of the main long chain fatty acids is an indicator of the lipolytic activity. LCFA's were not detected in the control experiment and detected only the first 11 days in L30. In L50, LCFA's concentration increased until day 22 following the same pattern found in lipolytic activity, to decrease to negligible values at the end of the process (Fig. 2).

From these results, it is probable that the addition of fats to a 30% total content results in a balanced environment for microorganisms responsible of organic matter degradation, and the necessary lipolytic activity, the consumption of LCFA and the degradation of the rest of organic matter are not limited, which provoked the highest lipolytic activity (Fig. 1) and no accumulation of LCFA. On the contrary, with a high fat content (50%) it seems that the microbial activity and lipolytic activity is hampered, which can be attributed to a combination of different phenomena such as nutrient limitation, insufficient moisture or low oxygen diffusion (Sasaki, 2003).

3.2. Pilot scale composting

A co-composting experiment with the recommended maximum fat content determined at laboratory scale (30%) was carried out at pilot scale (P30) to monitor the most significant biological activity parameters and to obtain an amount of compost that permitted to study lipase characteristics. Table 3 presents the initial parameters of the mixture which were again in the optimal range for composting (Haug, 1993). The LCFA profile of the fat content in the mixture, very similar to that of animal fat, is presented in Table 2.

Figure 3 shows the temperature, oxygen and CO_2 profiles obtained in the co-composting process, and the moisture content evolution. Periods with no data in Figure 3 (days 4 and 11) correspond to system failures with no oxygen control and no data acquisition. As can be observed, the process followed the typical temperature composting profile, with a thermophilic temperature reached at the first days of composting and maintained during a long period (temperature above 55ºC for a total period of 14 days). Therefore, it is confirmed that increasing the composting scale, the sanitation requirements are fulfilled. As can be observed in Table 3, total dry weight and total fat reduction in P30 experiment were similar to those found in the L30 experiment.

From day 28 to 33 and from day 38 to 42 temperature decreased due to the low moisture content reached (Fig. 3a). High amounts of leachate were produced during the process due to the high hydrophobic nature of fats (the total amount of leachate accounted for 25% of the initial weight of material in the composter). This fact produced a reduction of the moisture content of the composting material and limiting values for microbial activity were reached (< 40%), which produced a temperature decrease. When the material in the reactor was watered to reach a moisture content above 45%, the microbial activity and thus, temperature, recovered quickly (Fig. 3a). When fat content was below 15% (from day 40) the leachate generation decreased, which reduced the water requirements to maintain the optimal moisture level.

Oxygen content in interstitial air was maintained above 10% along the process, ensuring aerobic conditions (Fig. 3b). Respiratory Quotient (RQ) is the relation between CO_2 produced and O_2 consumed. Its value is approximately 1 under aerobic conditions although it depends on the oxidation degree of the organic matter to be degraded. Average value of RQ was 0.98 ± 0.21 (Fig. 3b) which indicated a relatively low oxidation degree of the organic matter composted. This is in accordance with the fact that fats are reduced carbon compounds. Moreover, the values obtained were lower than those obtained in previous composting experiments using only sewage sludge, were RQ was 1.09 ± 0.08 (Gea et al., 2004). Weppen (2001) reported a similar RQ decrease when fats were added to municipal solid waste (RQ decreased from 0.95 to 0.87). However, it must be pointed that the dispersion of RQ values was high, which was mainly due to the RQ reduction between days 15 and 30 (Fig. 3b). During this period, moisture content decreased (Fig. 3a) due to the high leachate generation. It has been reported that microorganisms under stress conditions may reduce the CO_2 production using oxygen mainly for maintenance operations (Priess and Fölster, 2001; Dilly, 2003). This hypothesis could explain the reduction observed in CO_2 emissions and thus, in RQ, and it is confirmed by the fact that no significant fat reduction was observed from day 22 to 32 (see Fig. 4b).

Lipolytic activity and total fat content are presented in Figures 4a and 4b respectively. Fat reduction was lower in the first two weeks of process (7.5% of total fat reduction). It is probable that during the first period, other more biodegradable molecules present in sludge are consumed, and the lipase production is delayed (Fig. 4a). However, it is also likely that the extraction of lipase from the compost matrix may not be complete for high fat contents since it is well known that lipases present a high affinity to be adsorbed on interphases (Boczar et al., 2001). The fact that lipolytic activity is observed at the end of the pilot composting experiment could be due to a release of the absorbed lipase at lower fat contents (Fig. 4a and 4b).

Figure 4b also shows the different LCFA concentrations measured during the composting process. The main organic acids detected (oleic acid, stearic acid and palmitic acid) followed the same profile. Their concentration slightly increased during the first 20 days of process to decrease thereafter until the end of the experiments, when they could not be detected. The initial accumulation of LCFA (Fig. 4b) is an evidence that some lipolytic activity was present in the composting matrix although it was not detected (Fig. 4a), which could be due to an insufficient extraction procedure. The oleic acid concentration detected was 4-7 times higher than palmitic acid concentration and 4-15 times higher than stearic acid concentration. As these ratios do not correspond to the initial composition (Table 2) it seems clear that there is a different biodegradation pattern for the LCFA present in the composted waste, presenting oleic acid a low biodegradability when compared to other shorter and saturated LCFA such as palmitic and stearic acid.

Maturity and stability tests (Self-heating Test and Respiration Index) were carried out in the final period of the process. Results are presented in Table 4. After 69 days of process the material presented a high maturation and stabilisation degree, which is in accordance with requirements for compost application to soil (Scaglia et al., 2000; U.S. Department of Agriculture and U.S. Composting Council, 2001).

3.3. Characteristics of lipases

As stated before, the lipolytic activity was followed during the co-composting process (Fig. 4a). In addition to standard measures at 30ºC, a thermophilic temperature (50ºC) was selected for lipolytic activity determination to obtain activity values according to real temperature conditions in the composting environment. Lipolytic activity detected at 50ºC was significantly higher than that detected at 30ºC but both activities followed the same pattern (Fig. 4a). Once it was detected, activity increased progressively to decrease on day 41 when moisture content reached values limiting for microbial activity and temperature decreased. Once the moisture content was recovered, lipolytic activity increased again. Again, this fact seems to confirm the intimate relationship between adequate moisture content and the microbiological activity related to the enzyme production.

A lipase sample extracted on day 34 of process (thermophilic period) was used to determine the properties and characteristics of the enzyme. Figure 5 shows the lipolytic activity of the sample at different temperatures of the activity test. As can be observed, activity was higher for thermophilic temperatures, which is in agreement with temperatures found in the composting environment at the moment of sampling.

Additionally, the combined effect of pH and temperature (T) on lipases stability was determined by means of a factorial experimental design. From the experimental design results (normalized data) the next equation was obtained to describe lipase stability (expressed as residual activity):

$$\text{Residual Activity (\%)} = 114.2 - 49.2T + 28.0pH - 42.9T^2 - 35.6pH^2 - 10.3TpH \qquad \text{(Eq. 2)}$$

Figure 6 shows the response surface obtained from the above equation. The lipase presented a high stability for most of the conditions tested, as can be deduced from the independent term over 100% (114.2%). It is evident that some of the conditions tested in the experimental design contributed to the lipase activation. The interaction term T-pH on the equation 1 indicated a simultaneous effect on lipase stability of both factors. The lipase sample was more sensitive to the effect of temperature than that of pH (temperature coefficients significantly higher than pH coefficients). The value of coefficients indicated that high values of temperature had a negative effect on stability whereas alkaline values of pH presented a positive effect on stability. These effects can also be observed in Figure 6. Optimal conditions for stability were found at 38.3ºC and pH 7.97 (136.5% residual activity for optimal conditions). Optimal pH corresponded to the values of the composting experiments, however, it was expected to find an optimal value of temperature within the thermophilic range according to composting conditions. However, our results are in agreement with stability results for other lipase not related to composting field (López et al., 2004) and other composting enzymes (Kim et al., 2004), where optimal values are found within the mesophilic range of temperatures.

3.4. Lipase identification

The sample used for the lipase stability study (thermophilic period of P30 experiment) was purified by anion exchange chromatography and a subfraction with lipolytic activity was eluted at a concentration of NaCl 0.6 M (data not shown). This procedure permitted to remove non-lipolytic proteins that are usually present in complex samples from composting environments or sludges (Boczar et al., 2001; Gessesse et al., 2003). SDS-PAGE of this fraction is presented in Figure 7. Two major bands were observed corresponding to molecular weights of 29 and 62 kDa. To identify these proteins, N-terminal sequence was determined on each of these bands. The N-terminal sequence of 62 kDa band could not be determined precisely, probably due to an insufficient amount of protein transferred in the blotting unit. This band may correspond to a fungi lipase since similar molecular weights are found in lipases produced by yeasts and fungi (Sánchez et al., 1999a; Sánchez et al., 1999b). The first amino acid of the N-terminal sequence of the 29 kDa band could not be identified. The following amino acid sequence was FELPALP or IELPALP. These sequences were analysed for similarities to other sequences in the National Center for Biotechnology Information (NCBI) database and no correspondences were found for known lipases. It is then likely that some non-identified microorganisms are responsible for the production of extracellular lipases in the composting environment.

4. Conclusions

Several conclusions can be obtained from this work:

1) Co-composting process of fats and sludge can be successfully carried out to obtain a sanitized and stabilized product. Addition of fats content up to 50% is possible although a maximum fat content of 30% is recommended to achieve a high fat content reduction (85%) and to avoid long composting periods at full-scale facilities.

2) Fat-enriched wastes can be added to low energy content wastes to fulfil the international requirements on compost sanitation. From this point of view, co-composting strategies are of special interest for plant managers.

3) Moisture content is a critical control factor in the composting of fat-enriched wastes, since it determines the biological activity of the process, as it is observed from the respiratory quotient and lipolytic activity.

4) Lipase from thermophilic composting environment showed a high stability for mesophilic values of temperature and slightly alkaline values of pH, however, the maximum lipolytic activity was observed at thermophilic temperature.

5) Major lipases involved in the composting of sludge:fat mixture could not be identified by N-terminal sequence analysis. Further research is needed in the field of enzyme identification in composting environments.

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Tables

Table 1. Main characteristics of the composted materials.

Parameter	Sewage Sludge	Wood Chips	Animal Fat
Moisture (%)	72.7	5.0	<1
Dry Matter (%)	27.3	95.0	>99
Total Organic Matter (% dry basis)	61.5	99.4	>99
Fat content (% dry basis)	13.0	-	>99
N-Kjeldhal (% dry basis)	2.6	0.1	<0.02
C/N ratio	8	500	>4000
pH	7.6	-	-

Table 2 Long chain fatty acids profile of the animal fat used as co-substrate and the initial mixture for pilot scale experiment (in parenthesis, number of carbon atoms and number of insaturations).

Material	Mystiric (C14:0)	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Other LCFA
Animal fat	3.0	30.0	17.0	38.0	6.0	6.0
P30 Initial mixture	3.7	27.4	20.7	36.1	2.1	10.0

Table 3. Initial properties of the mixtures co-composted in laboratory and pilot scale experiments and yields obtained in the composting process.

Parameter	Laboratory scale			Pilot scale
	Control	L30	L50	P30
Initial Moisture Content (%, wet basis)	55.7	48.2	35.0	54.4
Initial Total Fat Content (%, dry basis)	9.3	32.8	53.0	36.6
Initial pH	8.1	6.7	6.2	7.6
Dry Weight Reduction (%)	13.8	39.0	36.9	41.8
Total Fat Reduction (%)	50.2	85.3	68.5	83.4

Table 4. Stability assays: Respiration Index and Self-heating Test.

Day of process	Respiration Index (O$_2$ consumed per organic matter and time, mg g^{-1} h^{-1})	Self-heating Test (Maturity Grade)
20	2.4± 0.1	-
32	5.2 ± 2.5	-
44	1.2 ± 0.1	-
55	1.1 ± 0.1	III
62	1.3 ± 0.2	IV
69	1.1 ± 0.1	IV

Figure Legends

Figure 1: Laboratory scale composting: temperature (continuous line), total fat content (squares) and lipolytic activity (circles) for a) control experiment; b) L30; c) L50.

Figure 2: Evolution of the most significant long chain fatty acids in L50: palmitic acid (circles), stearic acid (triangles) and oleic acid (squares).

Figure 3: Composting experiment at pilot scale: a) temperature (continuous line), moisture content (circles) and water additions (triangles); b) oxygen (continuous line), CO_2 (dotted line) and respiratory quotient (open squares).

Figure 4: Composting experiment at pilot scale: a) lipolytic activity at 30ºC (circles) and at 50ºC (squares); b) total fat content (open circles) and LCFA concentration: palmitic acid (circles), stearic acid (triangles) and oleic acid (squares).

Figure 5: Effect of temperature on lipolytic activity.

Figure 6: Surface response corresponding to lipase stability for different pH and temperatures.

Figure 7: SDS-PAGE of lipase sample. Lane M: molecular weight standards; Lane 1: lipase sample from P30 experiment (thermophilic period).

Figure 1: Gea et al.

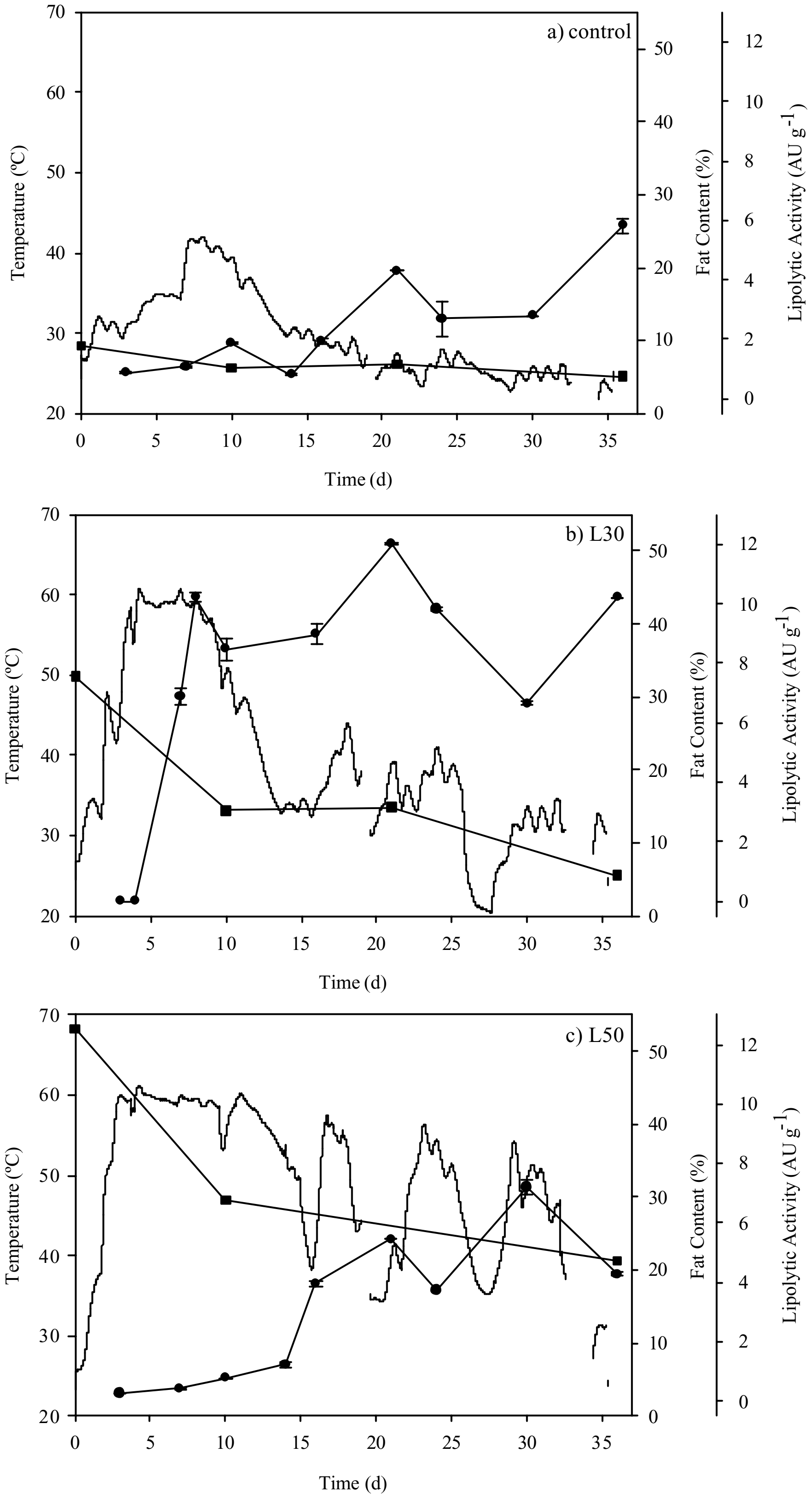

Figure 2: Gea et al.

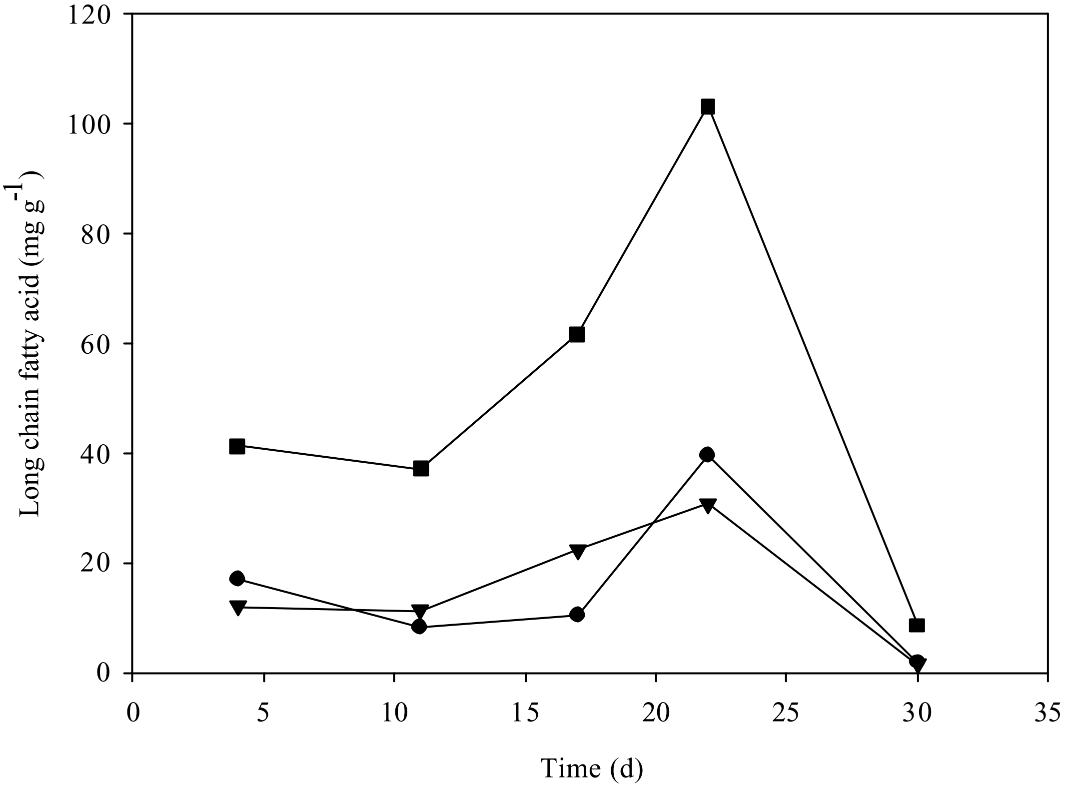

Figure 3: Gea et al.

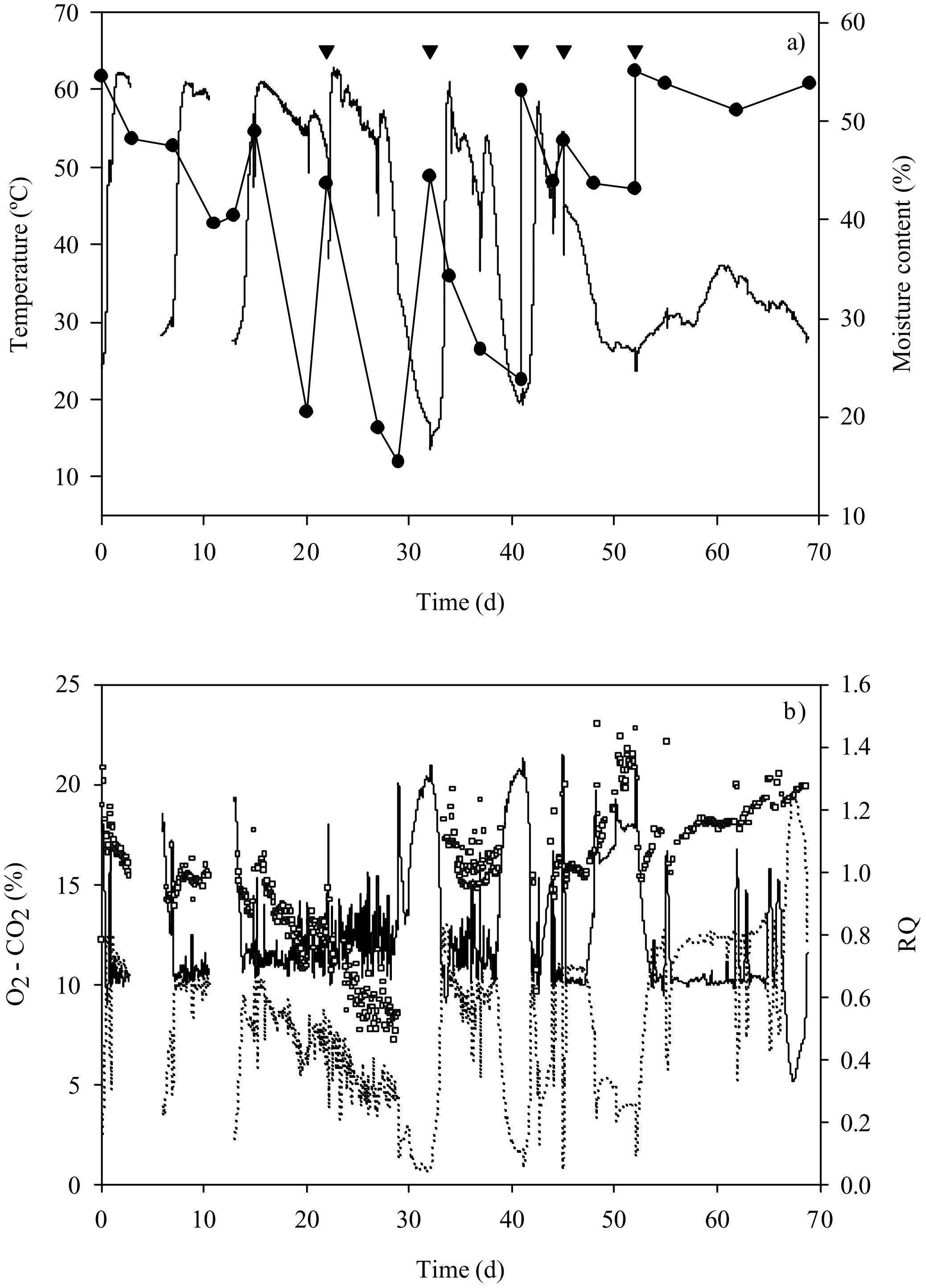

Figure 4: Gea et al.

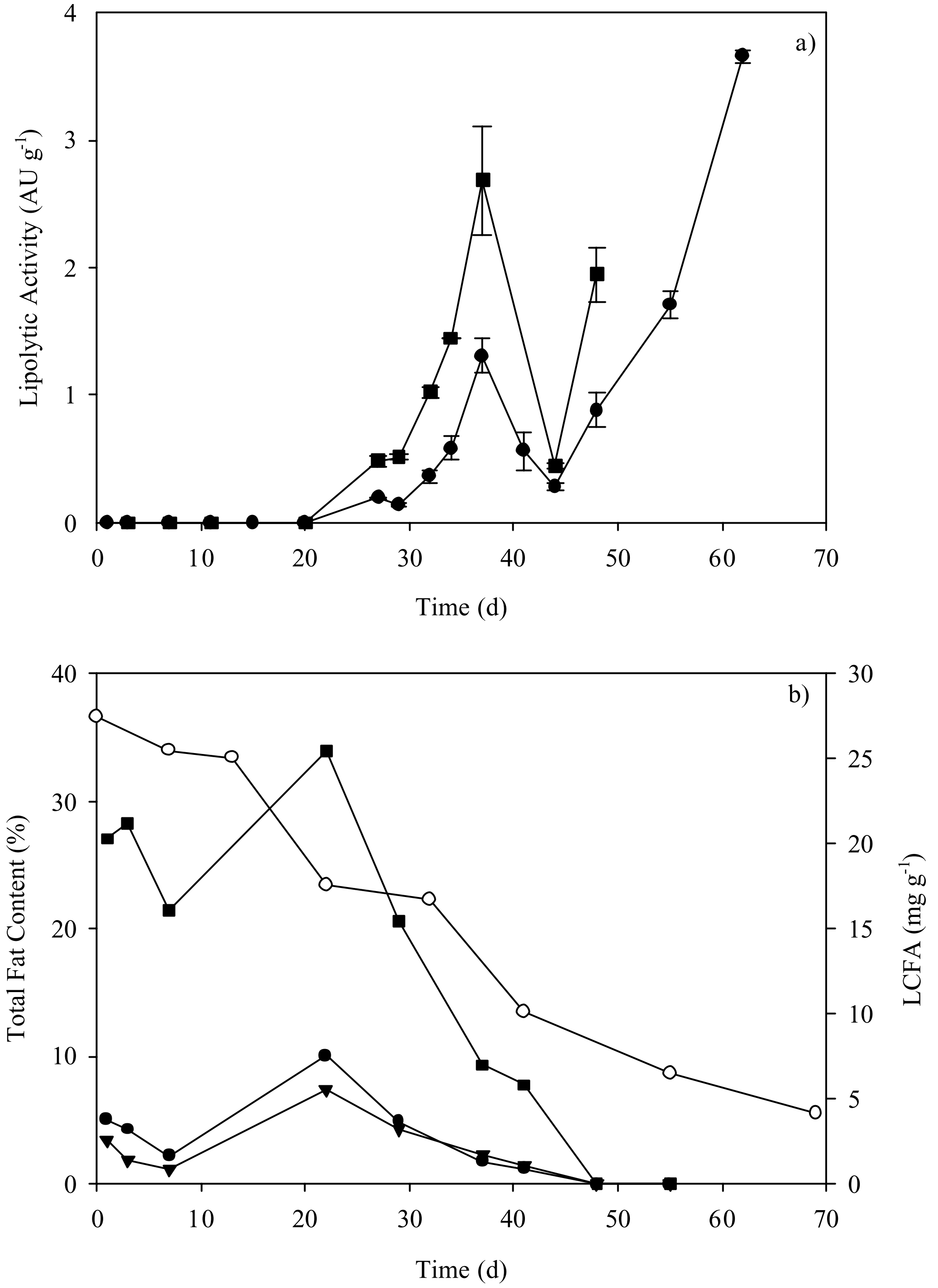

Figure 5: Gea et al.

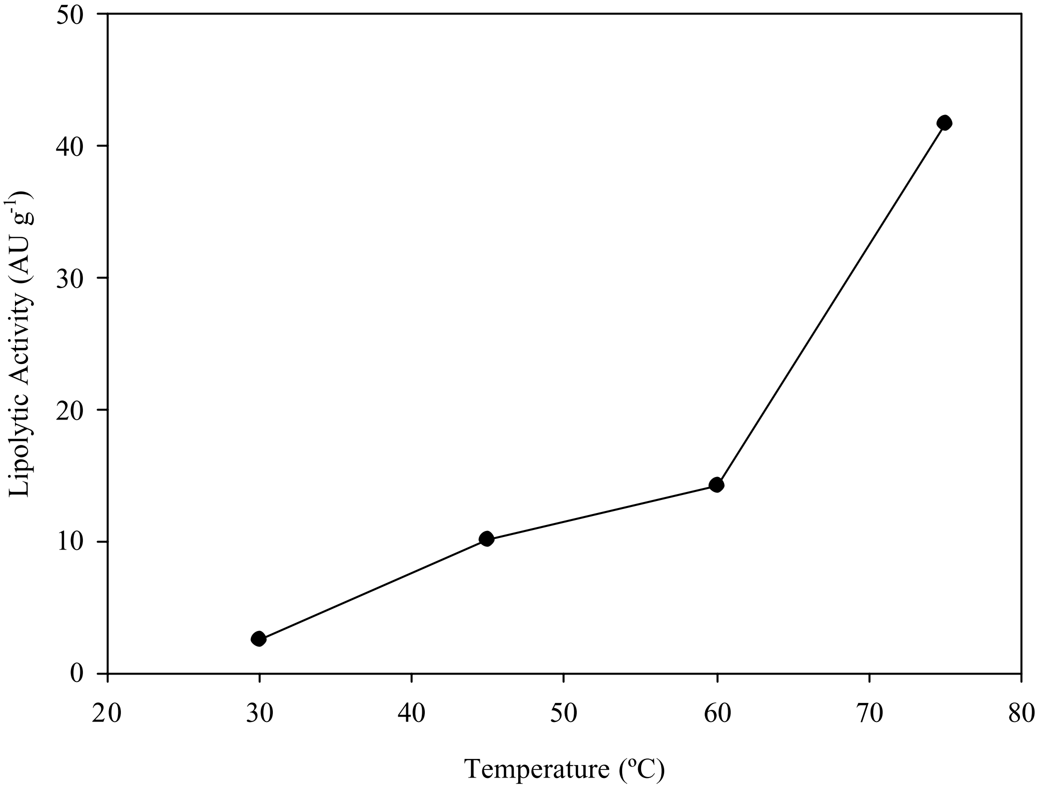

Figure 6: Gea et al.

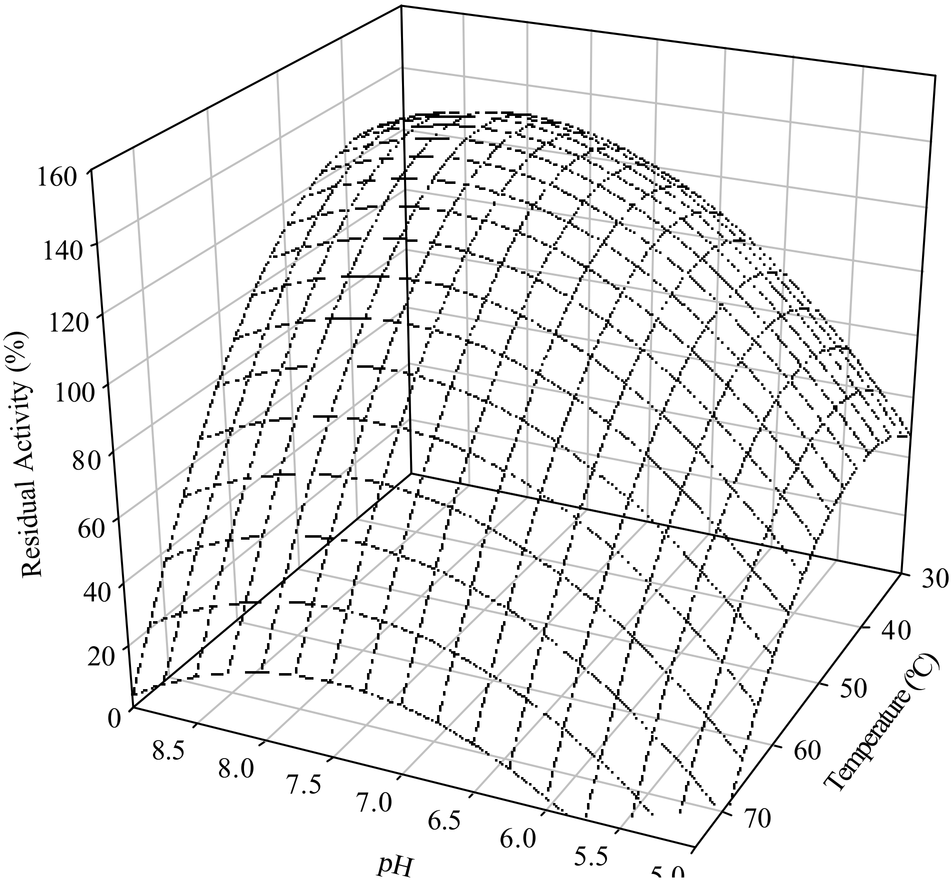

Figure 7: Gea et al.